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Volume 3

Sir Frederick S. Russell

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VOLUME 3

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VOLUME 3

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CONTRIBUTORS TO VOLUME 3

A. D. Boney, *Department of Botany, University College of Wales, Aberystwyth, Wales*

E. Naylor, *Department of Zoology, University College of Swansea, Wales*

Findlay E. Russell, *Laboratory of Neurological Research, Loma Linda University, Los Angeles County Hospital, Los Angeles, California, U.S.A., and Department of Zoology, University of Cambridge, England*

M. J. Wells, *Department of Zoology, University of Cambridge, England*

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LEARNING BY MARINE INVERTEBRATES

M. J. WELLS

Department of Zoology, Downing Street, Cambridge, England

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I. INTRODUCTION

It would clearly be ridiculous to limit a review of invertebrate learning to marine forms were the object to assess the relevance of research on invertebrates to the study of learning generally. This review does not attempt to do anything so ambitious. Its more limited objectives are to show how much marine forms have to offer for work on learning and how very little has been done with most of them. The hope in doing this is to persuade zoologists and psychologists that the existing literature is more than a mere anecdotage and that enough is now understood to justify much more intensive research; the range of material is vast, and much of it is very promising indeed.

In what follows the experiments surveyed are dealt with group by animal group, starting with the cephalopod molluscs which perform remarkably like the higher vertebrates in learning tests, and working through to the echinoderms and coelenterates where the very occur-

rence of " learning " depends on the definitions one cares to employ. This phyletic approach is more appropriate than a summary based on a classification of learning itself because of the diversity of the organisms concerned. Terms such as " habituation " or " trial and error learning " will be used, but it is intended throughout that these shall be understood to be descriptions of observations rather than implications that the phenomena depend in any sense on common mechanisms. For learning is just one of many ways in which animals adapt to their environment. In one species the ability to learn permits survival in more complex and diverse situations, in others it can be read as evidence of insufficient genetic variability. Learning implies mistakes and selection for appropriate innate responses is inevitable in any relatively unchanging habitat. Marine animals inhabit such a variety of environments that it would be absurd to regard them as faced with common problems in any but the broadest sense. Learning is not always the best solution in survival problems and it would be surprising to find these phenomena occurring other than erratically. Some animals learn only certain things under rather special circumstances; at other times their capabilities in this respect seem remarkably limited. Learning in invertebrate animals, in short, cannot sensibly be considered except in relation to their particular ways of life; the physiological background must be known and the capacities of each species investigated in relation to the things that the system is built to do. It is surprising how often this is forgotten, with the result that our knowledge of invertebrate learning is all too frequently based on their performance under conditions appropriate only for vertebrates. At best this can give only indirect evidence of the capabilities of the systems studied.

The present review is, therefore, in part a plea for a broad approach: learning cannot be studied in isolation. It is not intended as an exhaustive list of references. Some fields, cephalopod and crustacean learning for examples, have been the subject of comprehensive reviews within the last five years. Others have been practically neglected for decades. In general an attempt has been made to summarize the literature produced since the last substantial review, and where such is lacking, to cite key references with bibliographies that cover as many as possible of the experiments and observations that are not specifically mentioned.

II. Cephalopod Molluscs

More information is available about learning in cephalopods than for any other invertebrate group. For once, the primary concern is not whether the animals can learn or not, since they clearly do so whatever definition of learning one chooses, but rather what they can learn and

what are the mechanisms concerned. Most of the recent work has been done with *Octopus vulgaris* Lamarck.

Since Boycott and Young's first publication in 1950, there has been a rapid increase in the literature, so that at the present time something like ten or a dozen papers on learning in cephalopods appear every year. Two reviews are available, Wells (1962a), and Young (1961). Recent research has approached the subject by means of experiments on sensory integration, and experiments involving brain lesions in attempts to discover which parts are concerned in memory storage. It is convenient to subdivide the present account on this basis.

A. *Experiments on sensory integration*

Here the concern has been firstly, " What events in their external and internal worlds can octopuses detect?" And following this " What happens to sensory information as it passes from the sense organs towards those parts of the brain ultimately concerned with the accumulation of experience?" At the first level it is now clear that octopuses can be taught to make a wide variety of visual and tactile discriminations. They can taste things that they touch and they are sensitive to low-frequency vibrations, which they probably detect tactilely. They do not respond to sonic frequencies (Hubbard, 1960), although electrophysiological evidence (Maturana and Sperling, 1963) indicates that the statocysts can detect these, and they do not appear to have organs of electrical sense or anything comparable to the lateral line pressure receptors of vertebrates. They learn to recognize many patterns of stimuli very rapidly and the performance of individuals, despite their being taken from wild populations, is sufficiently consistent for the relative difficulty of discriminations to be compared from the number of trials required to attain given standards of accuracy of response.

1. *Detectable attributes of things seen*

Octopus vulgaris Lamarck of 200-800 g will live, feed, grow and even breed in aquaria, given a brisk circulation and some means of preventing the animals from climbing out. Experimentally, asbestos tanks 100 × 60 × 40 cm, with a " home " of bricks at one end, have proved convenient. The animals generally sit in the home, looking down the tank. Boycott and Young (1950) showed that it was possible to train octopuses kept in this way to distinguish between crabs shown alone and crabs shown together with plastic or metal shapes. The animals were allowed to seize and eat the crabs in the one situation and given small electric shocks (6–9 V A.C.) for attacking in the other. The result of a typical training experiment, with the two situations

presented successively at the end of the tank opposite the " home," is shown in Fig. 1. Subsequent developments of this technique, which has remained the basis of most training experiments carried out since, have led to the elimination of crabs in favour of discriminations between pairs of plastic figures, the animals being rewarded with small pieces of fish for attacking one and punished with shocks for attacking the other. Rates of training have varied between four and forty or more trials per day. Although there are obvious theoretical advantages in strictly randomized trial sequences, it has generally proved more practicable to

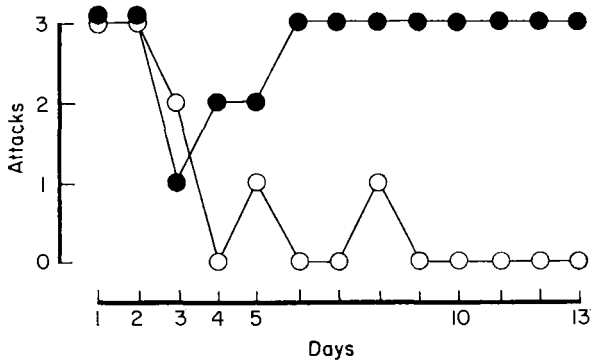


FIG. 1. Results of a training experiment with *Octopus*. The animal was trained to discriminate between a crab shown alone and a crab shown together with a 5-cm white square. There were three trials of each sort per day. Attacks on the crab alone are shown ●, attacks in the " crab plus square " situation ○. After the first 2 days the octopus was given a small electric shock each time that it attacked the crab shown together with the figure. (From Boycott and Young, 1955.)

arrange positive and negative trials in systematized orders, which ensure that groups of animals to be compared get comparable numbers of positive and negative trials; they commonly learn too rapidly to be sure of this occurring if random sequences are employed. So far there has been no evidence that octopuses can learn to recognize a trial sequence, or even learn to attack only at alternate trials.

2. *The discrimination of geometric shapes and the orientation of things seen*

Figures 2 and 3 show some of the shapes that octopuses can be trained to recognize, together with the scores made when groups of animals were trained to distinguish between pairs of these. It seems that octopuses do not take any of the obvious " short cuts " to visual discrimination, and in some manner recognize both the geometric shape

and the orientation of things that they see. The degree of transfer to similar shapes of different size depends on the size difference, and is made most readily to shapes somewhat larger than those used in training (Sutherland and Carr, 1962). Visual acuity is not a limiting factor in any of these experiments (Sutherland, 1963a), as might be

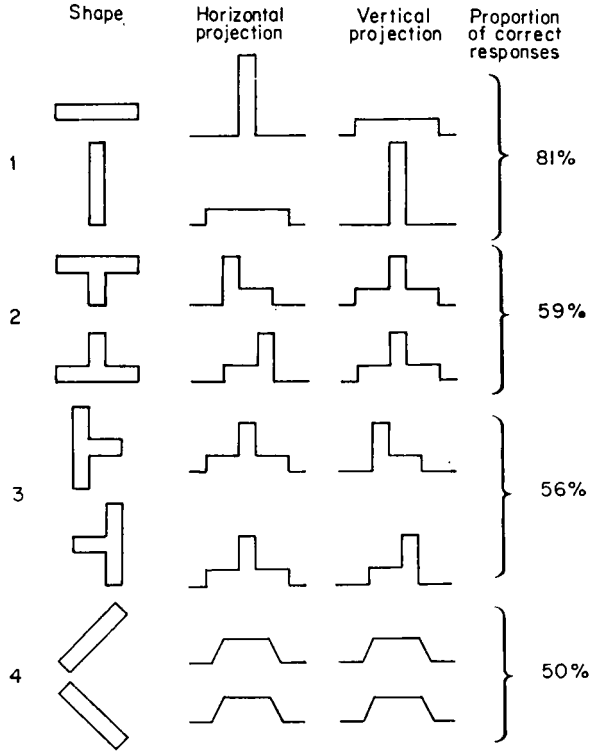


FIG. 2. Pairs of shapes that octopuses can and cannot learn to distinguish. Percentage figures show the proportion of correct responses made by groups of octopuses in the first 60 trials. Projections, see text. (From facts given in Sutherland, 1957.)

expected from the extremely close packing of the retinal elements and the very large numbers of optic nerves (Young, 1962, 1963b).

Differences in orientation are most readily distinguished when they are in the horizontal and vertical planes. Oblique rectangles are not distinguished at all under the same conditions. The results summarized in Fig. 2 and most other shape discrimination experiments can be summarized as showing that octopuses tend to classify the things that they see on a basis of their horizontal and vertical extents; by comparing the projections of any two shapes in these planes it is possible to

predict with a very fair degree of accuracy the proportion of errors that octopuses will make (Sutherland, 1957; but see also Deutsch and Sutherland, 1960; Dodwell and Sutherland, 1961).

There is evidence that horizontal extents are more accurately assessed than vertical extents (Mackintosh *et al.*, 1963). They also seem to be more important in determining behaviour. Thus the shapes shown in Fig. 3 are arranged in order of their similarity to octopuses. The animals were trained to distinguish between the cross and the

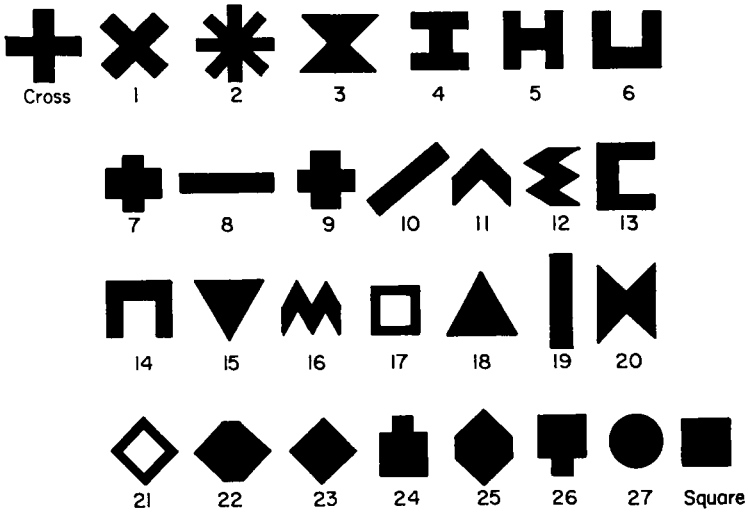


FIG. 3. Further shapes that octopuses can distinguish by sight. The order of the figures is determined by the number of attacks octopuses made on each in transfer tests following training to distinguish between the cross and the square. (From Sutherland, 1962.)

square, and subsequently tested with the other figures to determine, from the number of attacks made upon them, how like the original two figures they appeared to the trained experimental animals (Sutherland, 1962). The order of the figures appears to us to be determined along a dimension perhaps best described as " open " (large perimeter to area ratio) to " closed " (small perimeter/area). Some of the details of the order seem illogical unless one supposes that the animal pays more attention to horizontal than to vertical extents. It is otherwise not at all obvious why, for example, the horizontal rectangle 8 should be treated as so much more like a cross than the vertical 19, and shape 3 so differently assessed from 20, again the same shape in a different orientation.

These findings are particularly interesting in view of the anatomical studies of Young (1960a, 1962, 1964b) who has shown that both the retinal elements and the dendritic fields in the superficial layers of the optic lobes are arranged mainly horizontally and vertically. Moreover, that the number of vertically oriented dendritic fields is substantially less than those oriented horizontally, an observation clearly compatible with the more accurate estimation of horizontal extents. It has, moreover, been shown that octopus eyes are held in a fixed orientation with respect to gravity, so that the retinal and dendritic arrays indeed remain in a constant orientation with respect to things seen. When retinal orientation is upset by removal of the statocysts, visual dis-

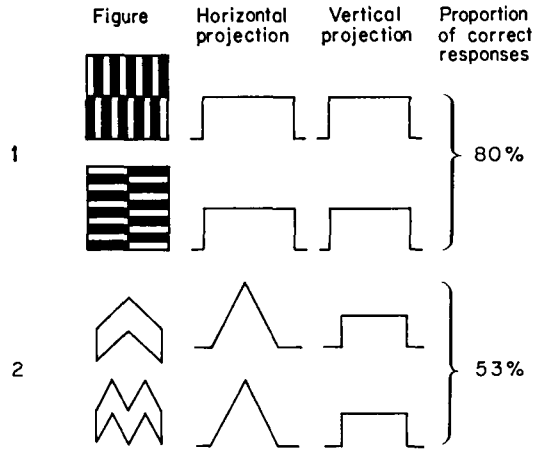


FIG. 4. Pairs of figures that octopuses can be trained to distinguish despite their having identical projections. The first pair are as readily distinguished as simple horizontal and vertical rectangles. Percentages show the proportion of correct responses in the first 160 (pair 1) or 240 (pair 2) trials. (From Sutherland, 1959, and Sutherland *et al.*, 1963.)

crimination of orientation fails, the animals continuing to behave as though the retinae were still held correctly (Wells, 1960). The implication is that for once the basis of an internal sensory classifying mechanism is both visible and to some extent comprehensible.

Perhaps unfortunately, it has since become apparent that classification in terms of horizontal and vertical extents is by no means the whole story, for octopuses can be trained (albeit sometimes with difficulty) to make a number of discriminations between objects that yield identical horizontal and vertical projections (Fig. 4). The horizontal and vertical axes in octopuses, as in most other animals (Sutherland, 1963b, 1964), are evidently important compared with other possible

visual axes; but the animals have alternative means of classifying shapes when consideration in these terms fails them. Indeed at the present time it appears possible that octopuses (again like some other animals, see Sutherland, 1964; Mackintosh, 1962, 1965) use a number of means of classifying the things that they see. It is argued that part of the problem of learning to make a discrimination is the selection of an appropriate analysing mechanism from the repertoire available. In reversal experiments, for example, octopuses, unlike certain other invertebrates, tend to improve in performance in successive reversals; ex-hypothesis, they start relearning with an appropriate analyser already " switched in." A similar explanation can be given of experiments in which learning one discrimination interferes with subsequent performance in others that have to be made on a different basis (Mackintosh and Mackintosh, 1963; Mackintosh, 1965).

Muntz (1961 a, b, c) has studied interocular transfer in discrimination experiments and shown that responses learned using one eye are also made when the animal is obliged to use the other eye. The degree of transfer depended on the degree to which the octopus had learned to discriminate before the test; it was almost complete for simple problems (Muntz, 1961a). Transfer is dependent on the integrity of the vertical lobe and the optic commissures linking the two optic lobes (Muntz, 1961b—see section B below).

3. *Discrimination of size and assessment of distance*

Octopuses attack small moving objects and retreat when confronted with unfamiliar moving objects larger than themselves. They can be trained to distinguish between geometrically similar objects of different size, and are not confused when the range of such objects is altered. Thus, octopuses trained to discriminate between squares of 5cm and 10cm side still discriminated when the 5cm square was shown at half the usual distance, and when the size of the tank was doubled to alter the scale of the objects relative to their surroundings (Boycott and Young, 1956). The fact that transfer to similar shapes of different size decreases with any increase in the size difference itself implies quite accurate recognition of particular sizes as properties of objects that octopuses have learned to recognize (Sutherland and Carr, 1962).

Recognition of size implies an ability to range objects seen, a capacity that has been independently demonstrated by Maldonado (1964) who showed that the jump of an octopus at its prey is determined before it takes off. The animal does not require to readjust its trajectory during the approach, and completes its jump accurately even if all light is extinguished as soon as it begins to swim forward. Decapods such as

Sepia, which strike prey with two long tentacles, rarely do so at an "incorrect" range; if too close to the prey, they back away before striking. There is evidence that the capacity to range prey and strike correctly is innate, since in decapods at least it does not measurably improve with practice, being as accurate immediately after hatching as subsequently (Wells, 1962b).

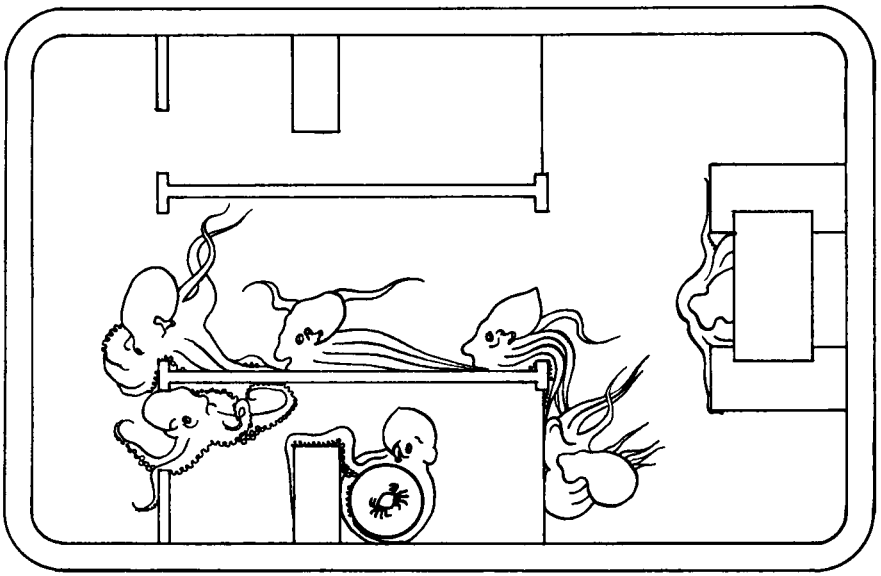
4. *Discrimination of wavelength and plane of vibration of light*

A number of experiments have been made on colour vision in cephalopods, and in some of these octopuses have been trained to respond to particular colours. The experiments, none of them recent, have been reviewed in Wells (1962a). They may be summarized as showing that cephalopods can, in all probability, learn to distinguish the wavelength of light reaching the eye; but none of the controls were sufficiently stringent to be certain of this.

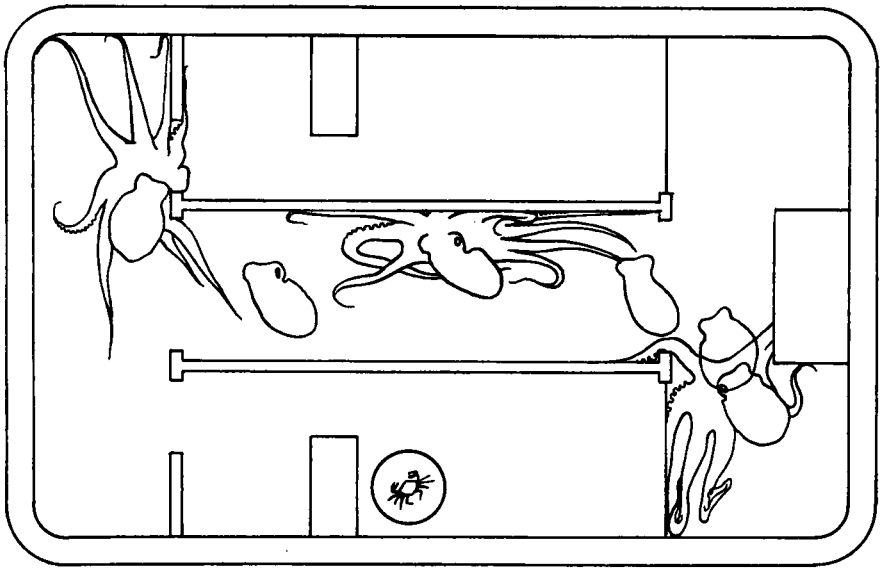
A number of more recent experiments have been concerned with the capacity to distinguish the plane of vibration of light reaching the retina. Octopuses, at least, can learn to respond to differences in the plane of polarization of light sources. As in shape discrimination experiments, they most readily learn to respond to horizontal and vertical differences; discriminations between obliquely polarized sources are learned less readily, a finding that appears to be related to the predominantly horizontal and vertical orientation of the individual retinal elements (Moody, 1962; Moody and Parriss, 1961). Further evidence that polarized light discrimination is based on intraocular detection of vibration plane rather than on patterns of reflexion in the experimental tanks comes from training experiments following statocysts removal. As in shape discrimination, recognition of orientation fails when retinal orientation is disrupted by the operation. Discrimination can be maintained only if the plane of the electric vector is matched to the position of the retina at each trial (Rowell and Wells, 1961). It is not known whether *Octopus* normally orients itself with respect to polarized light, but some squids do (Jander *et al.*, 1963) and it is at least possible that this is one of the means by which octopuses find their way about.

5. *Detour experiments*

Octopuses can be taught to make a detour in order to reach prey that they can see but cannot approach directly. In the apparatus shown in Fig. 5, crabs could be seen but not touched by octopuses confined to the home compartment. In order to get a crab, the animals had to go out of sight of their prey, into the corridor and along, making



(a)



(b)

FIG. 5. Detour experiments with octopuses shown a crab in one of two feeding compartments not directly accessible from the "home". (a) shows a run by an unoperated animal. At this trial the animal struggled against the transparent wall of the feeding compartment for 15 sec before going into the corridor; pictures of the animal show its position at 2-sec intervals thereafter. (b) shows an incorrect detour by an octopus blinded in the right eye. The position of the animal is again shown every 2 sec during the detour. (Tracings from film, from Wells, 1964b.)

appropriate left–right choices on reaching the end of the corridor. Most individuals had to be shown a crab and led round the maze on one or more occasions before they did it spontaneously, but once having made a successful detour, an octopus rarely failed. Thus eleven animals, each tested for twenty trials at four runs per day starting with the trial at which it first made a successful spontaneous run, together failed to detour or went into the wrong compartment only five times in 220 trials. Both the time spent trying to get directly through the glass, and the number of abortive entries into the corridor (animal returns to the attack without completing the detour) became less as training continued (Fig. 6). Having learned to detour to one side, the animals performed

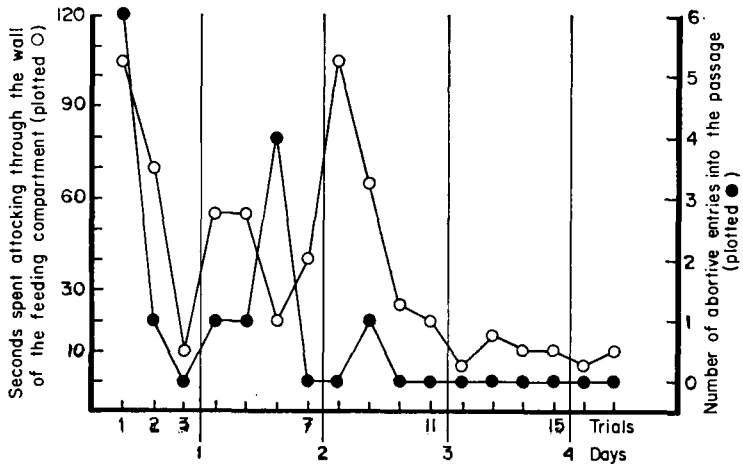


FIG. 6. A summary of the performance of an octopus in successive trials of a detour experiment in the apparatus shown in Fig. 5. Both the time taken to enter the passage and the number of abortive entries into the passage declined as tests proceeded. (From Wells, 1964b.)

faultlessly first time when tested in detours towards the other. There is evidence that the orientation of the animal towards its prey is maintained entirely visually. Thus unilateral blinding produces systematic errors, the operated octopuses going into the wrong compartment when required to detour towards the unblinded side, despite the positional disorientations that this involves (Fig. 5). Removal of the statocysts does not prevent successful detouring (Wells, 1964b).

6. *Learned and innate components in visual responses*

It seems to be particularly difficult to teach octopuses to ignore crabs, and certain brain lesions that leave the animals still capable of

learning other discriminations (see Section B, p. 15) appear to abolish altogether the capacity to learn to leave crabs alone (Boycott and Young, 1958). It would be nice to know whether this is merely due to long experience of good meals in the past, or because the response to crabs is truly innate. *Octopus vulgaris*, unfortunately, has very small pelagic larvae and while the responses of the related *O. briareus* Robson are potentially studiable from the moment of hatching, the animals have not so far been observed to attack (Messenger, 1963).

Sepia has proved more amenable to study. This animal lays large eggs, and the newly hatched young settle at once to bury themselves in the sand, whence they emerge, apparently just like adults, to attack

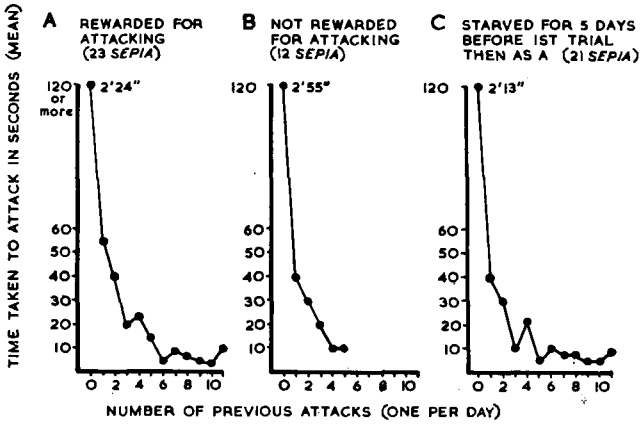


FIG. 7. Mean times taken to attack mysids in successive trials with young *Sepia*. The animals, newly-hatched in the laboratory, had never seen prey before the start of the experiment. The cuttlefish in the " B " series attacked but did not get mysids shown in a glass tube. It appears that the action of attacking is self-facilitating. (From Wells, 1958.)

moving prey. Naive *Sepia* will attack mysids, and occasionally models more or less like these. With further attacks the delay between observation and attack decreases, suggesting learning by experience. But at the same time the range of small moving objects attacked increases, suggesting that the action of attacking is in some way self-facilitating, so that the threshold for the response falls independently of learning anything from experience of which objects proved edible (Fig. 7). Within the first week or two of hatching *Sepia* appears to be virtually untrainable, a finding that is interesting because it is associated with the relatively slow post-embryonic development of the vertical lobe of the supraoesophageal brain, a part known from lesion experiments to be intimately concerned in visual learning (Wells, 1962b; see also IIB

p. 15). Later, the animals can certainly be trained to make simple discriminations (Sanders and Young, 1940).

It would appear from the *Sepia* experiments that at least some responses are in the first instance innately determined; this animal does not by any means attack indiscriminately, even when faced with potential prey for the first time in its life. It seems, however, exceedingly unlikely that innate mechanisms determine the responses of the animal towards its prey throughout life, if only because cuttlefish increase in size by a factor of a thousand or more during the first two or three years after hatching. The range of animals to be eaten or avoided must keep changing during this time, and adults, like newly hatched cuttlefish, are by no means indiscriminate. Innate prey recognition would imply a very elaborate size-dependent programme of maturation within the CNS. Knowing how readily cephalopods can be taught in the laboratory, it seems only reasonable to suppose that their responses in the wild are based largely upon individual experience.

7. *Detectable attributes of objects touched*

Octopuses handle things that they touch with the suckers, and animals blinded by section of the optic nerves can be taught to make a number of tactile discriminations on the basis of information from sense organs in the rims of the suckers. These latter are sensitive to both texture and taste, and in chemotactile training experiments octopuses have proved to be at least one hundred times as sensitive as the human tongue to substances such as quinine sulphate, hydrochloric acid and sucrose (Wells, 1963a).

Rather more extensive experiments have been made on the capacity of octopuses to distinguish the physical attributes of objects touched. It seems that they can be taught to distinguish textures, but not weights or shapes, except inasmuch as shape differences involve sharp corners which are detected as differences in texture (Wells, 1964a). Thus, Perspex cylinders made to differ in texture by cutting narrow grooves into the surface were regularly distinguished provided that the proportion of grooved to smooth surface was different; the distribution of the grooves—whether they ran up and down or around the cylinders—was irrelevant (Wells and Wells, 1956, 1957). In some more recent experiments, octopuses were required to distinguish between smooth cylinders of different diameter. The proportion of errors made was found to depend on the difference in surface curvature, that is on differences in the degree of distortion of the applied suckers (Fig. 8). The overall diameter of the cylinders was shown to be irrelevant by experiments with compound cylinders made up from rods of smaller

diameter; these were treated as being of the diameter of their components. In some transfer experiments it was found that textural differences could be substituted for diameter differences. Thus grooved cylinders were taken and smooth ones rejected where octopuses had previously been trained to take the smaller of two smooth cylinders,

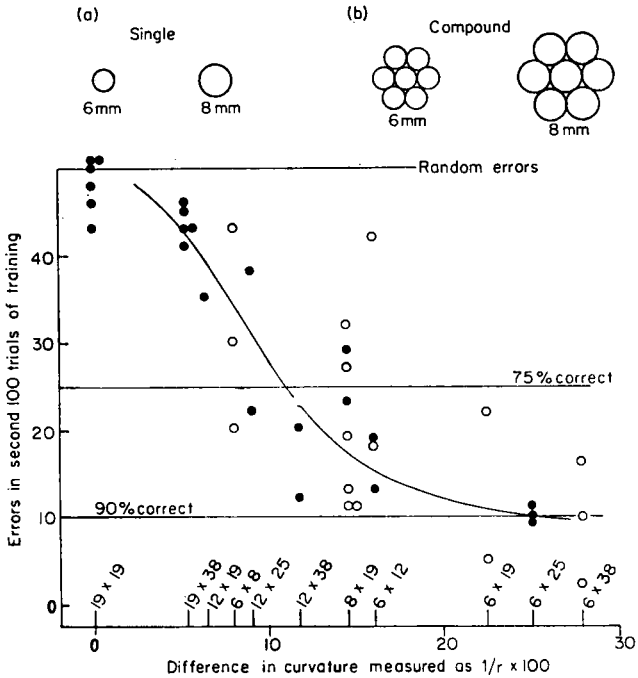


FIG. 8. Showing that the proportion of errors made varies with the difference in surface curvature of objects to be distinguished Each point plotted shows the result of an experiment in which an octopus was trained to discriminate between two cylinders of different diameter. The diameters of the pairs of cylinders are given (in mm) along the abscissa, which also shows the difference between the cylinders in terms of surface curvature. Plots ● and ○ respectively show the results of experiments with simple and compound cylinders ((a) and (b) above show the relative sizes of the 6 and 8 mm cylinders of the two types), the stated diameter in each case being the unit and not the overall diameter, which would appear to be irrelevant. (From Wells, 1964a.)

which implies that the receptors used for diameter assessment and textural detection are the same (Wells, 1964a). The anatomy of the receptors in the suckers has been described by Graziadei (1962, 1964) and a preliminary attempt to link these anatomical results with the experimental results has been made in Wells (1964a).

Octopuses cannot be taught to distinguish between similar objects

differing in weight. This is rather a surprising result, since octopuses quite obviously compensate by increasing muscle tension to support objects they pick up. Any observer, watching octopuses handling the test objects in experiments designed to train them to distinguish weights, can tell, simply by watching the animal, which object it is handling. An arm grasping a heavy object is passively extended as it takes the weight and contracts to take the strain before passing the object to the mouth or rejecting it in the normal way. The animals seem incapable of learning that one of the two test objects necessitates their doing this (Wells, 1961).

The results of texture, size and shape discrimination experiments all imply that octopuses never take into account the spatial distribution of the sense organs in contact with objects they handle. Together with the weight discrimination experiments they suggest that octopuses cannot use information about the position or movement of parts of their own bodies in learning. The tactile discriminations that they fail to make are those that would require integration of proprioceptive inputs with information from more superficial receptors. This finding is consistent with the consequences of statocysts removal in visual experiments. Once retinal orientation is upset the animal seems incapable of orientation discrimination, and indeed behaves as if the retinae were still correctly orientated: the implication is again that the animal cannot take its own bodily position into account (see II, A, 2, p. 7). It seems that in octopuses at least proprioceptive inputs are utilized for local adjustments to movement and that this class of information never penetrates to levels of the central nervous system concerned in learning. It can be argued that a hierarchic decentralized control of bodily movement is inevitable in animals where movement is unrestricted by joints, and that a similar state of affairs with failure to learn to recognize proprioceptive inputs may be expected in other animals with hydrostatic skeletons (Wells, 1963b, 1965b). It should be noted that a failure to use information from muscular stretch receptors is characteristic of vertebrates too (see Merton, 1964) and that the octopod failure to learn from the position of parts of its own body is associated with an absence of anything comparable with the vertebrate deep pressure, joint or tendon receptors (Wells, 1964b).

B. *Brain lesions and learning in cephalopods*

Since it was found that octopuses can so readily be trained to make visual and tactile discriminations, a considerable number of experiments has been made to identify the parts of the brain responsible. The brains of cephalopods are divided into about thirty anatomically

distinct lobes, each of which has a characteristic structure (Thore, 1939; Young, 1963a). Only a few of these concern us here, and the relative positions and some of the connexions of these are shown in Fig. 9. They are all parts characterized by very large numbers of small cells, none of which are final motor pathways (Young, 1963a).

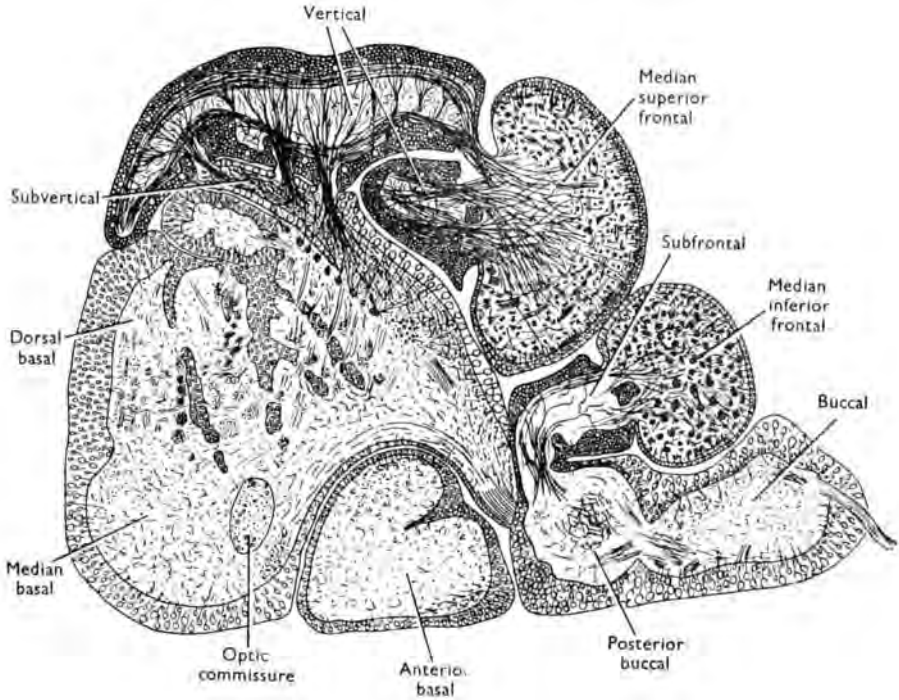


FIG. 9. A longitudinal section through the supraoesophageal lobes of the brain of *Octopus*, showing the relative positions and some of the connexions of parts mentioned in the text. This section is cut slightly to one side of the mid-line. More centrally the subfrontal would extend downwards to the region here occupied by the posterior buccal lobe, and the part of the vertical immediately behind the superior frontal would run backwards to join the rest of the lobe. The lateral divisions of the inferior and superior frontal lie to the sides and somewhat ventrally to the median parts of the same lobes. (From Young, 1963c.)

It has been known since the experiments of Sanders and Young (1940) on *Sepia* that the vertical and superior frontal lobes are in some way concerned in visual memory, and all the more recent experiments on *Octopus* have confirmed this. With the vertical lobe removed, the performance of octopuses in shape discrimination experiments is much impaired. The animals learn more slowly and may never achieve the standards attained by intact individuals. Otherwise they are indis-

tinguishable from normal octopuses. Removal of the superior frontal lobe has a similar effect. It is assumed that the residual capacity to learn is at least partly due to structures in the optic lobes, which cannot be removed without blinding the animals.

Similarly, it has been found that removal of the inferior frontal and subfrontal lobes abolishes touch learning whilst having no other detectable effect on the animals' behaviour. Partial lesions to the vertical and subfrontal lobes have effects proportional to the amount taken out, with, in the case of the subfrontal lobe, some indication that a minimum number of cells is required for satisfactory performance in any particular task. Approximately half a million subfrontal cells must be left if the animal is to discriminate effectively between a rough and a smooth perspex cylinder, while a few thousand suffice for learning to reject either when it is repeatedly presented (Wells, 1959). At the present time it appears that, while removal of the vertical and/or superior frontal lobes measurably slows touch learning, removal of the inferior frontal and subfrontal lobes is without any such effect on visual responses.

Reviews of the results of brain lesion experiments up to 1960–61 are included in Wells (1962a) and Young (1961).

Most recent work has been concentrated in an attempt to define more closely the function of the vertical and superior frontal lobes. Thus Maldonado (1963 a, b, c; 1964) has made a careful analysis of the effect of vertical lobe removal on learning to attack crabs and other objects seen at a distance. By accurate automatic measurement of the times taken to attack and the nature of the approach to the prey before and after the operation he showed (1) that both the time taken to complete an attack and the variability of this time from trial to trial increased as an immediate result of the operation, and (2) that after the operation details of the performance—the nature of the approach and the time delays—reverted to the condition seen in the same animals at the start of training; performance improved with further practice but they never became so swift and accurate as before. The effects were most marked in the animals with the largest lesions.

Maldonado's results show that the vertical lobe plays some part in positive learning as well as in learning *not* to respond. In this they confirm the results of a number of previous experiments in which vertical lobe removal has been followed by a drop in the number and speed of attacks on figures other than crabs which have hitherto appeared to constitute a special case (see II, A, 6, p. 12). It seems in general that vertical lobe removal reduces the probability of previous experience affecting responses. Individuals that attacked a lot before the

operation attack less afterwards and vice versa (Young, 1961, 1964a, b). Maldonado interprets these results as showing that the vertical lobe has a "general amplification" function, increasing the effects of commands to attack originating, presumably, within the optic lobes. He has developed an information-flow model, in which at least some of the components are identifiable with specific lobes and tracts in the supraoesophageal brain (Maldonado, 1963c).

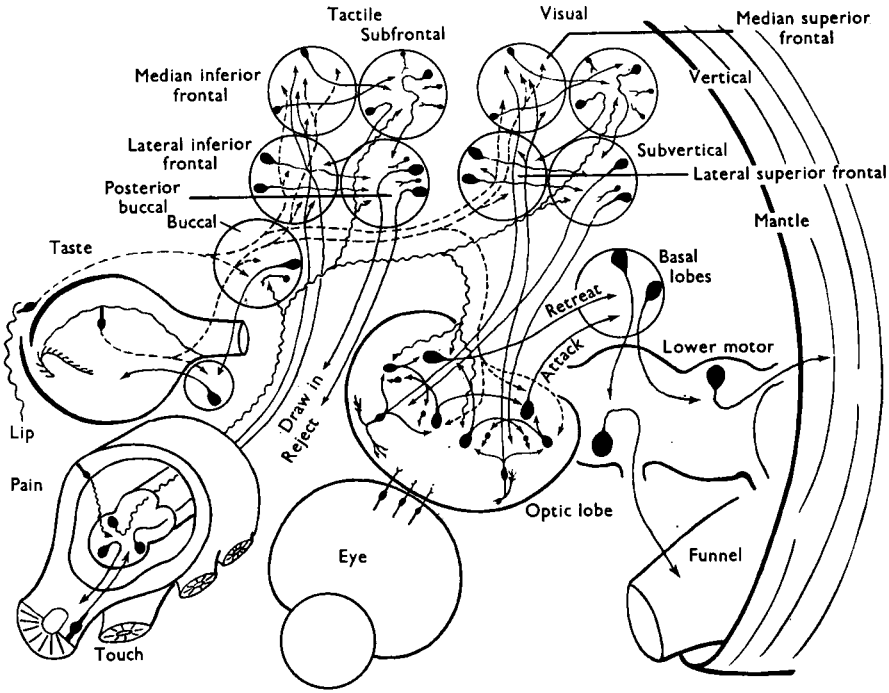


FIG. 10. Young's "paired centres" theory. A summary of the anatomical evidence; connexions known to exist in the nervous system of *Octopus*, which suggest a control system consisting of pairs of centres, each member of which might have an opposite effect on the sign of responses to things seen or touched. (From Young, unpublished.)

Young (1963c, 1964a,b) in a series of anatomical studies, has shown patterns of connexions within the supraoesophageal lobes strongly suggesting that several of the lobes already known to be concerned in sensory integration and learning function in pairs. Some of these connexions are shown in Fig. 10. Young suggests that in each case the first member of the pair serves to enhance and the second to inhibit positive responses to objects seen or felt. There is some experimental evidence for this. In the case of the median superior frontal-vertical pair, succes-

sive removal of one and then the other causes fluctuations in the number of attacks on crabs that certainly seem to support the hypothesis (Fig. 11). Discrimination experiments, however, give much less clear-cut results, with no consistent tendencies to make errors in particular directions as a result of median superior frontal or vertical lobe removals.

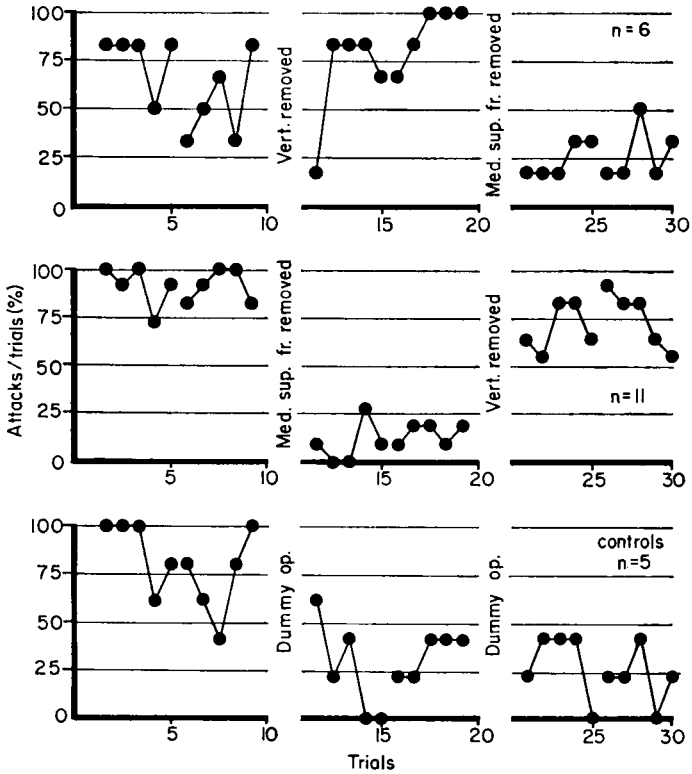


FIG. 11. Showing the effect of successive removals of the vertical and median superior frontal lobes on attacks on crabs by octopuses. There were ten trials per day, in two groups of five. Operations were done in the evening and the animals first tested next morning. Controls had dummy operations, the brain being exposed but nothing removed. (From Young, 1964a.)

It is not at present clear why discrimination experiments should give results so different from the " threshold for attack " experiments summarized in Fig. 11, and the possibility must be faced that the immediate results differ from those shown in relatively long-term experiments because the system in some way readjusts over a period of days following brain lesions.

In an analysis of the effects of vertical and median superior frontal removal on touch learning Wells (1965a) has shown that these operations increase the number but do not alter the pattern of errors in tactile discriminations, and concludes from a variety of training and retention experiments that vertical lobe removal has only quantitative and no qualitative effects. Wells's interpretation of the tactile results is that the amplification effect of the vertical lobe is due to the addition of further storage units to the learning system, which thus has a greater chance of dominating other factors determining the sign of response. In support of this he cites the survey of Muntz *et al.* (1962) of the effects of vertical lobe removal in visual experiments. These authors point out that the consequences of the operation are minimal in situations (massed trials, simultaneous discriminations, series of trials without rewards or punishments) where factors other than past experience of the shapes to be distinguished are minimally liable to cause fluctuations in the level of attack. At the moment there is little to choose between the various views of the mode of action of the vertical lobe, which in any case have much in common. This is an active field in which experimental results and anatomical findings are still rapidly accumulating.

III. Annelids

Some of the earliest experiments on invertebrate learning were made with tubiculous polychaete worms, because of their dramatic response to passing shadows. A number of papers on the subject had appeared by the end of that first decade of this century. Since then, interest in worm learning has been intermittent, with most of the literature concerned with T-maze learning by earthworms. Earthworms, since they normally live underground in the dark, do not perform well in open well-lit T-mazes, and the general impression created has until recently been that annelids learn rather slowly. An apparent exception was Copeland's (1930) training experiment with *Nereis virens* Sars, which learned to reverse a normally negative response to light, coming out of its tube to be fed after only five trials. After fifty trials the worm was retrained to emerge in response to a light-off signal in four trials, and in a succession of further reversals performance improved until one-trial reversals were achieved. Recent work has confirmed that nereid worms, and *N. virens* in particular, can sometimes learn quite rapidly. The exact conditions of the experiment are critical, and one is left with the impression that a great deal of time and effort has sometimes been wasted (particularly with earthworms) in attempts to make annelids generate data under inappropriate conditions.

A. *Habituation to repeated stimuli*

1. *Tubiculous polychaetes*

Nicol (1950) has summarized the literature and made experiments on *Branchiomma vesiculosum* Montagu with the results that in general confirm and extend previous work. *Branchiomma* withdraws suddenly into its tube when it is stimulated by passing shadows or a sudden decrease in light intensity. A sudden increase has no effect. Nicol found that the animal is sensitive throughout the human visual spectrum, minimally in red light and maximally at about 5500 Å. Dark-adapted

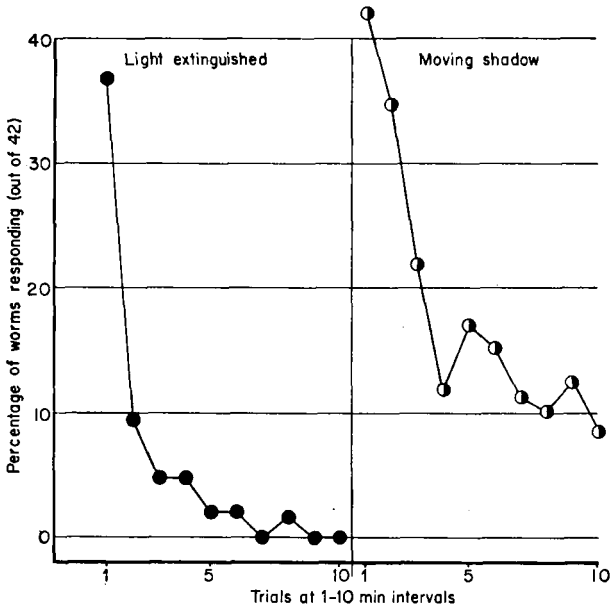


FIG. 12. Habituation in tube-dwelling polychaetes, 1. *Branchiomma vesiculosum*. Elimination of the response to a sudden decrease in light intensity is more rapid than the extinction of the response to passing shadows, a more complex stimulus. No trial was made until all the worms were expanded. (From Nicol, 1950.)

worms still made responses to shadows at intensities as low as 0·36 lux, which was the limit of his ability to observe them. This is many times the estimated daylight intensity at the depths where the worms live.

Habituation is quite rapid in *Branchiomma*; twenty-four out of fifty-six individuals (43% of those tested) withdrew into their tubes when the light shining into their aquaria was extinguished for the first time, but less than 10% of the worms responded at the fifth extinction. When the same worms were subjected first to a series of light

extinctions and then to passing shadows, habituation to the two stimuli did not follow the same course; the response to shadows was much more persistent than the response to a sudden decrease in light intensity (Fig. 12).

Rullier (1948) obtained essentially similar results with *Mercierella enigmatica* Fauvel, which habituates to moving shadows, mechanical shock and, more slowly, to a combination of the two (Fig. 13).

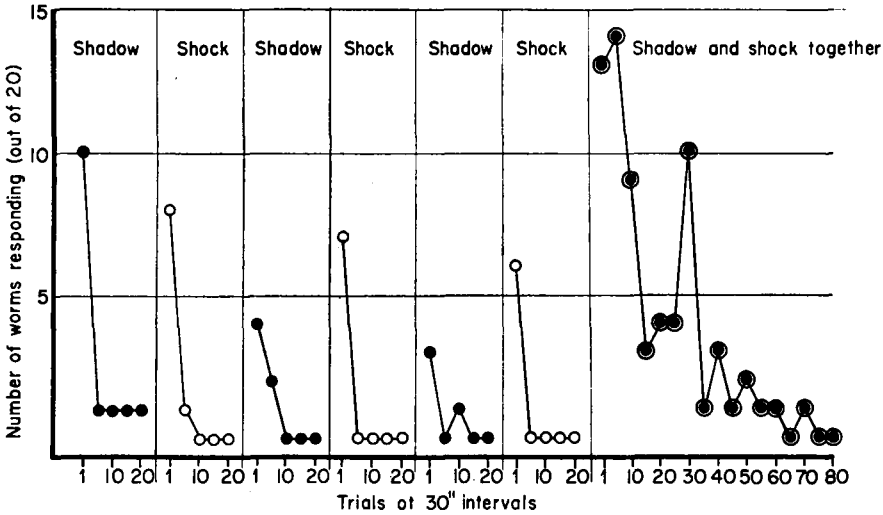


FIG. 13. Habituation in tube-dwelling polychaetes, 2. *Mercierella enigmatica*. Habituation to a complex of two simple stimuli (a passing shadow, and tapping the side of the tank) is much slower than habituation to either stimulus alone. The tests shown here were made in one continuous series, at 30-sec intervals; every fifth trial is plotted. The 20 worms had already been used in similar experiments with the simple stimuli on several previous days. At first 100% of them had reacted to the shadow, a response that was eliminated after twenty-five trials. By the day of the experiment shown here only 50% responded, even at the first occurrence of the stimulus. Either the effect of repeated habituation tests is cumulative over a period of days or the condition of the animals was deteriorating, an explanation rendered less likely by the very vigorous and sustained response to the combined stimuli. (Replotted from Rullier, 1948.)

Both authors point out (a matter that is also stressed by Clark (1960a, b) in relation to habituation experiments with errant polychaetes, see below) that individual worms vary a great deal, both in their initial responses and in the distribution and magnitude of subsequent responses. One possible explanation is that the extent of the response is at least partly controlled by the state of respiratory and feeding activity at the time. Many worms, including tube-living forms like *Sabella* and *Myxicola* (G. P. Wells, 1951, 1952), show rhythmic changes

in activity with cycle lengths varying from a few minutes to an hour or more; the worms are never inactive and their activities include spontaneous withdrawal into their tubes. Most of the training procedures used have involved training long enough to permit interference from cyclic fluctuations in the tendency to withdraw spontaneously. Thus, for example, Yerkes (1906), working on *Hydroides dianthus* (Verrill), comments that her worms, normally withdrawing for 25 sec or so after a tactile stimulus, periodically stayed inside their tubes for 4–12 min instead. She gives a table showing a typical series of results (it shows, incidentally, that *Hydroides* eventually habituates to touch as well as to light stimuli) and from this it appears that the unusually long periods of withdrawal occurred at intervals of 15–20 min, i.e. with a period comparable with the cycles since shown in other worms. Yerkes interpreted these results as periodic changes to alternative behaviour in the face of apparently unavoidable repeated stimuli.

2. *The electrophysiology of habituation*

The giant fibre responses of worms disappear when the evoking stimuli are repeated. It is relatively easy to record from giant fibres, and so discover whether habituation is a result of changes occurring before or after this stage in the action system. The results vary with the species. Thus it seems that habituation in nereids could be the result of non-transmission between the sensory elements and the giant fibres or between the latter and the segmental motor fibres (Horridge, 1959), while in tubiculous polychaetes such as *Branchiomma* and *Myxicola* it is certainly a property of parts of the nervous system involved before the giant fibres themselves. In *Myxicola*, where the giant fibres branch and run direct to the longitudinal muscles, more than 100 shocks to these nerves at 1 per sec are needed to produce an appreciable decline in the muscular response. With a longer time between shocks the number needed is further increased; clearly this is far too many for habituation to be accountable as a neuromuscular phenomenon (Roberts, 1962). In a further analysis of the situation in *Branchiomma*, Krasne (1965) has likewise ruled out sensory accommodation as an explanation of the failure to respond when repeatedly stimulated. Responses, having once been eliminated, are not restored when a different part of the crown is touched: the labile part of the system is after the sense organs themselves, and prior to the giant fibres. It seems that in *Branchiomma* withdrawal can be sudden, governed by the giant fibre system, or more gradual, the organization of the contraction involving other nervous pathways. With repeated stimulation the giant fibre response fails first (Fig. 14).

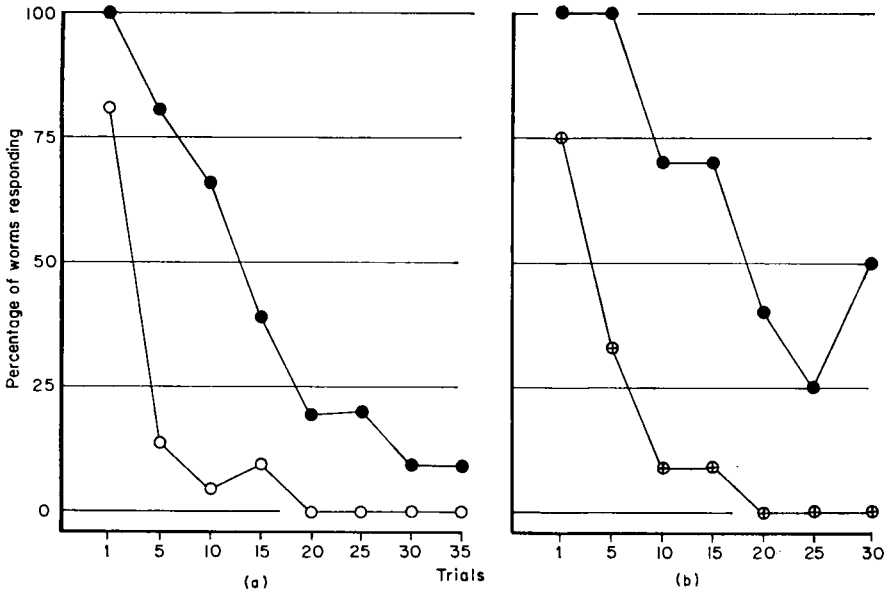


FIG. 14. Habituation in tube-dwelling polychaetes, 3. *Branchiomma*. (a) shows ● the proportion of twenty-one worms responding to touch, including slow withdrawals. ○ shows the proportion of these responses that were "immediate", i.e. in all probability mediated via the giant fibre system. (b) ● as in (a) except that the worms (n = 10) were removed from their tubes before the experiment. ○ shows the proportion of twelve worms, similarly treated, in which giant fibre responses were recorded electrically. (Data from Krasne, 1965.)

3. *Habituation in errant polychaetes*

The behaviour of the filter-feeding tube-dwelling polychaetes is naturally a great deal less complex than that of free-living predaceous and deposit-feeding forms. Nevertheless, the nereids so far studied behave in an essentially similar manner; the same stimuli are relevant to them and the same comments can be made about possible interference from biological clocks working independently of the experimenters' training programme (see G. P. Wells and Dales, 1951, on the rhythmic activities of *Nereis diversicolor* O. F. Müller). Clark (1960a, b) has made a detailed study of habituation in *Nereis pelagica* (L.). This worm normally lives under stones or in crevices; it does not make a burrow like *N. diversicolor*. In the laboratory *N. pelagica* will live in glass tubes. Most of the animal's time is spent driving a current of water through the tube by means of undulations of the body; there are periodic visits to the ends of the tube, where the worm may reach out to grasp food. Occasionally the worm will explore other tubes; fights ensue if there is already an occupant (Clark, 1959).

Nereis pelagica responds to shadows, to sudden changes in light intensity, and to mechanical shock, by a sudden contraction of the body, whether inside its tube or partially emerged from it. With trials at one minute intervals it rapidly habituates to any of these stimuli (Fig. 15). The number of trials required varies with the stimulus and increases with the interval between trials. Savings in retraining experiments indicate that the rate of decay of habituation depends more on the frequency and number of stimuli given than on the nature of the stimulus; with trials at 3-min intervals the effect of training not to

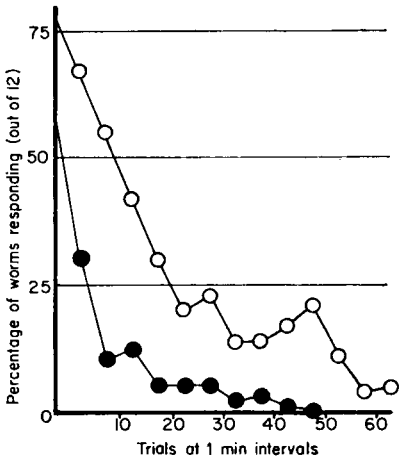


FIG 15. Latent habituation in *Nereis pelagica*. ○ shows the decline in response to a sudden increase in light intensity from 0·08 to 33·9 ft candles. Trials at 1-min intervals, light on for 12 sec at each trial. The worms were dark adapted for 2 h before the start of the experiment. ● plots the results of a similar experiment immediately following sixty trials (not shown here) at which no response was evoked by an increase in light intensity from 0·08 to 4·7 ft candles. The experiment plotted ● was done first, on worms not previously used for any habituation experiment; ○ follows 2 days later, with the same twelve worms. Points show the average percentage reaction per group of five consecutive trials. (From Clark, 1960a.)

respond to sudden decreases in light intensity had disappeared in 17 h, about three times as long as following similar training at 30-sec intervals (Clark, 1960a, b).

Clark (1960a) also studied the effect of habituation to one class of stimuli on habituation to a second. Habituation to mechanical shock, a sudden decrease in light intensity or to a moving shadow appeared to be independent, since the worms learned each at a different rate, but when the matter was examined in more detail complex interactions appeared between the various forms of light stimulus. It was found, for example, that habituation to a sudden decrease in light intensity

slowed subsequent habituation to a moving shadow and vice versa. Stimulation by sudden decreases in light intensity maintained habituation to moving shadows, but the reverse, with maintenance of the light response by shadows, was not found (Clark, 1960a).

Stimuli too weak to evoke a response may nevertheless alter the subsequent behaviour of the animals. In experiments on the habituation of *Nereis pelagica* to sudden increases in light intensity, an increase from 0·08 to 4·7 ft candles had no effect. But sixty such trials nearly doubled the rate of subsequent habituation to a larger increase from 0·08 to 33·9 ft candles (Fig. 15).

Clark (1960b) in reviewing his own and previous work on habituation in polychaetes, concludes that, although in general the rate of habituation to simple stimuli is related to their likely biological significance, learning not to respond to them is too quick and the effects too persistent for responses to these to form an effective safeguard against predators. In the sea most worms must be partly habituated to most simple stimuli most of the time. It is, however, noticeable that the fading of responses to more complex stimuli (a combination of mechanical shock and shadow, for example) is much slower than habituation to any of their components, and Clark suggests that nereids at least probably recognize the approach of potential predators from comparatively elaborate complexes rather than from simple "sign stimuli."

B. *Further experiments with errant polychaetes*

Evans (1963a) has shown that certain responses of *Nereis virens*, *N. diversicolor* and *Perinereis cultrifera* (Grube) can be reversed by training. Introduced into the narrow channel of the apparatus shown in Fig. 16, these worms invariably crawled rapidly to the far end. They were successfully taught not to do this by means of small shocks given through electrodes fixed in the sides of the passage. Of the three species *Nereis virens* learns most rapidly. With trials in quick succession 25 worms averaged 21·5 trials to reach a criterion of three refusals to run down the passage in three consecutive trials (individuals took from eleven to forty-nine trials). The other two species averaged 47 and 47·8 trials, with a similar spread in individual scores. Control animals were run down the passage without shocks, with the electrodes switched on between trials; they continued to respond. In some preliminary experiments, shocks given outside the apparatus did not appear to alter the tendency to run down the passage afterwards (Evans, personal communication).

Following up these results Evans (1963b) did experiments with the same three species in T-mazes, using shocks as punishment for turning

into the wrong arm of the T. His first maze is shown in Fig. 17a. Trials followed in quick succession, the worm being manoeuvred round to the entrance runway with a paintbrush as soon as it had emerged from the correct arm of the maze. As before, *Nereis virens* learned more rapidly than the others, but even this species made many errors, typically showing an initial improvement in performance followed by a reversion to random choices and finally to a refusal to run the maze at all (cf. results with flatworms, Best and Rubenstein (1962)). Evans

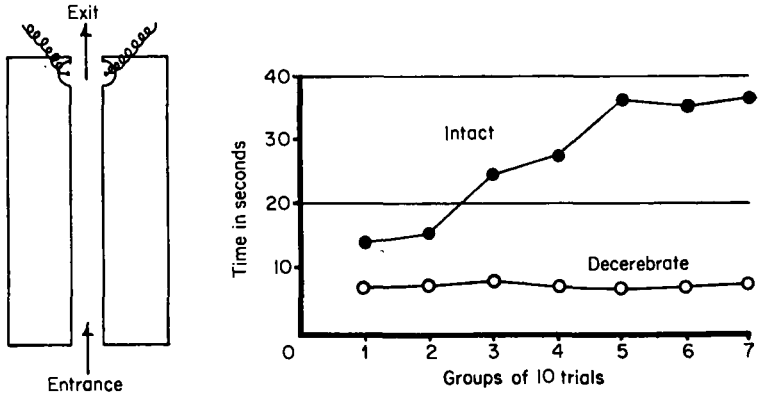


FIG. 16. Learning in *Nereis virens*. The worms were trained not to run down a corridor by giving them 6-V D.C. shocks when they did so. Trials were run at intervals of less than 1 min. At first worms regularly reached the end of the corridor in about 10 sec. If a worm did not reach the electrodes within 40 sec it was deemed to have " refused " to run—the correct response. Twenty-five worms reached a criterion of three successive refusals in an average of 21·5 trials. In the experiment plotted the performance of two intact worms is compared with that of four with the supraoesophageal ganglia removed. Trials were in groups of ten at 30-min intervals, except for two of the operated animals, which had groups at 15-min intervals—it made no difference. (From Evans, 1963a.)

concluded that handling the worms immediately after each correct response itself probably constituted some sort of punishment, and in some later experiments used the improved T-maze shown in Fig. 17*b*. Here the worms were allowed to remain in a darkened compartment for 5 min or more after each successful run. With the addition of this reward all three species learned more rapidly and performed more consistently than before, five *Nereis virens* reaching a criterion of ten consecutive correct choices in an average of sixty-eight trials (Fig. 17); *N. diversicolor* took eighty-six and *Perinereis cultrifera* eighty-five trials. Once the worms had learned, there were normally very few errors. *N. virens* apparently remembered the maze for at least 48 h (Evans, 1963b).

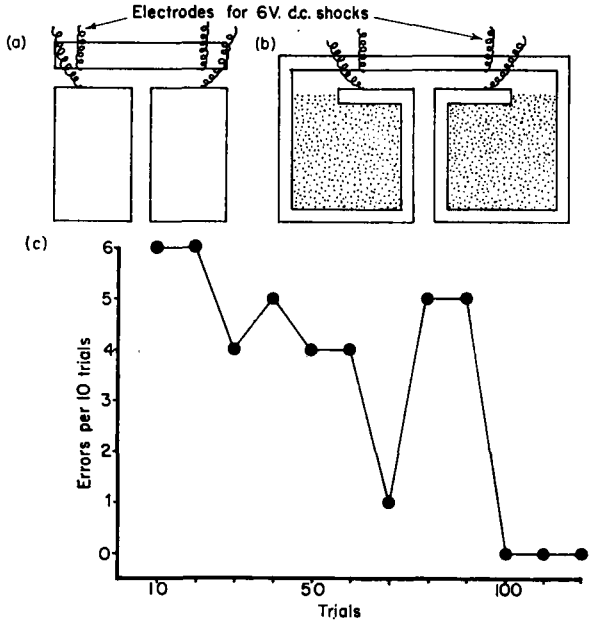


FIG. 17. Learning in *Nereis virens*. (a) shows a T-maze used in preliminary experiments by Evans (1963b). Performance was unsatisfactory, for reasons discussed in the text. (b) was a later modification, in which the worm was rewarded for a correct choice by being allowed to remain in a dark compartment for 5 min or longer. There were about twenty-five trials per day. (c) shows the performance of a typical individual under these conditions. ((a) and (b) from Evans, 1963b, (c) from Evans, personal communication.)

C. *Brain lesions and polychaete learning*

After removal of the supraoesophageal ganglion *Nereis virens* can still be taught not to run through a passage, provided that the trials follow in quick succession. With spaced trials the operated *Nereis* fail to learn. Thus seven operated worms reached a criterion of three consecutive refusals to run in an average of 29·7 trials, when the trials were in quick succession, a performance little different from the 21·5 trials averaged by twenty-five controls ($P = < 0{\cdot}05$). With trials in groups of ten at 15- or 30-min intervals, four operated animals failed to learn altogether, while the performance of two controls improved steadily (Fig. 16). If however the animals are first trained and then operated the acquired behaviour is retained (Evans, 1963a).

After brain removal *N. virens* seemed unable to learn the T-maze shown in Fig. 17. Trained *N. virens*, *N. diversicolor* and *Perinereis* showed no signs of retention when tested after operation. In both cases the experimental technique necessitated spaced trials. After the

operation, the responses of the worms were, however, by no means at random. Runs of trials at which individuals turned consistently in one direction were significantly more frequent than would be expected by chance (Evans, 1963b); the behaviour is perhaps comparable with that of cephalopods after analagous operations, where, too, consistent but perverse behaviour in discrimination experiments sometimes follows removal of parts of the brain known to be concerned in learning (Young, 1960b).

Flint (1965) has recently shown that *Nereis's* failure to learn T-mazes after removal of the supraoesophageal ganglion is at least partly due to sensory deprivation. The antennae, eyes, and palps are disconnected by the operation, as are the tentacular cirri if the circum-oesophageal connectives are cut close to the suboesophageal ganglion. If all these sense organs are removed or disconnected, leaving the brain intact, most trained worms revert to random choices. But naive worms, similarly treated, can be taught and, indeed, learn rather faster than unoperated controls; apparently the worms can learn to make the discrimination on the basis of a wide variety of sensory cues. Removal of the supraoesophageal brain, leaving the connexion between the tentacular cirri and the ventral nerve cord intact, had no effect on the learned behaviour of five out of ten *Nereis diversicolor*.

IV. Crustaceans—and *Limulus*

Arthropods are satisfactory experimental animals for a variety of reasons. They are tough and many of them apparently remain in good condition for long periods in the laboratory. They respond to visual stimuli, which we can ourselves measure. They have large neurones, so that electrophysiological analyses are possible. And perhaps most important, from the point of view of behavioural work, their very construction means that movements can be described with a detail and completeness that is simply not possible with soft-bodied animals. One result of this has been the attraction of ethologists, and the behaviour of some arthropods (fiddler-crabs and hymenopterans, for example) is now known in sufficient detail for individual learning to be recognized where it occurs in the normal behaviour of the animals. This means that information about learning in arthropods can come from two quite different sources, unlike evidence for learning in most other invertebrate groups, which is almost always derived from formal training experiments.

A. *The results of formal training experiments*

In aquaria, crustaceans soon become " tame " and cease to respond to stimuli that at first lead to withdrawal responses. A recent attempt

to quantify habituation in a marine crustacean is Fink's (1941) experiment on hermit crabs, *Pagurus longicarpus* Say. He showed that the animals, which initially respond to a tap on the side of their aquaria by withdrawing into the shell, soon cease to do so when the stimulus is repeated over a period of days (Fig. 18). Fink's results show that crustaceans, like annelids in similar experiments (see Section III, p. 21) are individually very variable, both in the number of trials required to eliminate a withdrawal response, and in the distribution of responses during the course of the experiments; in his experiments the most reactive crab withdrew 173 times during eighteen days of training, the

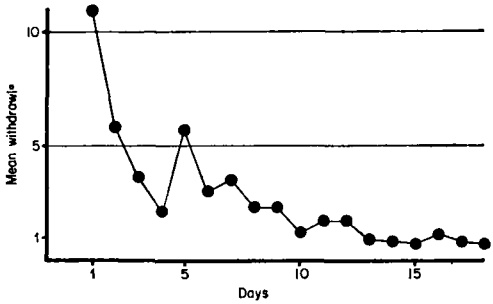


FIG. 18. Habituation in the hermit crab, *Pagurus longicarpus*. The crabs were caused to withdraw by tapping the sides of their aquaria. This was continued with each animal on each day until it failed to respond to three successive taps. The points plotted here show the average number of taps required, day-by-day, by thirty animals. There was considerable individual variation. (Data from Fink, 1941.)

least reactive only 6 times. It is not known whether these differences are due to individual experience, or to innate differences in disposition. A second matter that arises out of Fink's work is that hermit crabs do not respond equally vigorously at all times of day; crabs tested at mid-day responded twice as often as the same crabs tested 12 h before. As with worms, diurnal and shorter-term cycles in physiological state should be taken into account in assessing the results of learning experiments.

More elaborate training experiments with crustaceans have been concerned with their behaviour in choice situations and simple mazes. A number of these have been reviewed by Schöne (1961a); it seems that crustaceans can quite readily be trained to associate chemical and visual stimuli with food or shelter, and can learn to run simple mazes to attain these.

Since Schöne's review, only two papers have appeared reporting formal training experiments with marine forms. Both deal with simple choice experiments, the animals being motivated in the one case by a

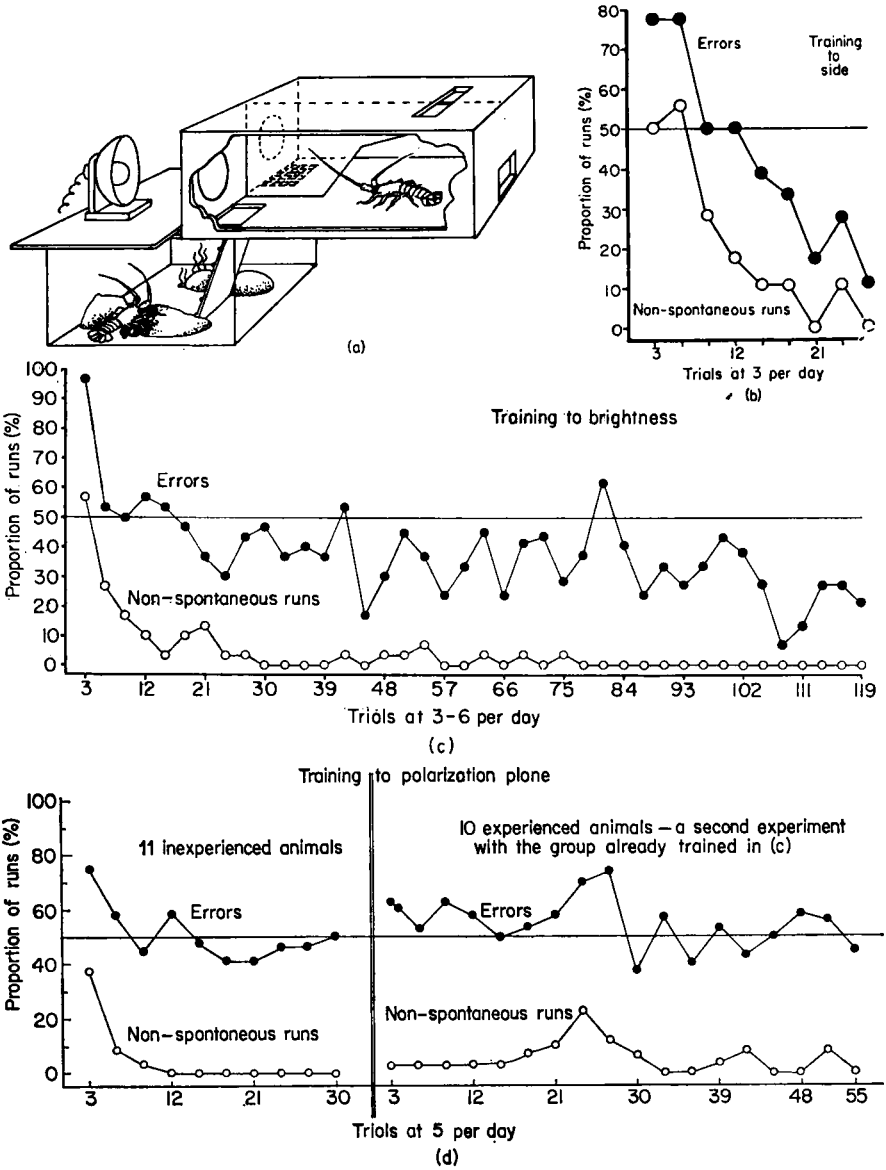


FIG. 19. Learning by the spiny lobster, *Panulirus argus*. (a) shows the apparatus. The animals had to choose between two compartments, one of which allowed return to the aquarium. If a lobster stayed at the starting end of the box for more than 5 min (a " non-spontaneous run ") it was prodded into activity and an error scored. An animal could thus make two errors in a trial (one by not moving, a second by then going into the wrong compartment) but only one correct response. (b) shows the results of training six animals to run to the left side of the choice compartment. (c) is a similar experiment with ten lobsters trained to select the brighter of two stimuli (3 × as bright for 9 days, then 5 × as bright). (d) summarizes an attempt to train animals to discriminate between two sources of plane polarized light. (From Schöne, 1961b.)

desire to get back into the water, in the other by the need to get out of it. In the former, Schöne's (1961b) study of learning in the spiny lobster, *Panulirus argus* (Latreille), the animals were tested in the apparatus shown in Fig. 19*a*. In order to get back into their aquarium the animals had to make a left-right or a brightness choice. The results of the two experiments are plotted in Fig. 19*b* and *c*, together

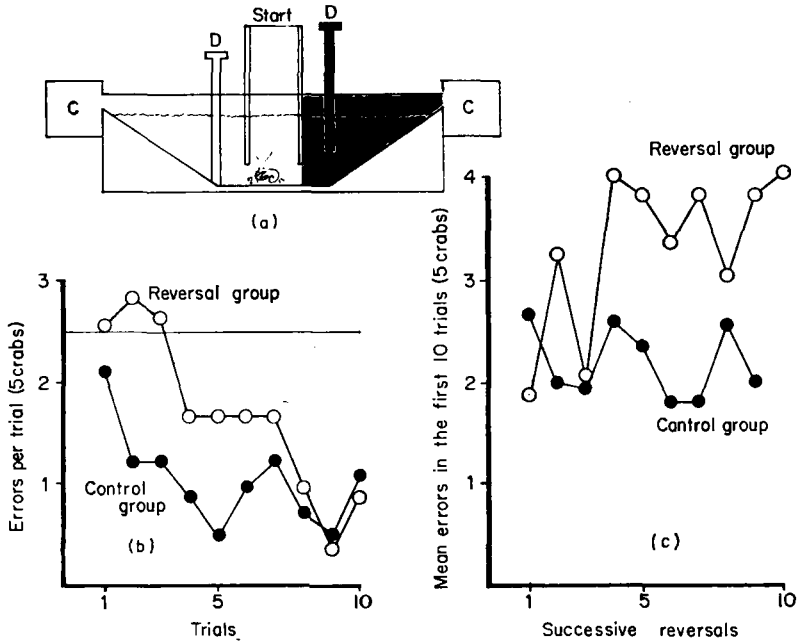


FIG. 20. Learning by the land-crab, *Gecarcinus lateralis*. (a) apparatus. The starting box walls were raised to allow the crab to see the two side passages, each blocked by a shutter the same color as the passage. If the crab passed out of the starting box into the correct passage, the shutter was raised, and the animal allowed to escape up a ramp into the home compartment, C. If the crab went to the wrong side, an error was scored; if it stayed there for 5 min it was pushed back into the starting position to try again. There were ten trials per day at 10–15 min intervals. The " reversal group " was subjected to a series of reversals in which the " correct " side and colour was switched every 4 days. Considering only the performance on the first day after each reversal (and the equivalent control trials), (b) shows the mean initial errors (first move into the wrong passage) at each trial of the ten-trial sessions and (c) the initial errors per ten trials over the whole series of reversals. (From Datta *et al.*, 1960.)

with the results of the third experiment, in which the lights differed in polarization plane instead of in brightness. Learning which side to go to is evidently easier for a lobster than learning to recognize a difference in brightness, which implies that kinaesthetic clues are relatively important in the normal life of the animal; this is not very surprising, for in

the sea *Panulirus* lives in holes among the rocks, and moves mainly at night. The animals failed to learn to discriminate between the polarized light sources, which is odd in view of the numerous cases of crustacean response to polarization plane that are now known (Waterman, 1961). Schöne's *Panulirus* experiment shows that under suitable conditions decapods rapidly learn by experience. In the choice of side discrimination an initial error score of more than 70% fell to less than 20% in twenty trials (in these experiments 50% + errors were usual at the start, since an additional error was scored if the animal had to be prodded into activity on failing to run spontaneously). Performance continued to improve over a period of weeks.

The experiments of Datta *et al.* (1960) with the land crab *Gecarcinus lateralis* Freminville (here considered a marine animal because it goes to the sea to breed) are less convincing. The authors' claim to have "achieved substantial control over the behaviour of *Gecarcinus*" appears to rest largely on the demonstration that an experimental group, subjected to a series of reversals in a choice situation, made substantially more than random errors (see Fig. 20). The control group, trained in the same situation with both intensity and directional clues, showed no cumulative improvement in performance over 32 days at ten trials per day. Both controls and experimentals showed some improvement in performance within each daily run of ten trials (Fig. 20). Altogether the performance of *Gecarcinus* is unimpressive compared with that of Schöne's *Panulirus*, a rather surprising result in view of the conditions of the lobster experiments (*Panulirus* lives below tide level) and other observations of land crabs in their natural habitats, which suggest that these animals are by no means slow to make individual adaptive responses. Datta *et al.* suggest that their animals were insufficiently motivated by immersion in freshwater.

B. *Other evidence that crustaceans learn*

Apart from formal training experiments, there is an increasing number of observations to show that learning plays an important part in determining the behaviour of individual crustaceans. Individual *Dromia*, for example, appear to have preferred ways of cutting out coverings for themselves (Dembowska, 1926), locomotion following loss of parts improves with practice, and crustaceans such as *Carcinus* and *Homarus* avoid traps in which they have once been caught (for references see Schöne, 1961). Wilson (1949) reports that aquarium specimens of *Palinurus*, having once taken to eating *Pagurus* during a period of temporary food scarcity, thereafter persisted with attacks on the hermit crabs which they had hitherto left unmolested.

Laboratory studies of groups of individuals show the evolution of stable dominance orders, which imply that the animals can both recognize their neighbours and remember the results of past fights. Dominance-subordinance relationships have been studied in most detail in freshwater crayfish (Bovbjerg, 1953; Lowe, 1956; Penn and Fitzpatrick, 1963), but a single account (Douglis, 1950) dealing with *Homarus americanus* Ml. Edw. indicates a similar state of affairs in marine forms. Bovbjerg (1960) reports that dominance orders in *Pachygrapsus* depend on size and sex; individuals fight and refuse to share shelters under water, but will aggregate when out of it. Among fiddler crabs, where the behavioural background is perhaps better known than in other crustaceans (*vide* Altevogt, 1957a, b; Crane, 1957, 1958) it appears that social dominance is mainly dependent on the behavioural phase of the individuals concerned, and since this changes over a period of hours or days, no stable relationships are built up. Occasionally, however, "an individual (*Uca maracoani* (Latreille)) just past a long-maintained display phase was still dominant to one or more displaying males which he had previously dominated" (Crane, 1958).

The holding of a sizable territory, or the return to a particular home or area after expeditions in search of food or mates are indications of individual learning inasmuch as the individual must recognize its own immediate surroundings. It is generally believed that many decapods have particular homes and that the males sometimes defend territories. In at least some cases this is illusory. Fiddler crabs, which at first sight certainly appear to stick to particular burrows, defend their holes only when in the active display phase. At other times they wander widely; there is no indication of permanent attachment to a particular area (Crane, 1958). In fact, information on homing is difficult to collect and the belief that individual decapods have lairs to which they return appears to rest mainly on cases where recognizable and usually large individuals have been found in the same crevice on successive occasions. It is not always certain that the animals were doing more than finding lodgement in the *only* suitable hole in the vicinity. Recent investigations have given conflicting results. Lindberg (1955), for example, in mark and release experiments with some 2000 *Panulirus*, caught and observed underwater, notes only two occasions on which lobsters appeared to be defending a territory; most of the animals of whatever size moved about in groups, aggregating where the shelter was big enough to hold more than one, and travelling mainly at night over considerable distances. Of 150 individuals marked and displaced 2–5 miles along the coast or out to sea, only two were sub-

sequently recovered from the catch area. Creaser and Travis (1950) reporting on a similar experiment, recaptured about 20% of their *Panulirus* following similar displacements from a reef area a quarter of a mile square. Clearly any proper assessment of these results depends on the chances of individuals turning up as a result of random move-

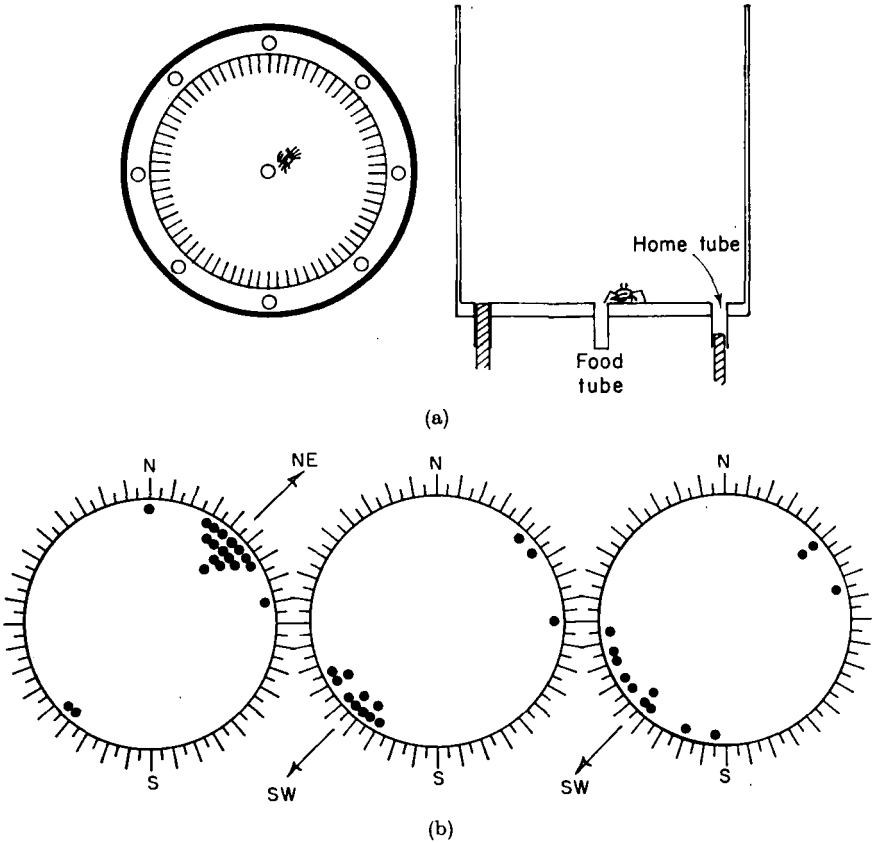


FIG. 21. Learning by *Ocypode ceratophthalma*. (a) the apparatus in plan and cross-section. All but one of the eight " homes " was blocked. A dead crab was placed in the central " food " tube. Out of a number of *Ocypode* tried, three, tested one at a time, settled down in the apparatus and regularly returned to the home tube after excursions to the food. After each had made at least ten such trips, tests were begun. As soon as an animal reached the centre, the apparatus was rotated through 45°. If the animal ran homewards during the rotation the trial was discarded. The direction of runs for home starting after the end of rotation was recorded. (b) shows the direction of runs for home made by the three individuals, together with the " training " direction in each case. All three ran mainly in the correct compass direction, to where the home should have been had no rotation occurred. Further experiments showed that the animals achieved this by orientation to the pattern of light polarization in the sky above. (From Daumer *et al.*, 1963.)

ments along the areas of suitable habitat available to them—an assessment particularly difficult to make for a marine animal. A number of decapods (including *Panulirus*, see e.g. Hernnkind and Cummings, 1964) are known to make or suspected of making seasonal migrations (Bainbridge, 1961) and this greatly increases the complexity of the problem.

Many crustaceans orient themselves with respect to the sun, either directly or from the polarization of sunlight in the sky (Waterman, 1961); one species, *Talitrus saltator* (Montagu) is also known to orient itself with respect to the moon (Papi, 1960). There is some evidence that appropriate responses in relation to these orienting stimuli are learned. Thus Pardi (1960) working with *Talitrus*, *Talorchestia* and *Orchestia*, showed that populations taken from different beaches all escaped from dishes in the directions of " their " seas when threatened with desiccation. Young animals, reared in the laboratory, showed a tendency to escape in the direction appropriate to the population from which they were reared—that is, the general escape direction is genetically determined for each population (cf. spiders, Papi and Tongiorgi, 1963). But the orientation of laboratory reared animals was inaccurate compared with that of " wild " controls, which implies that the response becomes refined by experience.

Schöne (1965) has reported similar experiments with the mangrove crab *Goniopsis*, in situations where the appropriate escape direction for individuals varies within a matter of yards along a highly dissected swamp coastline. Laboratory studies (Schöne, 1963) show that the animals can orient themselves with respect to polarized light and compensate for the time of day. The orientation must be learned, since *Goniopsis* has pelagic larvae.

A further laboratory experiment by Daumer *et al.* (1963) has shown that the shore crab *Ocypode ceratophthalma* (Pallas) can learn the direction of an artificial " burrow," and that it does this in relation to the polarization pattern of the sky. In the circular arena shown in Fig. 21*a*, crabs, having once established a " home " in one of the tubes, frequently ventured out to the centre hole, where there was food. The return home was always direct. In a series of test trials the arena was rotated 45° when the crabs were in the middle. The direction of their subsequent runs for home was recorded; the results are shown in Fig. 21*b*. Evidently the three animals had all learned the direction of " home " and the cue used was the sky, since rotation of the whole of the rest of the environment did not alter the compass-direction of the home runs. Further evidence was produced to show that the relevant cue was polarization and not brightness patterns in the sky.

C. *Learning by* Limulus polyphemus

An isolated classical conditioning experiment has been done with this arachnid. The animals ($n = 18$) were clamped in aquaria with seawater covering the gills but not the eyes. Kymographic records of tail movements were kept. The animals were first checked to see if they made tail movements in response to light alone (200 W at 8 in); two that moved more than twelve times in twenty trials were discarded. Eight experimental animals were then subjected to trials in which the light was switched on for 10 seconds every 100–250 sec (the interval was varied at random); in the last lit second a 9 volt D.C. shock was given. There were twenty trials a day. Controls had light alone ($n = 4$) or ten shocks followed by ten light-only trials every day ($n = 4$). The results are plotted in Fig. 22. The experimental animals reached a

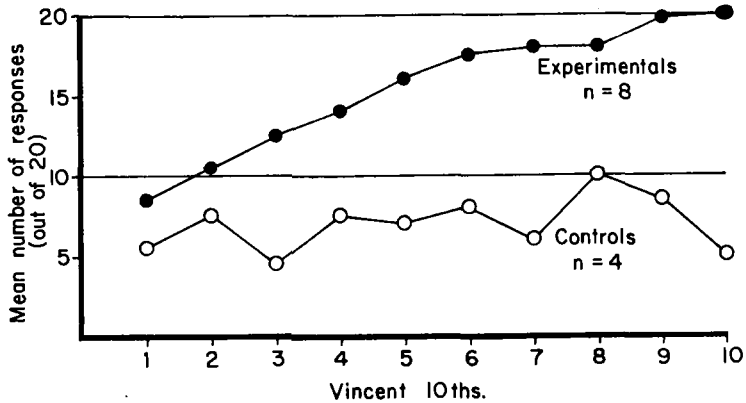


FIG. 22. Classical conditioning in *Limulus polyphemus*. Experimental animals were exposed to light for 10 sec at each trial; in the last sec a 9-V D.C. shock was given. There were twenty trials per day. This plot shows the number of trials at which tail movements (the unconditioned response) were made in response to the light alone. Training was carried on until each crab reached a criterion of seventeen or more tail flexing movements on 3 successive days. Individuals took from 4 to 24 days to reach criterion. Here each complete performance has been divided into 10ths before summing and averaging. (Data from Smith and Baker, 1960.)

criterion of seventeen tail responses to light alone on 3 consecutive days in an average of 11 days (individuals varied from 4 to 24 days); extinction of the response took from 2 to 7 days, with an average of 4·25 (Smith and Baker, 1960).

V. GASTROPODS AND LAMELLIBRANCHS

A. *Formal training experiments with gastropods*

Compared with cephalopods, other molluscs have received scant attention from students of learning. The number of formal training experiments made is small, and nearly all have been done with fresh-

water and terrestrial pulmonates. Thus Humphrey (1930) studied habituation in *Helix*—the animal ceases to respond to mechanical shock after two or three trials. Among marine forms Hirsch (1915) used *Natica* and found that the withdrawal response to a shadow failed after three trials; half an hour later the animal responded again. Arey and Crozier (1921) had similar results with the littoral pulmonate *Onchidium*. In more complex experiments, very variable results have been obtained. Fischel (1931), Garth and Mitchell (1926) and Thompson (1917) all tried to train pulmonates in T- and Y-mazes. Thompson's *Physa* did not learn at all. Fischel was more successful with *Ampullaria*, which appeared to master a Y-maze in about ten trials. The animal forgot, however, by next day, and could not be trained in a T-maze under the same conditions. Garth and Mitchell's experiment is perhaps more convincing, though even here there remains the possibility that their single *Ruminia* developed the habit of turning to the correct side of the T by chance rather than as a result of training. It should be noted that nearly all the animals that have been tested are terrestrial or freshwater herbivores, and that attemps to regulate their behaviour have been based on punishments for incorrect responses. In view of the results with annelids (see Section III, B, p. 27) it seems likely that better performances would have occurred had the animals been handled less and some system of rewards included in the training programme.

It is surprising to find that nothing has been done with the apparently far more promising marine prosobranchs. The neogastropods in particular are active predators and scavengers, seeking out their food in a purposeful manner which implies that it should be no great trouble to arrange for reward-motivated learning in choice experiments.

B. *Homing by limpets*

The lack of direct evidence of learning in marine forms is to some extent offset by a considerable body of information about the homing of limpets. There has been considerable argument as to whether limpets do in fact return to individual " homes " on the rocks. This has arisen because: (1) limpets at least in some habitats are comparatively immobile anyway, so that observation of individuals in the same place day after day is not good evidence of homing (Villee and Groody, 1940); (2) the same species may behave quite differently on different substrates; *Patella vulgata* L., for example, tends to be most active and to change its " home " most often on smooth damp rocks (Orton, 1929; Jones, 1948); and (3) there has been a tendency to compare findings from different species, which do not all behave in the same way even within the same habitat. Some species regularly return to a home,

others do not (Stephenson, 1936). On balance, there now seems to be no doubt that certain species, (*Patella vulgata*, *P. granularis* L. and *Acmaea scabra* Gould, for example) do regularly return to " scars " which remain their home bases for long periods of time. *Siphonaria*, among the pulmonates, appears to do the same (Ohgushi, 1955). The evidence for homing arises from direct observation of the tracks of individual limpets (Hewatt, 1940; Thorpe, 1963), from repeated observations of individuals found foraging away from home and subsequently on their bases (Stephenson—see Thorpe, 1963), and from the shape of the shell, which grows to fit the animal's particular home scar. (Hewatt (1940) filed away the edge of a limpet's shell and it returned to its old base, i.e. it seems not just to be a matter of the animal wandering about until it finds a place where it fits.) Return to a home base can be taken as evidence that an animal has learned, provided that it can be shown that the animal is not simply homing on a beacon (the sight of the home, or its smell) or returning along a track that it lays on the outward journey. The former type of explanation is difficult to eliminate experimentally, since to do so necessitates assumptions about the stimuli the animal is detecting. But it appears unlikely that limpets see or smell their home from a distance since their limited visual horizon must mean that they move rapidly out of sight of it and, at least for forms that normally move when covered by water, the usefulness of chemical cues must be very limited in an environment where current direction is so variable. Return along the outward track has often been observed but is by no means invariable (for references, see Fischer, 1950; Thorpe, 1963). Moreover, attempts to upset homing by scrubbing out the track (Davis, 1895) or altering the topography (Pieron, 1909; Hewatt, 1940) have not prevented return by most of the animals concerned.

Displacement experiments, mainly carried out with *Patella*, have given variable results, and in general, it seems that a *Patella*, forcibly removed from its scar and replaced more than 10 cm from home, has less than a 50% chance of finding its way home again (Fischer, 1898; Morgan, 1894; Pieron, 1909). Unfortunately these experiments have never been done with individuals whose normal feeding range was known or estimated from the condition of the algae around them.

The performance of the naked pulmonate *Onchidium*, which returns not to a scar but to a " nest," a hole in the rocks where a dozen or more individuals may live together, is evidently much more reliable than that of the prosobranch limpets or *Siphonaria*. Arey and Crozier (1921) report almost invariably successful homing in many experiments with individual *Onchidium* displaced 50–100 cm, including returns to

the nest from areas above highwater mark into which the animals did not normally penetrate. These authors believed that return to the nest was guided by " aerial dissemination of some guiding substance " on the following grounds: (1) that while the route out from the nest was normally irregular, the return was nearly always a straight line (i.e. the animal did not follow its outward track); (2) where the nest had more than one entrance, individuals did not always enter the way they came out; (3) the returns when displaced to unfamiliar territory, already mentioned; (4) the animals' only " home " in air; underwater, they appear to be lost; (5) homing sometimes involves complete reversal of the animals' otherwise regularly negative heliotropism; (6) individuals displaced into the area of another nest appear lost, rarely enter the new nest, and when they do, re-emerge rapidly.

In this very thorough study, Arey and Crozier tried also to reproduce field conditions in the laboratory, but though their animals continued to live and feed, they did not form nests or respond to artificial tides by the simultaneous emergence and withdrawal that is so marked a feature of *Onchidium* behaviour in the field. Arey and Crozier's explanation in terms of an aerial guiding substance emanating from the nest is probably wrong, as two of their own observations suggest. One of these is that a homing *Onchidium* removed and replaced on the substrate continues home, while one replaced on a sheet of glass laid on the substrate does not (it becomes negatively heliotropic and this may carry it away from the nest). The other is that interference with the entrance to the nest by chipping bits away upsets the animals, which wander about over the wreckage when they get there instead of entering the nest. Both observations argue against an airborne guide. Moreover, cutting off the oral lappets which are in contact with the ground disorients the animals; removal of the tentacles does not. Altogether it would seem more likely that the animals respond to a gradient generated by their own repeated excursions from the nest, the main arguments against this being that they do not obviously react to recent trails and that they apparently home successfully when placed close to the nest but outside their normal area of operations.

Whatever the explanation, it seems that the capacity to return home is lost if the animal is kept away from its nest for any length of time. Individuals kept in the laboratory for 24 h failed to return home when replaced in their normal foraging ground. While this forgetting is perhaps explicable in terms of physiological state (it would appear, on the face of it, that the animals home when replete), their former specificity is most unlikely to be genetic; one might compare it with the learning of nest smell by hymenopterans.

The *Onchidium* experiments do not necessarily invalidate explanations of homing by other species based on topographical learning. *Onchidium* is a pulmonate of a rather specialized type and is thus related only remotely to *Siphonaria* and hardly at all to the other, prosobranch, limpets. It is highly probable that the homing habits of these forms have developed independently. A reinvestigation of the whole subject in terms of the information potentially available from orientated features of the environment (polarization of the sky, brightness, slope and humidity gradients) is clearly needed at this stage.

C. *Other evidence of learning by molluscs*

Chiton, like the gastropods, seems to have some sense of the geography of its immediate surroundings. Crozier (1921) showed that individuals did not always return " home " to the underside of rocks by their outgoing paths, and considers their performance an " incipient stage in a kind of homing instinct." Bounlangé (1927, cited in Pelseneer, 1935—a source often quoted because it contains names and dates but no further details of references) apparently did similar experiments.

Among the gastropods, Verlaine (1936) has shown that the performance of the predaceous *Natica*, which bores holes in the shells of bivalves, gets better as the animal grows up. Young individuals as often as not bore their holes in unsuitable places where the shell is thick or the soft parts withdrawable; larger *Natica* make their incision over the gonad in nearly every case. A similar state of affairs has been recorded for *Nucella lapillus* L., which mainly feeds on barnacles. Fischer-Piette (1935) records a remarkable series of behavioural changes in a whole population of these whelks, following the overrunning of their barnacle beds by mussels. Apparently the whelks not only took to feeding on the mussels, but also got more adept at it as time went on. Fischer observed that the proportion of shells found with holes drilled in obviously unsuitable places, such as from the inside of empty valves, fell progressively during one generation of the whelks, and remained low throughout the next generation, by now wholly devoted to mussel-eating. With the subsequent elimination of many of the mussels, barnacles were able to re-establish and the whelk population reverted to its original food.

Leaving aside a matter of the inheritance of acquired characters that would seem to be implied by some of Fischer's observations, it is clear that neither this, nor Verlaine's report is necessarily indicative of learning. Both are, however, well documented accounts of behavioural changes that might well form the basis of work on the effect of individual experience on feeding.

Bivalves in general do not seek their food. It comes to them. They have a rather complete and simple means of responding to enemies. Not surprisingly, the only aspect of learning that has been reported in lamellibranchs is habituation. The animals respond to simple stimuli, such as passing shadows (Patten, 1886; Hecht, 1924; Kennedy, 1963) and generally cease to do so when these are frequently repeated. Where the matter has been specifically investigated this change in behaviour would seem attributable to sensory adaptation. Thus Hecht (1919, 1924) working with *Mya arenaria* L. showed that the mobilization of photosensitive substances in dark adaptation takes a matter of hours, and argues that it is more reasonable to attribute the failure of the siphon-withdrawal response to exhaustion of photosensitive substances in the receptors rather than to " learning ". In the absence of experiments with a variety of stimuli, or localized stimuli—studies comparable with those done on annelids—there would seem no reason to dispute this view.

VI. Platyhelminthes

The current outbreak of work on flatworm learning has so far been based entirely on freshwater triclads, notably *Dugesia* (= *Planaria*). A recent review of this subject is included in Jacobson (1963). Information about marine flatworms is limited to Hovey's (1929) study of learning in *Leptoplana*, a polyclad. Though by no means recent, Hovey's work is included here for comparison with learning by other marine animals, and because it is in some ways more convincing than much of the *Dugesia* work.

Hovey trained *Leptoplana* to remain still when a light was switched on, thus reversing a normal response of the animal, which is negatively phototactic. After 12 h in the dark, the animals ($n=17$) were exposed to a bright light (300 W at 25 cm!) for 5 min. Every time a worm moved it was touched on the snout, and the number of touches counted. After a 5-min training period the light was switched out for 30 min before a further training session, followed by another half hour in the dark and so on. The results of this experiment are summarized in Fig. 23; the number of touches needed to keep the worms immobile fell progressively, though they never ceased to respond altogether. Explanations based on sensory adaptation, motor fatigue or physical damage to the snout were eliminated by control groups subjected to light alone, or trained after surgical removal of the snout. The control group of twelve worms used to test the effect of light alone was subsequently trained in the same manner as the rest. They learned more slowly than the other experimental animals, having to be touched on average

72·2 times during the first five 5-min sessions, nearly twice as often as the original experimental group which were touched on average 40·6 times.

Six of the worms had the cerebral ganglia removed after training. A 2-h break in training was required to cool the worms to 0°C,

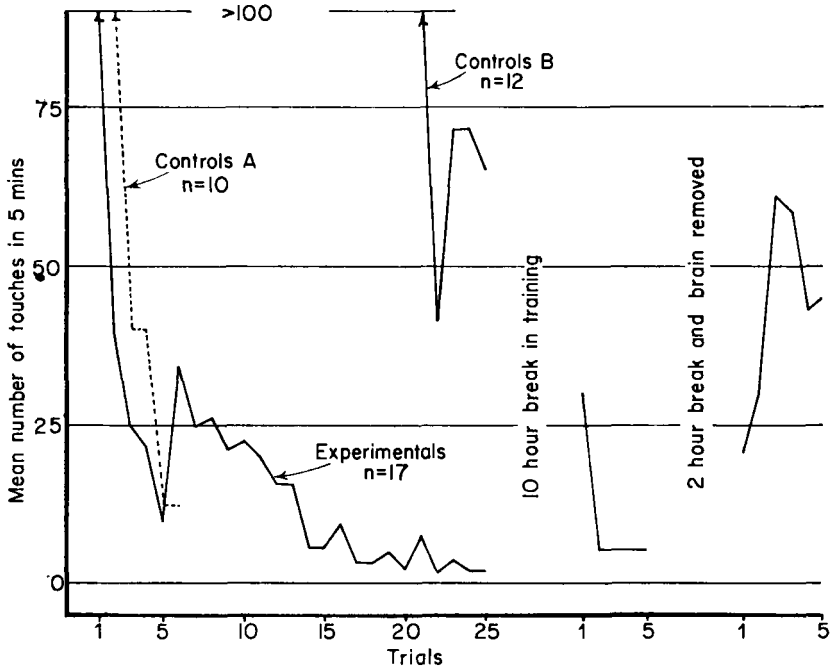


FIG. 23. Learning by *Leptoplana*. This polyclad is negatively phototactic. At each trial a bright light was switched on and the worm under test touched on the snout whenever it moved. Trials lasted 5 min, and were separated by 30-min periods in the dark. Controls were made for snout damage (controls A, with plot displaced one trial to the right) and for the effect of light alone (controls B, which were lit, but not touched during the first twenty trials). After twenty-five trials there was a 10-h break after which a single 5-min training period returned performance to the pre-break level. Removal of the brain was followed by a reversion towards the untrained condition, and the worms did not appear to relearn. (From Hovey, 1929.)

operate upon them, and return them to room temperature. After the operation the worms responded more often than before; in the first five post-operational trials they averaged 41·6 touches, against a score of 35·2 touches for the same individuals in their original training. Previous tests had shown substantial retention of the non-moving habit over 10 h (Fig. 23).

VII. Echinoderms

Starfish and sea-urchins have no centralized grouping of sense organs, and no obvious " brain." Movement can be in any direction and although individuals commonly have a preferred radius, they can readily be reversed and are apparently as well equipped to respond in one direction as another. Starfish can right themselves if turned upside down and individuals achieve this end in a variety of ways that may differ widely from trial to trial. The things echinoderms can most

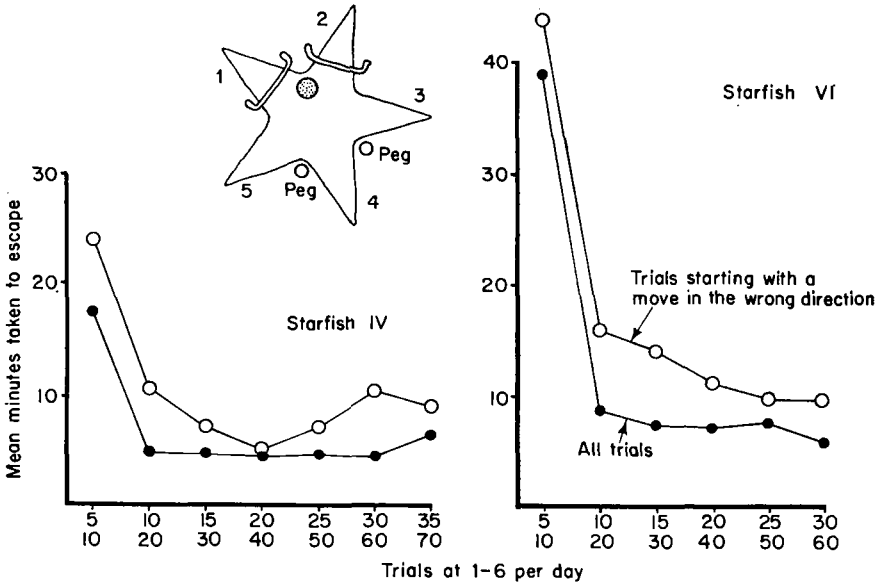


Fig. 24. A training experiment with *Asterias*. The starfish was trapped on a board placed on the floor of its aquarium by means of hoops over the two arms on either side of the madreporite, and pegs between arms 3 and 4, and 4 and 5. The time taken to escape off the board was recorded. The performance of two individuals is shown. In each case ● shows the mean escape time (animal gets off the board) for each ten trials and ○ the mean escape time for those trials at which the starfish began with a move in the wrong direction, leading with one of the arms trapped under the hoops. In this case means are for every five such trials. (Facts from Ven, 1921.)

readily be shown to do can, in short, generally be achieved in a number of ways. This sets a problem in assessing whether echinoderms learn, because criteria based on the animals acting in ways that they did not do before tend to presuppose only one correct type of response. The observation that a starfish switches over from one series of movements to another in the course of an experiment is not proof of learning; it might not, if one may put it this way, matter to the starfish how it

achieves the result it gets, and selection of one set from a whole range of appropriate movements may be a matter of chance.

Attempts to show that performance improves with practice have mainly centred round the animals' righting reactions and their capacity to escape from obstacles restricting their movements. In most cases the time taken to turn over or escape was measured. Thus Preyer (1886) showed that ophiuroids could extract arms from rubber tubes placed around them. He interpreted the changes in the movements that the animals made as a sign that the animals were in some way aware of the problem and intelligently struggling to free themselves. Neither he nor Glaser (1907) who repeated the experiments (but disagreed with the interpretation) could detect any improvement in performance with practice. In a further series of experiments (for references see Thorpe, 1963) Preyer and others studied the attempts of ophiuroids and asteroids to escape when confined by means of pegs driven in around them, between the rays. The latest and most thorough of these works is Ven's (1921) series of experiments with *Asterias*. He showed first that these animals most commonly lead with the arms on either side of the madreporite (an observation since confirmed by Kerkut, 1955). By trapping these two arms under hoops on a board (see Fig. 24), Ven obliged his animals to lead with other arms in order to escape. Trials were at 1–6/day; as tests continued " the animals fatigued less easily " and the larger number of trials became possible. Considerable improvements in the time taken to escape were found, both for trials beginning with a move in one of the right directions and for those starting with a move in the direction of one of the trapped arms (Fig. 24).

It is a little difficult to interpret these experiments. Ven himself states that the improved escape time was due: (1) to less time being spent on false starts; (2) to more activity on the part of the untrapped arms; and (3) to an increased suppleness, enabling the arms to bend up over the pegs more readily. Taken together with the statement that the animals fatigued less easily as tests continued, statements 2 and 3 make one wonder whether (for example) a starving starfish moves more vigorously than a fed one. Of Ven's six animals two died after showing some improvement in performance and one failed to learn, " pour lui la puniton et la recompense étaient trop faibles pour vaincre sa nature trop indolente." Are the other results explicable as changes in the general condition of the animals? Against this conclusion are various observations. First, there are occasions when training was resumed after a 48-h break; performance after the breaks in the single experiment that Ven plots out in full tended to be unusually poor: one would not expect this result if improvement in performance was just a

function of the time spent in the aquarium. Secondly, it is stated (though no data are given) that performance improved during days when several trials were given. Thirdly, it was noticed that the animals, escaping off their test board onto the floor of their aquarium, soon ceased to pause at the edge of the board. All imply that the animals were actually learning something about the test situation. A more convincing proof comes from tests made with the three successful starfish at the end of their training experiments. These three, trained not to use arms 1 and 2 on either side of the madreporite, were after-

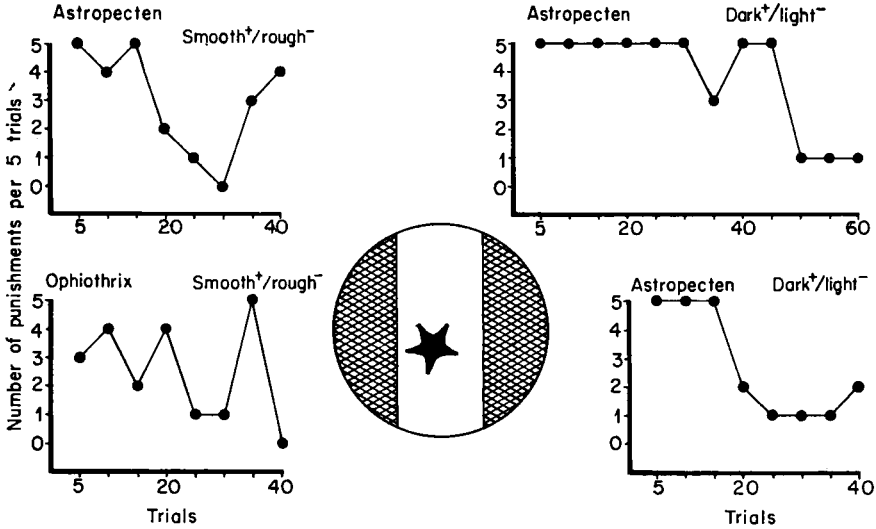


FIG 25. Discrimination experiments with echinoderms. These plots show the performance of individual animals in the apparatus shown in the inset, a glass dish with a central strip defined either by rough varnished emery paper stuck to the bottom, or by light shone from beneath. Any arm leading the animal out of the strip was punished by touches on the tip with a group of *Asterias* pedicellariae (causing reversal of the movement) as soon as one-third of the arm had crossed the boundary. The number of spontaneous reversals (trials at which the animals reversed without punishment) generally increased with training. (Data from Diebschlag, 1938.)

wards tested with 2 and 3 under hoops instead of 1 and 2. In thirty-six trials, arms 1 and 2, the dominant arms before the experiment, led only six times.

Similar results have been obtained in righting experiments. Thus Jennings (1907) showed that starfish which are repeatedly prevented from using any but one particular pair of rays in righting themselves, continue to use this pair when unrestricted. His results imply retention of such learned habits for at least 5 days, and would appear to

show that starfish can be trained to use or not to use particular arms.

Discrimination experiments with *Astropecten*, *Ophiothrix* and *Psammechinus* have been made by Diebschlag (1938). The experiments were carried out in a dish with a difference in surface or illumination defining a 6-cm wide strip across the centre. The animals, about 3 cm in diameter, were punished for transgressing the edges of the strip, by touching the tube feet of the leading arm with a group of pedicellariae held on the end of a dissecting needle. This rather curious " biological " stimulus was sufficiently unpleasant to cause retreat, the animal generally reversing until it overstepped the opposite edge of the central strip, whereupon it was again touched with the pedicellariae. After a few punishments, contact with the edge of the strip was followed increasingly often by spontaneous reversal. A variable number of these trials was made, continuing generally until the animals stopped moving about. Similar results were obtained in some of the experiments where the dish was divided into halves with a central boundary. Some of the results are plotted in Fig. 25. Diebschlag made no attempt to check the possibility that he was sensitizing his animals by punishment and it is possible that his results are accountable on the grounds that the repeated punishments merely made the animals more responsive to any change in stimulation.

Coordination of the five arms depends on the circumoral nerve ring. When this is cut in two places integrated stepping by the tube feet ceases. Those on each of the two groups of arms disconnected by the cuts remain coordinated; those on the separate groups do not. The two halves of the animal may eventually pull apart (Moore, 1939). In this situation it is not surprising to find, for example, that the animals fail to right themselves as usual and that the effect of central nervous lesions on learning has not been investigated; with the training techniques so far adopted everything depends on movements by the animal as a whole.

Works on the nervous organization of starfish and the coordination of the tube feet have most recently been reviewed by Smith (1950) and Kerkut (1954).

VIII. Coelenterates

Whether a coelenterate learns or not depends very much on your definition of learning. The animals have a considerable repertoire of patterns of activity, and in anemones at least some of these (the cycle of events that follows feeding, to take an example) continue for many hours. As a result of these very slow reactions, it is sometimes difficult to separate what is and what is not an immediate response. *Metridium*,

for example, may move away from a place in which it was given a series of electric shocks hours later, during the following night. From the time scale, with any other type of animal, it would be not unreasonable to suppose that the move was a result of association of area and shock; the animals found the region inhospitable and moved on. Yet because anemones respond so slowly, the possibility remains that the shocks do no more than cause a switch in long-term patterns of activity, with results that show up hours later as an apparently adaptive reaction to remembered circumstances (Pantin, 1952). The very variety of the long term activities of the animals means (as with echinoderms) that it is difficult to distinguish whether they are selecting one of a series of possible action patterns as a result of experience, that is learning, or simply switching to any of the available alternatives when the first doesn't work.

Bearing this in mind and without, at this stage, prejudging the issue by defining what would constitute evidence of learning in a coelenterate, it is worth examining the experiments that have been held to demonstrate learning in the past. These are:

1. Cessation of responses to repeated stimuli that at first cause withdrawal.
2. Cessation of responses to inedible objects (usually bits of filter paper soaked in meat juice) repeatedly presented.
3. Persistence and gradual fading of tidal and diurnal rhythms in anemones brought into constant conditions.
4. Position habits in individuals.

The first type of experiment is clearly a study of habituation. *Aiptasia*, for example, normally withdraws when it is suddenly stimulated by a drip falling into the still water of its aquarium. If the drip occurs repeatedly at intervals of a minute or so (long enough for the anemone to expand its tentacles again) the response fades after two to three trials. If the drips are more than about 5 min apart, it does not (Jennings, 1905). Such habituative phenomena must be general; anemones moved from a quiet environment to aquaria with a brisk circulation do not remain permanently withdrawn. What is not known is whether one is dealing with sensory accommodation, or changes sited more deeply in the nervous system. Most definitions of learning would exclude the former.

Nagel (1897) appears to have been the first to note that anemones (he used *Adamsia*) soon cease to accept pieces of filter paper soaked in fish juice, though they will at first grasp and pass this to the mouth just like other food. A number of workers have confirmed this, using a variety of species (for references see Thorpe, 1963). There has been

some conflict about the interpretation of the results. Thus Jennings (1905) found that *Aiptasia*, having learned to reject the paper, generally refused real food as well, and attributed the whole phenomenon to a difference in the degree of stimulation. Allabach (1905), using *Metridium*, suggested that the cessation of response could be due to accumulation of mucus on the tentacles. Neither of these, however, seems a likely explanation of the results of Fleure and Walton (1907) who found, with *Actinia* and *Tealia*, that the change of the response to filter paper not only occurred in experiments at one trial per day (surely long enough for a reversal of sensory accommodation or the dispersion of mucus?) but remained detectable up to a week after the end of training. Their animals learned not to pass the paper to the mouth within 3–6 days and 2 days later refused even to take hold of it; *Tealia* learned more rapidly than *Actinia*. Fleure and Walton found, moreover, that the effect of the training was limited to the tentacles immediately involved; other tentacles would take the filter paper once or twice when first tested, a result that shows at least that their results are not attributable to a steady decline in the condition of the animals. Clearly these experiments should be repeated, under conditions designed to show: (1) that the animals remain equally responsive to other stimuli at the same time as the decline in response to imitation food; and (2) that there are no alternative explanations of the change in behaviour in terms of trial timing in relation to tidal or other rhythmicities that the animals may exhibit. Until this is done, the experiments can only be summarized as appearing to show learning by experience.

Like many other animals, coelenterates commonly show long-term rhythmic behaviour when brought into the laboratory from the sea. Diurnal and tidal rhythms sometimes persist for many days under apparently constant conditions. Thus *Cavernularia*, an alcyonarian "sea-pen," expands at "night" and closes up during the "day" for at least 100 days in constant laboratory conditions (Mori, 1959). *Actinia* in contrast, shows tidal rhythms that die away within 2–3 days, or sooner, according to the markedness of tides in the places from which they were collected. A number of other examples are known (for references see Harker, 1958). Taken collectively, there seems to be little doubt that environmental effects can influence rhythms in coelenterates. Whether their influence is superimposed on innate tendencies to cycle with a circadian or tidal periodicity is another matter, not known for coelenterates.

The position habits of individual anemones would appear to indicate learning by experience. Two sorts have been noted. One, discussed by Jennings (1905), is the tendency of individual animals to

adopt characteristic postures with the column bent in ways that remain constant over considerable periods of time. When an individual contracts and re-expands, it returns to a shape that it held before the contraction, often a shape that happens to fit the available space in its own immediate surroundings; one is reminded of Teyrovsky's (1922) experiments with tube-worms. In the case of the anemones, however, there is no need to postulate habit formation, for it seems that the essential elements of the individual's characteristic shape are retained as local differences in muscle tone even when it is largely contracted. After any maximal contraction, the " habit " is lost. Animals can be " trained " to adopt a new position by any form of punishment that eliminates this " muscular memory " (Jennings, 1905). Anemones placed in aquaria are said to select the under or upper sides of surfaces depending on the site from which they were collected. If true, this position habit could be interpreted as associative memory (Thorpe, 1963). The experimental evidence is, however, unconvincing because the numbers of animals involved have always been small and because lighting effects, which are known to be important, have not always been adequately controlled. The most complete account is by Ghinst (1906) who did a series of experiments with *Actinia equina* placed upright or upside down between two horizontal glass sheets. Ghinst, who did his experiments in a darkroom, recorded how long the animals took to attach and whether they ended up facing upwards or downwards. He was able to conclude only that the " position memory " accelerated or retarded attachment, depending on whether their new position was the same or different from their position when collected.

Much more recently Ross (1965) has reported two further experiments that appear to show conditioning in sea anemones. In the first of these, an attempt was made to link the mouth opening of *Metridium* with low frequency electric shocks. The unconditioned stimulus was squid-juice, applied while the anemone was receiving small electric shocks (condenser discharges) at intervals of several seconds. Shocks separated by more than 3 sec normally have no effect. Forty-five anemones were trained. Twenty-seven of these had to be discarded in the course of the experiment because they ceased to respond to the squid or covered the mouth with the tentacles. Of the rest, seven began opening the mouth wide in response to shocks alone after ten to twenty trials, three made smaller responses of a similar kind and eight showed no changes in behaviour. In a repeat series no positive results were obtained at all.

More promising perhaps are experiments recently begun with *Stomphia*, which responds in a remarkable way by expanding, detach-

ing the pedal disc and swimming away when touched by the starfish *Dermasterias* (Yentsch and Pierce, 1955). In training *Stomphia* escape was prevented by prodding the anemone, causing it to close up at the same time as contact was made with the starfish. After ten to twenty such trials the normal response to *Dermasterias* was lost (Ross, 1965).

How many of the coelenterate results one regards as indicating learning depends, as pointed out at the beginning, on one's definitions. And thereby raises the more fundamental problem inherent in attempts to define learning anyway. The fact of the matter is that at present we label as " learning " all individual adaptive phenomena for which we have no adequate physiological explanation. Where it is possible to give an explanation, as for example in Jennings' (1905) analysis of position habit learning in *Actinia*, the phenomenon is struck off the " learning " list. Sensory adaptation or sensitization are further types of explanation that, when proven, withdraw the phenomenon from the list. In effect this means defining learning in terms of central nervous activity. We do not know the mechanism of adaptive changes in the CNS and, indeed, have no good reason *a priori* for thinking in terms of one mechanism only. All animals, so far as we know, have nerve cells with rather similar properties. There are a number of theoretical ways of connecting these up to provide circuits capable of holding a record, even supposing that one is considering only explanations in terms of synaptic connexions. When explanations in terms of local dendritic potentials or chemical coding are added the range of possibilities is still further increased, and it may well be that different groups of animals have exploited different nerve cell characteristics. The requirements of a starfish are very different from those of an octopus. In evolving towards their respective ways of life, echinoderms and cephalopods may have advantageously developed two quite different computer systems and it would be remarkable to find that both had exploited the properties of nerve cells in exactly the same way.

IX. Discussion

This section would perhaps be better entitled " concluding remarks and apologia". When I agreed to write this account, I had in mind a critical review of formal training experiments, with careful examination of experimental techniques. It very soon became obvious that this would produce a very one-sided view of the situation and in any case lead to the omission of much of the very matter from which the most important criticisms arise. So I have tried to cover not only formal training experiments but also other evidence of individual adaptive phenomena—a much more heterogeneous collection of material.

To have dealt adequately with all this would have led to an indefinite expansion of the review. The result is a compromise and suffers from the defects inherent in compromises. On the one hand a more detailed criticism of the experiments would have been desirable. On the other a further expansion of the physiological background and behaviour of the animals would have made it easier to assess the status of learning in the various phyla.

Admitting all this, and agreed that the present survey pursues none of these topics in depth, it seems that the following generalities can usefully be made about the study of learning in invertebrate animals:

1. The phenomenon called " habituation " by vertebrate workers seems to be universal. Animals do not continue to respond to irrelevant stimuli and in general there seems to be a neural mechanism for ensuring that the response stops before either the sense organs or the muscular system is fatigued. Escape responses to simple stimuli disappear within three or four trials in a wide variety of organisms, and return only after a matter or minutes or hours. Such rapid failures of the response occur, however, only when very simple stimuli are used; in any more complex situation habituation is liable to be much slower. One is led to suspect mechanisms for ensuring that even the simplest animals remain unusually wary of complex stimulation and that many of them may be protected by highly specific and virtually unhabituatable responses to particular patterns of stimuli. Systematic studies of the interacting effects of simple stimuli in habituation could well be used to discover facts about the neural organization of some lower animals, just as discrimination training experiments have been used to study sensory integration elsewhere.

2. Most invertebrate animals continue to show rhythms of activity under constant laboratory conditions. The pacemakers for these cycles are sometimes known, and are very commonly not immediately related to the highest parts of the CNS. It would be remarkable to find that learning proceeds equally readily at all phases of these cycles, some of which are of short (minutes rather than hours) duration. Their effect must clearly be taken into account before changes in behaviour associated with training experiments can reliably be classed as the result of the animals' experience. Undirectional responses (animal " learns " to escape more rapidly or to respond to a stimulus that did not elicit a response before) are particularly dangerous as indicators of learning for this reason, and even in discrimination experiments some control is necessary to ensure that the chance of a choice in a particular direction does not fluctuate in time. Very little has been done to quantify the effect of such cycles on learning by

marine forms. Young (1958) has shown that octopuses are more liable to attack objects seen in their aquaria in the evening than in the morning, a finding that correlates with activity records (Denton and Gilpin-Brown, 1960; Wells, unpublished) which show that at least some cephalopods are more active at night. In *Octopus* the diurnal fluctuation in level of attack does not seem to be great enough to effect the results of discrimination experiments. But this is not the case in some of the terrestrial and freshwater animals that have been studied (Arbit, 1957: earthworms learn more rapidly in evening trials; Best, 1965: planarians learn best at periods in phase with lunar cycles). Tidal periods might well be critical in the case of many marine forms.

3. Tested under appropriate conditions, most animals can be shown to learn something sometime. In assessing their capabilities in this respect it has to be remembered that learning by experience is a wasteful process and that selection for innate responses is particularly liable to take place in marine animals, many of which live in restricted environments that have probably remained practically unchanged over long periods of time. It seems reasonable to suppose that the capacity to change with experience is a primitive feature of all but the simplest nervous systems and that the degree to which learning is restricted or developed in each species depends on a nice balance between the advantages of knowing the answers at the outset and the extravagance of a genetic programme sufficiently elaborate to give a wide-ranging animal a reasonable chance of survival. Learning may indeed be a more reliable means of ensuring a high proportion of correct responses in situations where the programme would be elaborate, simply because of the uncertainties produced by genetic variation. No animal can start life without any built-in experience and few live in sufficiently simple and stable conditions for all responses to be innate. Somewhere between the two are most marine invertebrates. For each species there are bound to be areas where individual experience has been sufficiently constant generation by generation to have favoured the development of innate responses. It is not suprising that the occurrence of learning is erratic and the absence of the capacity is not remarkable in any instance unless it can be shown that the survival of the species suffers in consequence. The idea that it would be possible to trace the evolution of learning mechanisms from a survey of invertebrate animals is a sound one only if the series selected is comparable in respect of the things that each system has to do.

The ideal approach to learning is, therefore, in the context of the ethology of the animals under study. In practice, work has usually started with experiments on learning and bogged down when it became

apparent that the results were difficult to interpret in the absence of this background. The few really successful experiments—successful in the sense that it has been possible to use them as tools for further investigation of the animals' nervous systems—are those that by chance or design happened to mimic some natural learning situation. Thus, for example, the reward and punishment visual discrimination technique devised by Boycott and Young (1950) for training octopuses is successful probably because it tests the animals under conditions similar to those that they would have to contend with in the sea. Octopuses normally live in "homes" from which they emerge to attack passing prey. The tank situation teaches them which prey is worth attacking, a type of discrimination they would probably have to learn anyway. In the sea they must relearn repeatedly as they grow up and become capable of preying upon animals at first too large and dangerous for them to tackle.

4. It is possible, though the data are at present scanty, that animals can be divided into two great categories in respect of the things that they can learn. The distinction rests on whether they can or cannot take into account the positions of parts of their own bodies when they learn. Octopuses at least, which have an otherwise impressive performance, seem unable to do this. Arthropods, by contrast, seem to learn to make discriminations based on kinaesthetic cues relatively easily. It is very noticeable that arthropods and vertebrates are the only animals that have been shown to learn mazes beyond the complexity of a straightforward T. It has been argued elsewhere (Wells, 1963b, 1965b) that this may be because the movement of soft-bodied animals, unrestricted by joints, is necessarily organized hierarchically, so that proprioceptive inputs never penetrate to the uppermost parts of the CNS. With the restrictions imposed by joints, central control of movement becomes feasible and with this the possibility of taking details of movement into account in learning. In this respect the annelids may represent an intermediate condition in which segmentation has produced pseudoarticulation and with it the possibility of some central control of movement on a kinaesthetic basis. If these speculations are correct, the range of things that it is reasonable to try and teach a soft-bodied animal is limited.

5. The more one learns about invertebrates the more it becomes apparent that many, perhaps most of them, arrange their activities mainly in response to chemical cues. These, then, are the stimuli that should be used for most invertebrates in experiments on learning. And these, unhappily, represent the one class of stimuli that we ourselves find hard to measure and impossible to classify.

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ADDENDUM

The electrophysiology of molluscan learning has been approached with *Aplysia*, which has the advantage of giant nerve cells and the disadvantage that it is a comparatively inactive animal. Recording from the giant cell on the right side of the abdominal ganglia, Hughes and Tauc (1963) recorded a fall in response that paralleled the decline in the whole animal's response to prodding. Similar reductions in the size of the synaptic potential were found in response to electrical stimulation of the left and right giant cells, the fall (of the order of 50%) being very marked even between the first and second shocks (Hughes, 1965). Further studies (Bruner and Tauc, unpublished) are in progress to determine the site of the habituation process.

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EFFECTS OF HEATED EFFLUENTS UPON MARINE AND ESTUARINE ORGANISMS

E. NAYLOR

Department of Zoology, University College of Swansea, Wales

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I. INTRODUCTION

The largest single industrial use of water is for cooling purposes (Cairns, 1956) and vast quantities of water heated in this way are discharged into natural bodies of water. Balchin (1958) states that the electrical supply industry is the biggest user of water, taking upwards of 15 000 million gallons a day in Britain, and figures are available for this industry which enable the heating problem to be quantified. It is known, for instance, that a conventional coal-fired generating station of 400 megawatt (MW) capacity has to dispose of about 2000 million British Thermal Units (Btu) of heat per hour (Ross, 1959). This quantity of heat is sufficient to raise the temperature of a flow of water of 15 million gal/h from 10°C to 15°C, and 15 million gal/h is about twice the summer rate of flow of the River Thames at Teddington (Ross, 1959). In total the Central Electricity Generating Board had 22 264 MW of installed generating capacity in 1956 and it added 1788 MW in 1957. Such a rate of increase of 7–8% compound would mean a doubling of the 1956 output capacity by the late 1960's (Ross, 1959), by which time the heat output by electrical power stations alone in Britain would be about 2225×10^8 Btu/h. In the United States too, it is pre-

dicted that there will be a similar rate of increase until 1970, by which time output capacity will be about 250 000 MW, compared with about 85 000 MW in 1954 (Cairns, 1956). By 1970, therefore, the United States will be disposing of about $12\,500 \times 10^8$ Btu of heat/h, much of which will be discharged as heated water into natural water bodies. Cairns (1956) calculates that, assuming a doubling of output every 15 years, in the United States the total heat output by the end of the present century would heat the total minimum run-off of 220 000 ft^3/sec through 50 Centigrade degrees.

Increased utilization of cooling water in the electricity generating industry and in other industrial processes suggests that in future the emphasis will be away from the use of inland waters, as now, towards the utilization of estuarine and marine resources. Evidence of this shift of emphasis is to be seen in information and predictions which are available for the River Thames (Ministry of Housing and Local Government, 1961). In the 1950's 1000 million gallons of water were taken from the Thames each day and heated through 7–8 Centigrade degrees, thus raising the temperature of water at London Bridge by about 4 Centigrade degrees above normal, and at a point 26 miles downstream by rather less than 1 Centigrade degree above ambient. However, by 1960 the increase of heated effluents lower down the estuary resulted in an increase of average temperatures to more than 1 Centigrade degree above ambient at the station 26 miles below London Bridge. By 1967, at the same station, it is predicted that the temperature differential will be increased to 1·7 Centigrade degrees, whilst at London Bridge it is expected that the differential will decrease to less than 2 Centigrade degress. The siting of nuclear power stations in estuarine and marine localities also reflects this trend (Balchin, 1958; Mounfield, 1961; "Atlas of Britain and N. Ireland", 1963) and these stations produce at least twice the amount of heat generated by conventional power stations of similar capacity (Ross, 1959), one of 500 MW generating capacity requiring 840 million gal of water each day (Balchin, 1958). More specifically, the Hunterston Nuclear Generating Station in Scotland was expected to discharge up to 20 million gallons hourly at about 10 Centigrade degrees above ambient sea temperatures (Scottish Marine Biological Association, 1961). The possibility of extensive deployment of nuclear power stations (see Fig. 1) together with possible increased discharges of other industrial heated effluents, makes it worth while to consider effects which these effluents might have upon communities of marine organisms by collating such literature as is already available on the subject.

The precise biological effects of heated effluents in marine localities

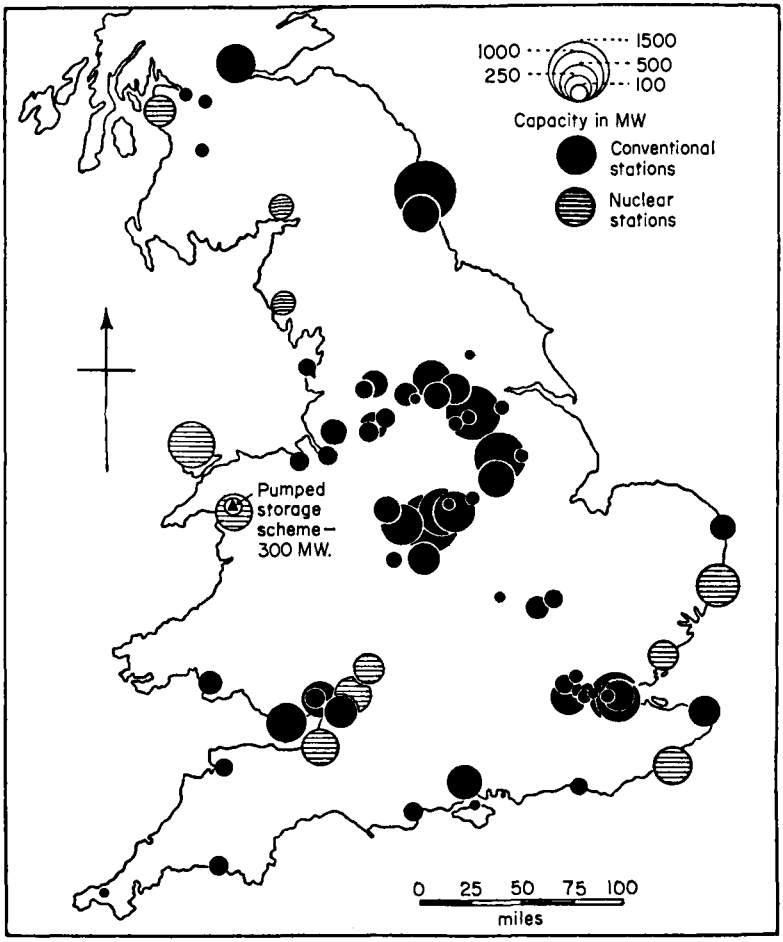

FIG. 1. Location of modern generating stations which supply or are intended to supply electricity to the British Central Electricity Generating Board, showing deployment of large nuclear stations to coastal areas. (After Mounfield, 1961 and "Atlas of Britain and N. Ireland," 1963.)

will obviously be expected to vary according to the extent to which the discharge area is enclosed as a cooling pond or is flushed by tidal streams or other water movements. Rarely, however, will temperatures be as high as those in non-tidal rivers. For example, van Vliet (1957) and Coutant (1962) recorded temperatures of up to 37°C and 40°C respectively in the Delaware River where summer temperatures normally reach about 27°C, Wurtz (1961) reported temperatures of

33–46°C in various Pennsylvanian rivers, and Cairns (1956) records exceptional heating to 60°C in Mahoning River at Youngstown, Ohio. The importance of continuous measurement in determining these maxima in heated areas is emphasized by Gameson, Gibbs and Barrett (1959) who demonstrated considerable diurnal fluctuations in temperature up to 5½ miles downstream owing to fluctuations in output at a power station. For marine localities such figures as are available indicate temperatures of up to about 40°C for water in the actual discharge ducts at power stations. Thus Fox and Corcoran (1957) record summer values of about 35–41°C in discharge tunnels of installations in Southern California and Naylor (1965) records temperatures taken at a power station ranging from about 23°C in winter to about 37°C in summer in a locality where mean sea temperatures range from about 7–17°C. Heat is, of course, dissipated very rapidly from such discharges, the average rate of heat loss from water being 9·7 Btu/ft^2/h/°C excess temperature (Gameson *et al.*, 1959), so that temperatures in the vicinity of the latter outfall were about 10 Centigrade degrees lower than those recorded in the effluent duct, though they were, of course, still higher than normal sea temperatures (Naylor, 1965). That author showed that in an almost completely enclosed system of docks used as a cooling pond by a continuously operating coal-fired generating station all the water in the docks was at least 5 Centigrade degrees higher than outside sea water. Temperatures near the outfall in this case were at all times between 7–10 Centigrade degrees above those of outside sea water and often reached nearly 30°C (Naylor, 1959, 1965). Crisp and Molesworth (1951) also recorded high temperatures of 25°C or more near an effluent in a marine harbour where temperatures normally ranged up to about 18°C, and Wolff (1954) recorded water temperatures of 6°C near factory outfalls in Copenhagen Harbour at a time when the harbour was normally icebound. In Southampton Water, too, Pannell *et al.* (1962) recorded a temperature rise of nearly 3°C for distances of up to a quarter of a mile away from heated outfalls, at one of which temperatures averaged about 8 Centigrade degrees higher than at the intake (Ansell *et al.*, 1964). In more open areas heating effects perhaps fall off more quickly away from outfalls, as Gilet (1960) found near Marseilles where heated effluents were quickly mixed with Bay of Marseilles water and heated areas were localized in fairly shallow surface layers or in isolated parts of the dock system. In open areas off Southern California, however, heating appeared to be more widespread, for temperatures higher than normal were recorded for distances of up to " a few thousand feet " away from the industrial outfalls (Resig, 1960).

In an estuary it has been reported that even during periods of low

flow temperatures below a power station outfall were only 1½ Centigrade degrees above those which would be expected without warming (Swain and Newman, 1962). However, heated water and other types of pollution may be patchily distributed in estuaries owing to the complexities of flushing and layering which may be encountered (Ketchum, 1951a,b; Newell, 1959; Croft, 1960), so heating might be locally severe. For instance, Pannell *et al.* (1962), Hockley (1963) and Raymont and Carrie (1964) have reported that in Southampton Water a salinity gradient due to the River Test produced layering in the water so that a generating station effluent of warm saline water was held between a surface layer of cold fresh water and a bottom layer of cold saline water. In such conditions, Pannell *et al.* (1962) point out that dispersion of heat will be by mixing and not by cooling to the atmosphere. As a result of this the effects of the heated effluent are felt at a greater distance from the outfall than if the heated water spread out and was cooled at the surface (Raymont and Carrie, 1964; Ansell *et al.*, 1964a).

II. *A Priori* Considerations

Before considering the direct effects of heated effluents it is useful first to consider very briefly what is known of experimental and ecological effects of temperature upon marine and brackish-water animals. There are innumerable papers on this subject which has been extensively reviewed in recent years, notably by Bullock (1955), Prosser (1955), Precht *et al.* (1955), Gunter (1957), Crisp (1959, 1964a), Brett (1960), Pennsylvania Dept. of Health (1962) and Kinne (1963). From a study of this literature it is possible to consider a variety of effects which heated effluents might be expected to bring about.

A. *Heat death*

Perhaps the first of many effects which should be considered is that of heat death, for it is clear that many organisms are killed at temperatures not far in excess of those to which they are accustomed (Gunter, 1957; Kinne, 1963). This is particularly true for tropical animals, which often live at temperatures very near to their thermal death points, whereas some arctic forms may live in water as much as 13 to 16 Centigrade degrees below their death temperatures (Mayer, 1914). In addition, despite the fact that acclimatization may take place (see p. 72), another relevant factor is that within any limited geographical area temperature tolerance may vary according to the habitat of particular species. Thus intertidal molluscs show correspondingly greater tolerance of high temperatures the higher up the shore they are

found and the longer the periods they are normally exposed to air (Fig. 2) (Vernon, 1899; Gowanloch, 1926; Gowanloch and Hayes, 1926; Broekhuysen, 1940; Evans, 1948; Bullock, 1955; and Segal *et al.*, 1953), whilst sublittoral species are much less tolerant than any intertidal forms (Henderson, 1929). Indeed, littoral organisms in general are more eurythermal than sublittoral species (Gunter, 1957; Southward, 1958)

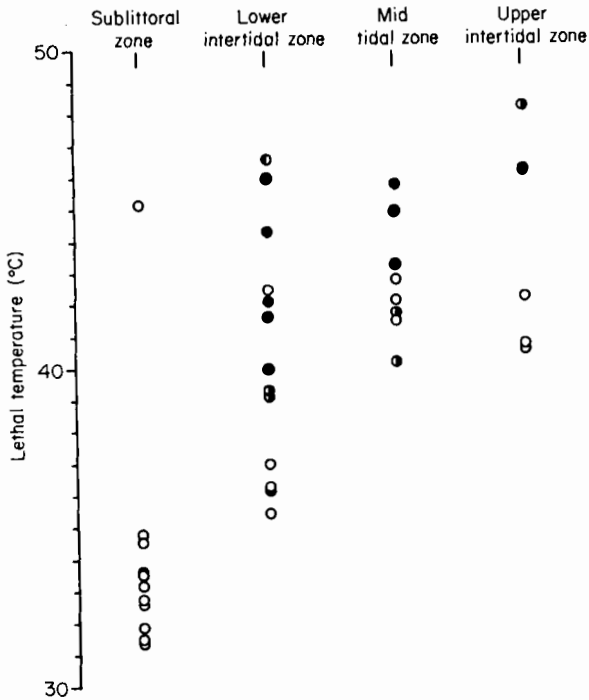

FIG. 2. Temperatures at which various marine molluscs from different tidal levels were killed, mostly in water heated at the standard rate of 1°C/5 min. Each symbol represents the lethal temperature of species from different localities as follows:— ○ St. Andrews (New Brunswick), ◐ Halifax (Nova Scotia), ◑ False Bay (S. Africa), ● Aberystwyth (Wales). (From data in Gunter (1957), after Gowanloch and Hayes (1926), Henderson (1929), Broekhuysen (1940), Evans (1948).)

amongst which, off the coast of Peru, it is reported that irregular appearances of the warm Equatorial Counter-current cause considerable mortality (Gunther, 1936). According to Schott (1913) the temperature of the counter-current averages about 25°C, which is about 7 Centigrade degrees higher than normal.

Estuarine species, too, may be expected to have a greater range of temperature tolerance than sublittoral marine species, since tempera-

tures like other physical factors fluctuate to a greater extent in estuaries than in the sea (Kinne, 1963; Hedgpeth, 1957; Moore, 1958; Naylor and Slinn, 1958; Tarzwell, 1962). Thamdrup (1935), for instance, showed that optimal temperatures for respiration were higher in upper-shore and brackish-water species than in lower-shore forms where less extreme temperatures are encountered. Waede (1954), too, demonstrated that the flounder (*Pleuronectes flesus* (L.)), a brackish-water species, tolerated high temperatures for longer periods than the plaice (*P. platessa* L.), a marine species, even at high salinities (see Fig. 3).

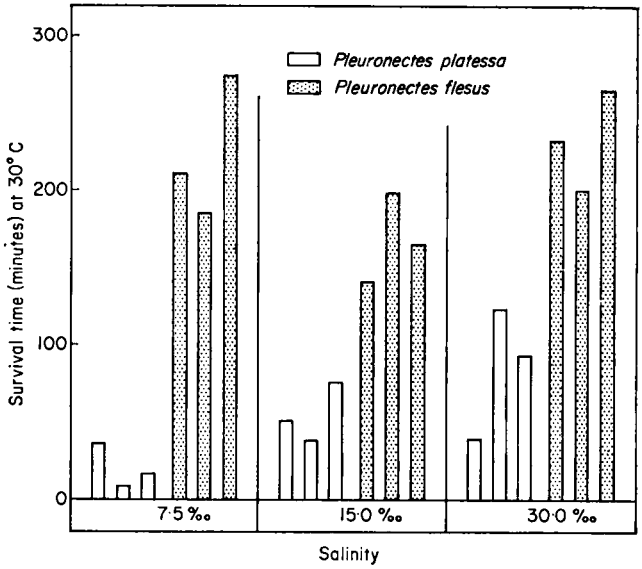

FIG. 3. Heat resistance, measured as survival times at 30°C, of the brackish-water species *Pleuronectes flesus* and the marine species *P. platessa* kept at various salinities. (From Remane and Schlieper, 1958; after Waede, 1954.)

There is similar evidence also for two species of *Idotea* (Naylor, 1954) in which specimens of similar size of *I. emarginata* (Fabricius), a sublittoral species, and *I. chelipes* (Pallas) (= *viridis* Slabber), a brackish-water species, were heated in sea water from 11°C at the rate of 1°C every 15 min. In these experiments *I. emarginata* died at about 33°C whereas *I. chelipes* survived even at 35°C and recovered to feed normally at 16°C 4 hours later.

B. *Metabolism, growth and form*

In temperate seas most poikilothermic animals have reduced food requirements in winter and cease growing then (Kinne, 1963). Heated

effluents might therefore serve to increase feeding rates in winter and also increase the growth rates of affected species, as Weymouth (1918) has shown for crabs and salmon which grew faster and attained greater size at comparable ages in years when the growing season was warmer and longer. Kinne (1960) too, has demonstrated that the fish *Cyprinodon macularius* Baird and Girard grows fastest from hatching to maturity at fairly high temperatures, with growth rates decreasing in the order of the following temperatures: 30°, 25°, 35°, 20° and 15°C. However, in *Cyprinodon* the high growth rates at high temperatures were not maintained later in the lives of the experimental fish and initially slow growing individuals at lower temperatures reached a greater final length and age. This indeed seems to be a fairly general phenomenon since many marine and brackish-water animals attain a larger final size in the colder parts of their distributional area (see for example Wimpenny, 1941; Sverdrup *et al.*, 1942; Gunter, 1957). Some exceptions to this rule are given by Moore (1958), but in general one might presuppose that heated effluents would lead to increased initial growth rates and precocious maturity, resulting in a decrease of adult size and perhaps shortening of the life cycle of at least some species.

C. *Reproduction*

Without entering into a full discussion of the effects of temperature upon reproduction in marine organisms (see Gunter, 1957) it is of particular relevance here to distinguish between the "vegetative temperature range" and the "reproductive temperature range" of the organisms likely to be affected by heated effluents. The fact that many apparently eurythermal species breed within a narrower range of temperatures than that which they tolerate as adults (Gunter, 1957; Kinne, 1953a, b,c, 1956a,b,c, 1959; Brett, 1960) makes it clear that survival of animals at temperatures simulating those near heated effluents does not preclude the possibility that the species may be prevented from breeding in such a locality and therefore would persist there only if continuously recruited from outside. Organisms which have already been shown to grow at temperatures higher than those at which they reproduce include the Pacific salmon (Fig. 4), *Gammarus duebeni* Liljeborg (Kinne, 1953a,b,c, 1959), *Crassostrea virginica* Gmelin (Gunter, 1957) and some hydroid coelenterates (Kinne, 1956c,d,e). Conversely, artificial warming might encourage the breeding of non-native species in areas which receive warm water species through the agency of polewards-flowing currents. Natural occurrences of such non-breeding warm water immigrants are reported in the Bay of Fundy (Huntsman and Reid,

1921; Fish and Johnson, 1937) and in areas affected by the Gulf Stream and North Atlantic Drift (Bigelow, 1926; Naylor, 1957a,d).

A further *a priori* consideration of the effects of temperature upon breeding concerns temperate regions where sub-polar and sub-tropical species may occur together. In such regions there is considerable evidence to show that high-latitude species breed in winter and low-latitude species breed in summer (Apellöf, 1912; Orton, 1920; Runnström, 1928; Thorson, 1946; Crisp, 1954; Qasim, 1956), so heated effluents might be expected to have differential effects upon these two groups of animals. Thus heating might raise temperatures sufficiently

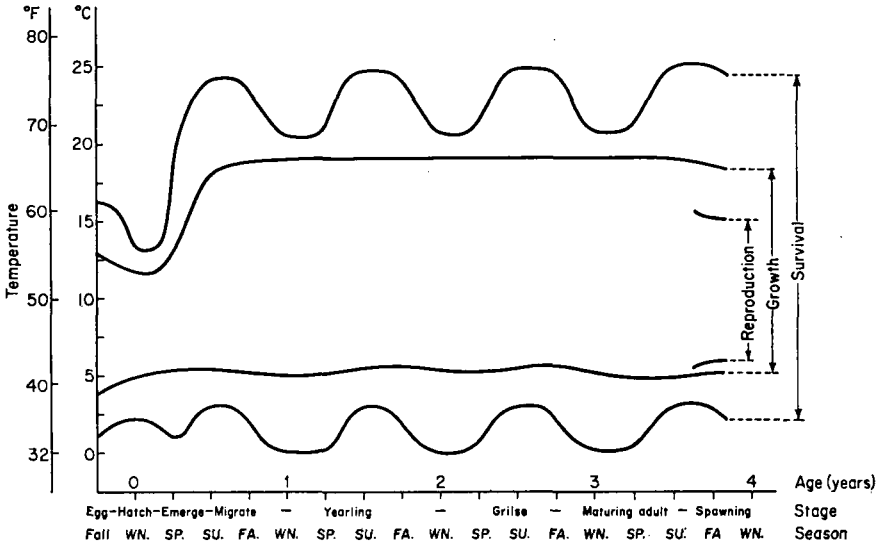

FIG. 4. Schematic representation of temperature limits for different life processes in Pacific salmon. (After Brett, 1960.)

to prevent breeding in high-latitude species, whilst at the same time inducing low-latitude species to become more prolific. For instance, fertilization can be delayed indefinitely in British specimens of the winter breeding boreal-arctic barnacle *Balanus balanoides* (L.) by maintaining temperatures above 10°C (Crisp, 1957, 1964b). Conversely, several low-latitude species of molluscs and barnacles have been induced to breed in the laboratory during their non-breeding period by raising water temperatures and providing sufficient food (Loosanoff and Davis, 1950; Patel, 1959; Patel and Crisp, 1960). In the field, Southward and Crisp (1954, 1956) have suggested that even quite minor changes in average sea and air temperatures affect the ecological

equilibrium between the competing barnacles *Chthamalus stellatus* (Poli) and *Balanus balanoides* in Britain. Such changes, if sufficiently prolonged, cause the boundaries of distribution of each species to alter considerably, so that slight increases in temperature result in a northward spread of the low latitude species, *Chthamalus*, at the expense of the high latitude species, *Balanus*, and vice versa. As Southward (1958) points out, the evidence for a causal relation between temperature and distribution in this way must be sought in non-lethal terms such as debilitating effects, or indirectly through competition, or in combination with other factors.

Finally, if electricity generating stations tended to produce more power in winter and consequently discharge greater quantities of heat at that time, their effluents would result in a narrowing of the annual range of temperatures in the discharge area. Extensive narrowing of the seasonal range of temperature would probably have considerable biological effects since it is known that a constant temperature of a particular value and fluctuating temperatures of that average value do not necessarily have the same biological effects (Kinne, 1963). For instance, the brackish-water amphipod *Gammarus duebeni* exhibits normal growth rates, reproduction rates, sex ratio and life span only under conditions simulating annual fluctuations in the environment (Kinne, 1952, 1953a,b,c).

D. *Behaviour*

Many inshore marine species and estuarine forms undertake migrations to deeper water in winter. Such migrations are well documented in Crustacea (Bainbridge, 1961; Naylor, 1962; Kinne, 1963) where salinity and temperature are controlling factors. Temperature changes, too, govern the seawards and riverwards migrations of salmon (Foerster, 1937), which in rivers are sometimes killed by heat during hot summers (Huntsman, 1942). In considering the biological effects of siting heated effluents in estuaries the possible long-term effects upon migratory species should, therefore, not be overlooked.

E. *Acclimatization**

Despite the sensitivity of many species to high temperatures there is nevertheless accumulating evidence that many poikilothermic

* The term "acclimation" is avoided here. It is sometimes used to indicate experimentally induced and fairly rapid thermal adaptation, as distinct from slow, naturally occurring adaptation (see Erichsen Jones, 1964). Other authors, on the other hand, still use "acclimation" synonymously with the term "acclimatization" as used here (see Kinne, 1963).

species are capable of considerable acclimatization over a wide range of temperatures. This follows from numerous observations that the rates of various physiological activities within members of one species or between closely related species vary predictably with seasonal, spatial and experimental variations in temperature (Bullock, 1955). Mechanisms of acclimatization are reviewed and discussed by Kinne (1963) and are beyond the scope of the present discussion. Here it is relevant to consider the effects which a rise in water temperature might have upon different systematic groups of animals and upon different marine communities.

In coelenterates latitudinal acclimatization has been demonstrated in *Aurelia aurita* (L.), which show maximal swimming activity at higher or lower temperatures according to the latitude from which they are collected. Thus specimens from Halifax, Nova Scotia, collected from water at 14°C, showed maximal pulsations of the bell between 0° and 27°C whereas specimens from water at 29°C in Tortugas, Florida, showed maximal activity over the temperature range 13–35°C (Mayer, 1914). Similarly, *Cyanea capillata* (L.), a cold-water stenotherm, pulsates at about the same rate at 15°C as *Cassiopeia xamachana* R. P. Bigelow, a closely related tropical form, does at 29°C (Mayer, 1914). Geographical temperature compensation of this kind, in metabolism, development and activity, has also been demonstrated in a variety of poikilotherms, particularly polychaetes and crustacea (Fox, 1936, 1938, 1939; Fox and Wingfield, 1937; and Wingfield, 1939) and, more recently, in a shore crab *Pachygrapsus crassipes* Randall (Roberts, 1953, 1957), the amphipod *Gamarus duebeni* (Kinne, 1953b) and *Balanus balanoides* (Crisp, 1964b). *Pachygrapsus* appeared to show no acclimatization to monthly changes in temperature, but short-term unseasonable changes in temperature seemed to be reflected in metabolism and behavioural activity (Roberts, 1957). Amongst other crustacea changes in metabolism associated with seasonal variations in temperature have been demonstrated in the sand crab *Emerita* (Edwards and Irving, 1943a), the beach amphipod *Talorchestia* (Edwards and Irving, 1943b) and probably also in the copepod *Calanus finmarchicus* (Marshall *et al.*, 1935). Halcrow (1963) confirmed that the acclimatization takes place in *Calanus finmarchicus* (Gunnerus) but showed that it was possible only within the normal seasonal range of temperature in the water from which specimens were collected. This ability to compensate for changes of temperature within the normal seasonal range of variation has also been observed in various rhythmic activities of decapod crustacea. These include rhythms of chromatophore expansion and contraction in the fiddler crab *Uca* (Brown and Webb, 1948), the blue crab *Callinectes*

sapidus Rathbun (Fingerman, 1955) and the green shore crab *Carcinus maenas* (L.) (Powell, 1962), respiratory rhythms in *Uca* (Brown *et al.*, 1954) and rhythms of locomotor activity in *Uca* (Bennett *et al.*, 1957) and *Carcinus* (Naylor, 1958, 1963). Experimental acclimatization to high temperatures, as measured by altered death temperatures, has been demonstrated in *Homarus americanus* H. Milne Edwards (Mcleese, 1956), *Ligia* (Edney, 1960), *Gammarus* (Kinne, 1953b,c) and *Artemia* (Grainger, 1958).

Amongst molluscs *Mytilus* has been shown to have considerable powers of acclimatization (Rao, 1953a,b). Thus *M. californianus* Conrad from higher latitudes in North America exhibits consistently higher rates of ciliary pumping than low latitude species at low temperatures. Moreover, specimens of this species and those of *M. edulis* (L.) collected low down on any one shore have lower pumping rates than specimens of the same species collected higher up the shore. It seems therefore that the high tide specimens are adapted to the high average temperatures prevalent in the upper shore, and, in one case, the difference in pumping rates of specimens collected only 75 cm vertically apart was equivalent to the difference between specimens collected at the same tidal level in two localities about 330 miles apart in a north-south direction. Adaptive changes associated with varying temperatures are also evident in *Mytilus* from further observations by Rao (1954) who demonstrated a temperature independent tidal rhythm of pumping activity. Latitudinal acclimatization has also been demonstrated in a variety of other molluscs, including oysters which spawn at different critical temperatures according to the latitude at which they occur (Stauber, 1950; Loosanoff and Nomejko, 1951). Similar phenomena have been demonstrated too in three species of gastropod molluscs, *Thais emarginata* Deshayes, *Crepidula nummaria* Gould and *Lacuna carinata* Gould, in which the growth of larvae within the egg capsules was shown to be from two to nine times faster in Alaskan specimens than in Californian specimens of the same species at any given temperature between 10–16°C (Dehnel, 1955). Physiological differences according to tidal level have been demonstrated in the limpet *Acmaea limatula* Carpenter in which at a given temperature rates of heart beat are higher in specimens taken from low levels of the shore than in those from higher levels, the higher tide specimens apparently being adapted to higher average temperatures prevalent in that habitat (Segal *et al.*, 1953). Transplantation of limpets between levels suggests that complete acclimatization is possible within a few weeks (Segal, 1956). In earlier experiments too, various workers have compared the thermal death points of specimens of the same species or of

closely related species of molluscs from different tidal levels, and all support the view that temperature acclimatization takes place in this group. Thus, Vernon (1899), Gowanloch (1926), Gowanloch and Hayes (1926), Henderson (1929) and Broekhuysen (1940) all concluded that gastropods and lamellibranchs inhabiting lower levels of the shore were less tolerant of high temperatures than upper shore specimens. More recently, Evans (1948) was unable to confirm this in some British species, but that author was able to show differences in thermal tolerance according to the particular microhabitats of specimens within any one species.

Amongst other groups there is evidence for acclimatization and resistance to high temperatures in marine fishes (Doudoroff, 1942, 1945; Morris, 1961), so it is clear that a wide variety of marine animals show considerable powers of temperature compensation. Some exceptions to this general conclusion are given by Fox (1938, 1939), Fox and Wingfield (1937) and Wingfield (1939), but Berg (1953) recalculated Fox and Wingfield's data and concluded that of the exceptions which they listed only the crinoids *Antedon petasus* (Dübon and Koren) from West Sweden and *A. bifida* (Pennant) from Plymouth showed no apparent acclimatization. More recently too, Farmanfarmaian and Giese (1963) have reported that another echinoderm, *Strongylocentrotus purpuratus* Stimpson, showed no acclimatization beyond the upper limit of its temperature range of 5–23·5°C. Temperatures of 25°C were found to be lethal and the apparent thermal tolerance of the species in having a wide geographical distribution seems to be related to a change of habitat rather than to acclimatization. Whereas the species is intertidal in the northern part of its range on the Pacific coast of North America, it is sublittoral on Mexican coasts, presumably owing to the high average temperatures of the intertidal zone in that locality. These examples suggest that echinoderms have little power of temperature acclimatization and would therefore be very susceptible to the temperature change.

In conclusion the evidence available so far concerning the temperature relationships of marine species suggests that in artificially heated areas several types of effect could be observed. Cold water stenothermal forms might be eliminated or they might be incapable of breeding in the heated locality and would survive only by recruitment from outside. Some more eurythermal species might become warm-adapted by the process of acclimatization and survive to breed in the heated locality, perhaps even evolving into separate races or species from native forms. Also, immigrant warm-water stenothermal species might be encouraged to breed there.

III. Observed Effects

In a general paper concerning the effects of power station cooling water on aquatic communities Markowski (1959) showed that some animals were alive even after being circulated through the cooling condensers and he found no difference in faunal composition between the intake and outfall areas. However, in the first place Markowski's main conclusions apply to organisms in brackish regions which might be presupposed to be rather more tolerant of heating than fully marine species (see p. 68). Also, though some small, hardy, brackish-water species were shown to survive for a few days after being circulated through power station condensers, longer term effects upon the individual animals were not considered, nor were the possible effects upon breeding (see p. 70). Moreover, from a number of other papers upon the effects of heated effluents in both marine and estuarine waters, several types of change have been observed which agree closely with the conclusions drawn from *a priori* considerations discussed above. There are known to be examples of the elimination of some native species and of considerable effects upon the breeding and general biology of others, some of which are of commercial importance. Also, there are many examples of the introduction of immigrant species to artificially heated localities.

A. *Elimination of native species*

Effects are most striking in the immediate vicinity of outfalls, particularly in summer. Thus in the Delaware River van Vliet (1957) reported the absence of Tardigrades from heated regions and Trembley and his collaborators have shown an almost complete elimination of most species of fish from the maximum heated zone in summer (Trembley, 1960, 1961, 1965). In the same river Coutant (1962) has shown that in summer there is a substantial reduction in the number, diversity and biomass of macroinvertebrate riffle organisms in that portion of the river lying in the path of water discharged from the power station. In the Schuylkill River (Pennsylvania) Wurtz and Dolan (1960) report the elimination of thermally intolerant species below a power station effluent and a differential increase in the numbers of tolerant species which remain. Fish are sometimes killed by heated effluents (Alabaster and Swain, 1962) but such forms usually avoid death by swimming to cooler water nearby. The general problem of heated effluents in relation to freshwater fish is discussed by Erichsen Jones (1964) who doubts whether significant numbers of such fish are killed by thermal pollution.

For freshwater organisms in general the normal population structure is maintained only up to a tolerance limit of about 32°C and extensive loss in numbers and diversity of organisms occurs above that temperature (Coutant, 1962). Some genera were shown to be more tolerant than others of temperatures higher than 32°C, but all species were limited by temperatures of 40–43°C (Coutant, 1962). Cairns (1956) concluded that to maintain survival in temperate streams large areas should not be heated above about 30°C for long periods. This conclusion is also supported by results of work by Alabaster (1963) who showed that though coarse fish are attracted into water heated to about 26°C, temperatures of above 30°C were avoided.

All this work, including some in Poland (Stangenberg and Pawlaczyk, 1961) and in the U.S.S.R. (Luferova, 1960; Mikheev, 1962; Rutkovskiy, 1962) has been concerned with freshwater localities. In strictly marine or estuarine localities effects are less striking since effluents are usually immediately diluted in larger volumes of cooler water. However, Chadwick *et al.* (1950) have pointed out that whereas the intake tunnels of power stations are often choked with fouling organisms, no serious fouling occurs in discharge tubes. The elimination of fouling species was attributed by these authors to differences in temperature of the water in the two conduits, and indeed they, like Ritchie (1927), describe a system for controlling troublesome growths of sessile marine organisms by periodically reversing the flow of discharged water down alternative intake tunnels. Experimental work on the thermal tolerance of fouling organisms, particularly *Mytilus*, coupled with trials at the Westbank Electricity Station, Portobello (Ritchie, 1927) and at the Redondo Power Station (Chadwick *et al.*, 1950; Fox and Corcoran, 1957) showed that fouling by *Mytilus edulis* and *M. californianus* could be completely controlled in this way. Thus in Southern California, where these species normally breed and settle from mid-March to mid-October when sea temperatures are about 16°C and above, mussel fouling was prevented by tri-weekly reversals of discharge water either for periods of 1 h at 38–41°C or for 7 h at 34·5°C (Fox and Corcoran, 1957). In Scotland Ritchie (1927) showed that *Mytilus edulis* could be controlled by flushing the intake ducts with effluent water at a minimum temperature of about 43°C once every 4 weeks during the spatting season from March to October.

In the vicinity of ocean outfalls off Southern California, Resig (1960) has shown that heating effects are apparent up to a few thousand feet away from discharge areas and that in the heated areas species of Foraminifera with calcareous tests are less abundant than those with arenaceous tests. However, though it is known that Foraminifera are

indicators of temperature-depth variations, the presence of sewage may be a more limiting factor for calcareous species than high temperatures. Industrial outfalls of mixed pollutants including heat are also reported to bring about the elimination of species by Reish (1960).

Enclosed areas, such as docks used as cooling ponds for power stations, might be expected to provide particularly striking examples of the elimination of native species. Thus though Markowski (1959,

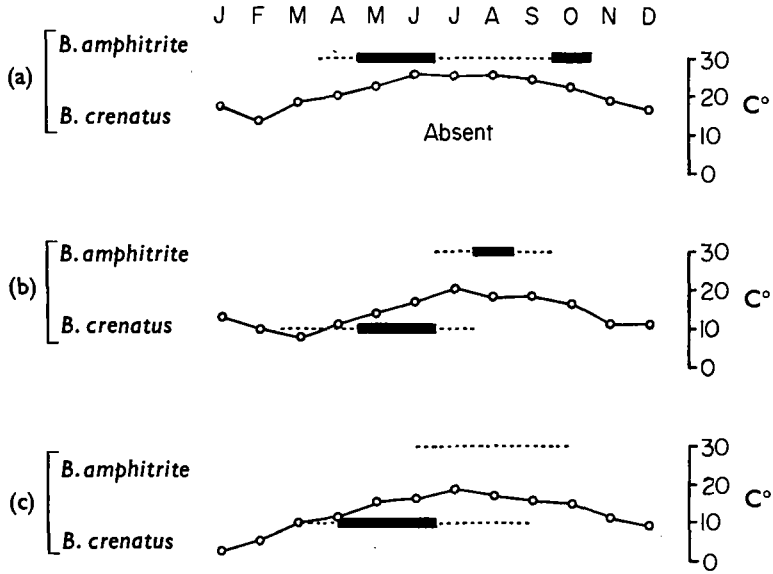

FIG. 5. Results of experiments carried out in a heated dock showing settlement on test panels of the warm water immigrant barnacle *Balanus amphitrite* and the native barnacle *B. crenatus* (a) in 1957 when temperatures were 7 to 10 Centigrade degrees higher than normal and (b,c) in 1962 and 1963 when temperatures were less than 7 Centigrade degrees higher than normal. (From Naylor, 1965, and additional unpublished data kindly supplied by Mr. D. W. Pearce.)

1960, 1962) records no detrimental effects upon native species in predominantly brackish areas, Naylor (1959, 1965) has recorded considerable effects in an almost fully saline locality. Two factors probably contribute to the different conclusions of these authors, firstly that brackish-water animals perhaps show more tolerance of heated conditions than marine species (see p. 68) and secondly that the heating effect was probably slightly greater in the marine locality. Mean temperatures taken by Markowski (1960, 1962) near the outfall of a power station in Cavendish Dock, Barrow-in-Furness, ranged from about 12°C in winter to about 28°C in summer, and those taken by Naylor

(1959, 1964) in Queen's Dock, Swansea, ranged from 14–28°C. Though these ranges appear very similar it does seem that the temperatures in Queen's Dock were taken rather farther away from the outfall than those in Cavendish Dock. Moreover, in contrast to Queen's Dock, temperatures in Cavendish Dock periodically fell to near ambient sea temperatures owing to intermittent working by the power station, so perhaps the heating in Queen's Dock was more prolonged. At any rate the biological effects of heating in Queen's Dock were most striking (Naylor, 1959, 1965). In particular, British native species of barnacles were excluded from the sampling area, either directly owing to the high temperatures or indirectly through competition with the artificially introduced subtropical species *Balanus amphitrite* var. *denticulata* Broch (see p. 87). Other evidence that high temperatures appeared to exclude some species was also apparent in observations in the same area during a period when the dock was cooler than before, owing to a reduction in power output at the generating station and a corresponding fall in the volume of the heated discharge (Naylor, 1965). During that period the native barnacle *Balanus crenatus* Brugière occurred in the dock alongside *B. amphitrite* (see Fig. 5), and *Elminius modestus* Darwin also extended its range towards the heated effluent. In addition, other common native species such as *Gammarus locusta* (L.), *Aurelia aurita* and *Cryptosula* were recorded for the first time near the outfall, and it seems reasonable to suppose that the apparent absence of these native species during extensive observations when the dock was at its warmest was due simply to the effects of the heated discharge (Naylor, 1965).

B. *Effects upon breeding and general biology*

In addition to the experimental and field evidence that many species tolerate higher temperatures than those at which they breed (p. 70), *Carcinus maenas* is an example of a cold-temperate water species which appeared to thrive abundantly in an artificially heated locality without apparently being able to breed there. At a time when the seasonal range of temperatures in a heated dock was from 14–28°C *Carcinus* was found to be very abundant and apparently did not breed, for specimens moulted throughout the winter months (Naylor, 1965) when moulting normally ceases and females carry eggs (Naylor, 1962). Presumably under those conditions of heating the species was recruited from unheated areas outside the dock, for during a period when dock temperatures were lowered to near ambient sea temperatures breeding was observed to take place (Naylor, 1965; Pearce, 1965). Markowski (1962) also recorded the absence of breeding amongst *Carcinus* in a heated dock, but in this case high temperatures may have been less

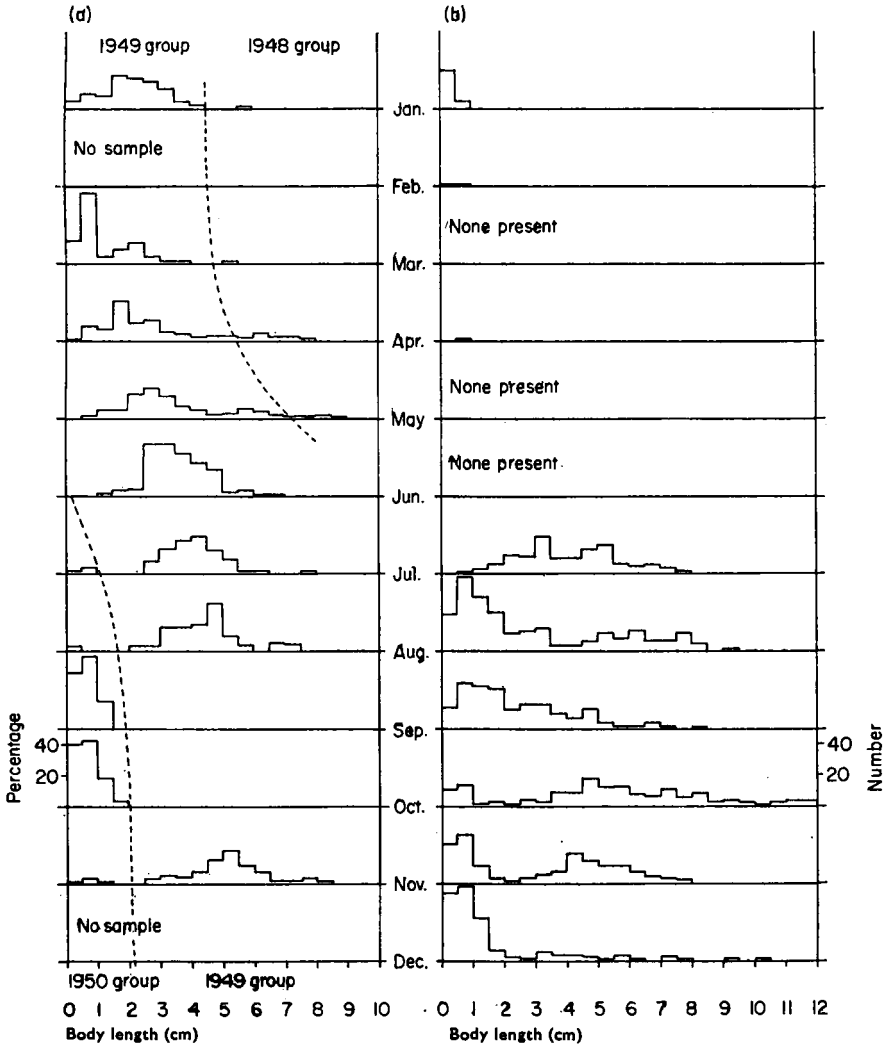

FIG. 6. Growth of *Ciona intestinalis* (a) during 1950 in an unheated dock in Scotland (After Millar, 1952) and (b) during 1957 in a heated dock in S. Wales (After Naylor, 1965).

limiting than low salinities which were also recorded, for it is known that female *Carcinus* from estuaries tend to migrate to deeper more saline water where egg development takes place (Broekhuysen, 1936). So far there is no experimental evidence to support the view that raised winter temperatures might prevent breeding in *Carcinus maenas* and the conclusion itself might seem surprising in view of the fact that the species

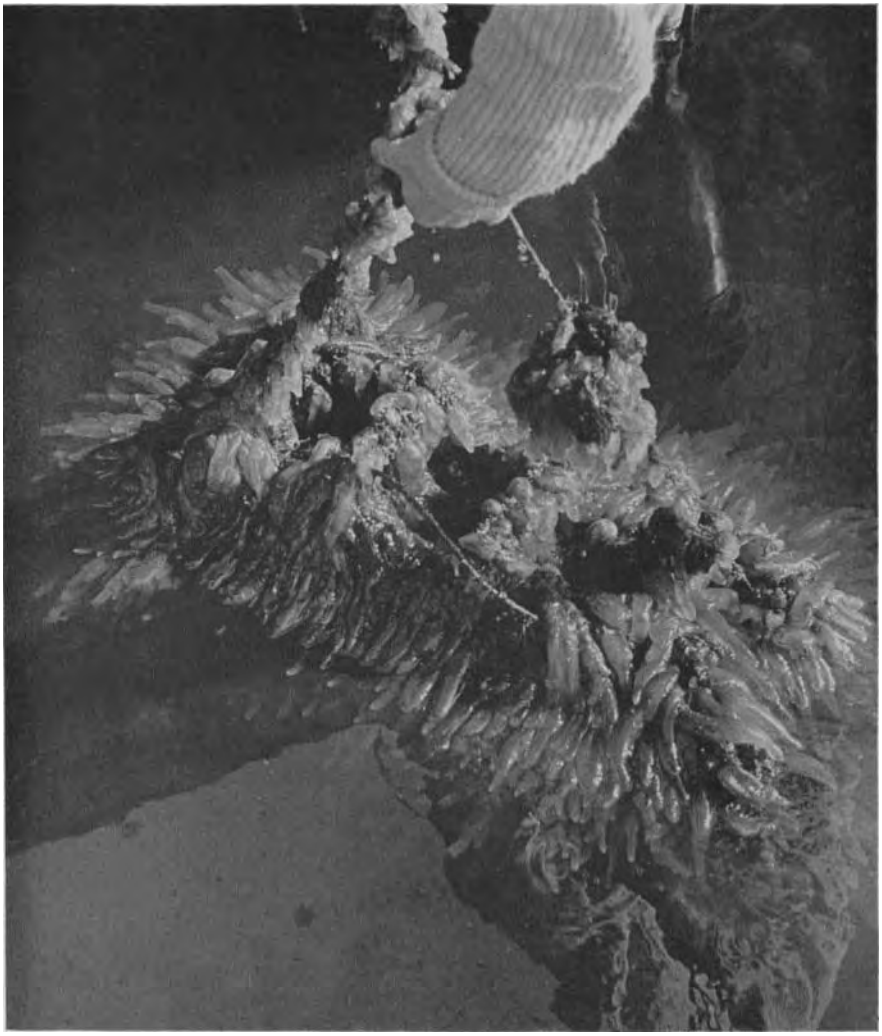

FIG. 7. Dense growths of rapidly growing *Ciona* on test panels exposed in the heated dock at Swansea. Specimens were about 2 months old and measured up to 20 cm in length. (*Photo:* C. Stockton).

is often regarded as a cosmopolitan form which ranges into subtropical areas. However, there are known to be physiological races within this species (Veillet and Demeusy, 1953; Demeusy, 1958; Carlisle, 1957) and Mediterranean forms at least have recently been reinstated as a separate species (Holthuis and Gottlieb, 1958).

The effects of heating on the breeding of lower latitude species in

temperate marine waters are better documented. As in freshwater localities (van Vliet, 1957; Coutant, 1962; Mann, 1965) there is evidence that some organisms show an increase in numbers and their seasonal cycles of abundance are shifted or extended. In Britain, Crisp and Molesworth (1951), for instance, recorded breeding and settlement of *Balanus amphitrite* from March to September in artificially heated areas, and the absence of breeding for the same species within the normal range of sea temperatures (see also Fig. 5). Another barnacle, *Elminius modestus*, also showed a more extended breeding period in heated parts of Southampton Water (Pannell *et al.*, 1962), whilst in the same area a predominantly low latitude estuarine copepod *Acartia tonsa* Dana has increased in abundance (Conover, 1957; Raymont, 1964) and a related warm water species *Acartia grani* G. O. Sars has appeared for the first time (Raymont, 1964). In addition, Ansell (1963b), Ansell and Loosemore (1963) and Ansell *et al.* (1964a) have shown that *Venus mercenaria* (L.) in heated localities in Britain had advanced spawning times, protracted growing seasons and showed significantly greater total annual increments in shell length and total weight than specimens found in unheated areas. More prolonged breeding periods have also been reported for a number of species, including fouling and boring forms, in heated docks at Swansea, S. Wales. These include two species of the bryozoan *Bugula* (Ryland, 1960), *Balanus amphitrite*, *Corophium acherusicum* A. Costa, *Limnoria tripunctata* Menzies, *Botryllus schlosseri* (Pallas), *Diplosoma listerianum* (Milne Edwards), *Ascidiella aspersa* (O. F. Müller) and *Ciona intestinalis* (L.) (Naylor, 1965). The patterns of growth and reproduction of *Ciona* (Figs. 6 and 7) and *Ascidiella* were of particular interest, for normally in Britain these two species often occur together and reproduce as annuals. At Swansea, however, they each produced more than one generation each year and in the warmest parts of the dock competition between the two seemed to be such that *Ascidiella* was most abundant in winter and spring whilst *Ciona* was more abundant in summer and autumn (Naylor, 1965). The maximum size reached by *Ascidiella* was also much smaller than would be expected in Britain (see p. 70).

The extension of breeding periods and alternation of abundance of species in this way not only leads to changes in the relative abundance of different species but also demonstrates that the problem of fouling on dock structures and ships could be considerably worsened by heating. For instance, barnacles and mussels appear to have increased in density in Southampton Water as a result of only slight heating (Pannell *et al.*, 1962). In Copenhagen Harbour, too, it has been shown that the growth rate of *Mytilus* is linearly related to the age of the animals expressed in

" day degrees ", where " day degrees " are defined as mean temperatures × days (Boëtius, 1962). Reish (1964), also, has demonstrated a direct relationship between the number of species in a *Mytilus edulis* community and the temperature of the water, such that in higher temperatures a greater diversity of organisms was observed. Furthermore in heated areas there is evidence of more protracted periods of colonization of new timber, as well as of breeding, by the wood-boring isopod *Limnoria* (Eltringham and Hockley, 1961; Pannell, 1962; Jones, 1963; Hockley, 1963; Naylor, 1965), and by *Teredo* (Pannell *et al.*, 1962). There is too some evidence that heating resulted in increased depredation by *Teredo* in docks at Swansea (Bell, 1949), such that it became necessary to replace wooden jetties with structures of reinforced concrete. It seems, also, that after the early 1930's when the docks were first used as cooling ponds it soon became uneconomical to use the small timber boats which carried mooring ropes of docking vessels owing to increased depredation by wood borers. At any rate, at that time the timber boats were replaced by metal punts and many dock timbers were replaced by concrete piles. Not all the effects of heating are detrimental since, for the River Thames (Ministry of Housing and Local Government, 1961), it has been concluded that there are only localized effects from even the largest heated effluents, and that some miles downstream some benefit might be felt. The beneficial aspects of heated effluents have also been stressed by Markowski (1960) on the basis of higher densities of organisms nearer outfalls than intakes. In addition Iles (1963) and Markowski (1965) have reported increased rates of growth of fish in the cooling ponds of power stations, which clearly have beneficial implications (but see p. 70).

Apart from the direct effects of heated effluents several possible indirect effects should be considered, since temperature also influences the effects of other environmental factors such as oxygen concentration, CO_2, pH, dissolved solids and toxins (Pennsylvania Dept. of Health, 1962). The fact that high temperatures may be associated with low concentrations of dissolved oxygen, particularly in areas receiving oxidizable effluents as well as heat, has been confirmed by Alabaster and Wellcomme (1962). This occurs despite the fact that high temperatures increase the rate of oxygen uptake by water at any particular level of oxygen deficiency, owing to the facts that solubility is reduced and rates of oxygen utilization by bacteria are increased (Ministry of Housing, 1961). Thus even though Swain and Newman (1962) report that oxygen variations in a heated tidal river were related to variations in freshwater flow and pollution, not to heating, nevertheless it has been recommended (Ministry of Housing and Local Government, 1961) that

heated discharges should be avoided in areas of low oxygen concentration unless compensatory measures can be taken. Freshwater fish are known to show reduced toleration of high temperatures in lowered oxygen tensions (Downing and Merkens, 1957; Alabaster and Wellcomme, 1962) and the marine serpulid *Hydroides norvegica* (Gunnerus) settles less abundantly in harbour areas where oxygen concentrations are low (Reish, 1961), thus no doubt avoiding polluted areas. Another possible side effect is the reduced tolerance of toxic substances such as might be discharged in or alongside heated effluents. This has been demonstrated mainly for freshwater organisms by a number of authors (Ministry of Health, 1949; Cairns and Scheier, 1958, 1962; Doudoroff and Katz, 1953; Hynes, 1960). The inter-dependence of several types of pollution, including heat, is also illustrated by the work of Resig (1960) (see p. 77), and also by Milne (1940). The latter author showed increases in abundance of *Jaera albifrons* Leach in areas of the intertidal zone of the Aberdeenshire Dee which were affected by effluents from a food factory and from a power station. The relative effects of the two sources of pollution were not investigated.

In estuaries, apart from the interplay of temperature and the factors mentioned above, the effects of heating may also extend to changes in the osmoregulatory capacity of the organisms concerned. However, though the combination of temperature and salinity is of basic importance (Kinne, 1960, 1964), opinions vary as to the precise effects which are to be expected. Pearse and Gunter (1957) review this subject and conclude that osmoregulation is facilitated by higher temperatures, which may be one reason to account for the abundance of organisms in tropical estuaries compared with those in cold-temperate regions. On the other hand Kinne (1963) concluded that in general the osmoregulatory capacity of a given species appears to be greatest at or just below optimum temperatures and to decrease in supra-normal temperatures. At any rate, in practice it seems that estuarine forms are more tolerant of heated effluents than marine species (see p. 68).

There appear to be few observations on the effects of heat upon marine and estuarine plants and microorganisms. Most fixed marine algae are said to be eurythermal (Pruvot, 1896) and have even wider ranges of distribution than their ability to reproduce would indicate (Setchell, 1920). In freshwater localities too, it is reported that thermal pollution does not effect plant communities (Rees, 1954), but Hynes (1960) has recorded the occurrence of an immigrant flowering plant species *Vallisneria spiralis* L. near heated effluents in freshwater. In addition, effects upon the growth of various stream algae have been demonstrated by Cairns (1956). He showed that diatoms were most

abundant at 20°C, that green algae were most abundant at about 32°C and blue green algae most prolific at 40°C. Blue green algae therefore appear to be indicators of extreme thermal pollution as they also seem to be of organic and chemical pollution (Cairns, 1956; Trembley, 1965). Amongst brackish-water forms, too, *Nitzschia filiformis* (W. Sm.) grows best under conditions of slight heating at 26°C. Indeed, in marine and estuarine localities unicellular algae grow prolifically where there is local warming in areas which are well supplied with nutrients, often resulting in " red tide " and other forms of algal blooms (Brongersma-Sanders, 1948; Hayes and Austen, 1951). Artificially heated regions in temperate waters would therefore be more prone to occurrence of such blooms. In Britain it has been shown that heated effluents do result in increased total plankton production (Pannell *et al.*, 1962; Hockley, 1963) and that the spring outburst of phytoplankton may occur earlier than in unheated areas (Pearce, 1965).

C. *Replacement faunas*

In general discussions of the effects of pollution Hynes (1959, 1960) distinguishes two types of pollution, " man-made " and " natural ". The first of these usually results in the elimination of some species, often accompanied by a corresponding increase in those that remain. The second type on the other hand may result in the substitution of " replacement " organisms which are pre-adapted to the peculiar conditions of the polluted area. This effect is produced by types of pollution which closely parallel naturally occurring conditions, such as pollution by organic matter, mineral salts and reduced salts of iron. Thermal pollution is also a "natural" type of pollution and might be expected to be tolerated by a replacement warm water fauna, provided such forms had a suitable means of dispersal. Indeed, efforts to find indicator species for marine pollution both in the United States and in Japan suggest that the polychaete *Capitella capitata* (Fabricius) is particularly abundant in areas affected by mixed domestic pollution including heat (Filice, 1954, 1959; Kitamori and Kobayashi, 1958; Kitamori *et al.*, 1959; Kitamori and Funae, 1959; Kitamori and Kobe, 1959; Reish, 1955, 1957). In addition, the crab *Rhithropanopeus harrisi* Gould also occurs in greater abundance around mixed industrial outfalls than in unpolluted areas nearby (Filice, 1954, 1959; Reish, 1955, 1957, 1960). However, whereas replacement faunas for most types of natural pollution can occur in the same geographical areas as species which are eliminated, heat-tolerant species would normally be expected to occur only in low latitudes. An important prerequisite, therefore, if thermal pollution is to result in the substitution of replacement animals, is that

heat-tolerant species should be accidentally or deliberately introduced from remote areas, for they will rarely spread by natural means. In temperate fresh waters the introduction of warm water immigrants is relatively rare, though Mann (1958, 1965) has documented the occurrence of a tropical tubificid *Branchiura sowerbyi* Beddard in British waters and Hynes (1960) records the occurrence of an immigrant plant *Vallisneria spiralis* near heated effluents. In marine areas, on the other hand, interchange of animals between geographical areas is more prevalent, largely since animals are transported by ships. However, even in those

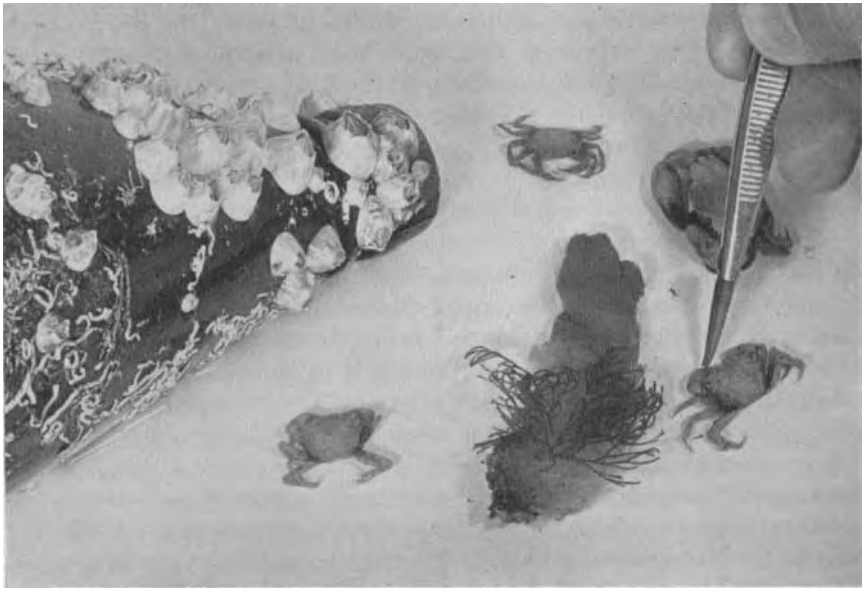

FIG. 8. Some warm-water immigrant species from Queen's Dock, Swansea: many *Balanus amphitrite* and *Mercierella enigmatica* (on bottle), colonies of *Bugula neritina* (on *Ciona*), three *Brachynotus sexdentatus* and one *Neopanope texana*.
(*Photo:* C. Stockton).

areas it is possible that the chance importation of a few specimens may not be sufficient to establish breeding communities, as in the case of the immigrant Australasian barnacle *Elminius modestus* in Britain which must build up to a critical density by repeated introduction from outside before it is capable of maintaining itself as a breeding population (Crisp, 1958). Therefore, the areas in which immigrant species are most likely to accumulate are in shipping terminals such as docks and estuaries. Heated effluents situated in these areas are more likely to support replacement faunas of heat tolerant species than those on open coasts.

Apart from temperature tolerant species which have been trans-

ported (Naylor, 1957a; Elton, 1958) warm water immigrants which have been recorded from artificially heated conditions in temperate European waters (see Table I and Fig. 8) include the crabs *Rhithropanopeus harrisi* (Wolff, 1954), *Brachynotus sexdentatus* Risso (Naylor, 1957b,c) and *Neopanope texana sayi* (Smith) (Naylor, 1960), the barnacle *Balanus amphitrite* var. *denticulata* (Crisp and Molesworth, 1951; Naylor, 1958, 1965), the kamptozoan *Loxosoma kefersteinii* (Claparède) (Ryland and Austin, 1960), the bryozoan *Bugula neritina* (L.) (Ryland, 1960) the mollusc *Venus mercenaria* (Heppel, 1961; Ansell, 1963; Ansell *et al.*, 1964a), the isopod *Limnoria tripunctata* (Eltringham and Hockley, 1958; Jones, 1963), the serpulid polychaetes *Mercierella enigmatica* Fauvel (Fauvel, 1922; Monro, 1924; Tebble, 1956; Naylor, 1959, 1965; Markowski, 1962; Gee, 1963) and *Hydroides incrustans* Monro (Monro, 1938; Naylor, 1965, Gee, 1963), and perhaps also the fish *Atherina boyeri* (Risso) (Bowers and Naylor, 1964). The methods by which various species are transported will clearly vary according to their habits. Apart from deliberate introductions such as oysters and clams, and species introduced accidentally with those species (see Elton, 1958), sessile species could also travel amongst ship fouling (Bishop, 1951; Kuhl, 1963). However, large free living forms would only be able to travel on the hulls of ships if fouling was very thick and provided a suitable foothold. Alternative methods of travel for these species might be as larvae or adults in seawater ballast tanks (Peters and Panning, 1933; Rees and Cattley, 1949; Wolff, 1954) or as adults under the grids covering sea inlet valves (Naylor, 1957b). There is no reason to suppose that tropical or subtropical species could not tolerate at least a summer voyage into temperate waters and become established by any of these methods. Warm-water species which have also been introduced into Europe by human agencies, but which do not appear to have become established in heated localities, include a cladoceran *Penilia* (Cattley and Harding, 1949), the crabs *Pachygrapsus transversus* Gibbs (Bertelson and Ussing, 1936; Bull, 1962), *Pilumnoides perlatus* (Poeppig) (Marine Biological Association, 1957), *Planes minutus* Leach (Wolff, 1954) and *Callinectes sapidus* (Wolff, 1954). Whereas such species apparently died off during the winter months they could perhaps have thrived near warm water effluents and should be considered as likely replacement species in addition to those known to occur already. Similarly several warm stenothermal species, introduced naturally by warm currents, should also be considered as possible replacement species. For example, Huntsman and Reid (1921) and Fish and Johnson (1937) have shown that certain fish, medusae, amphipods and chaetognaths seldom spawn in the Bay of Fundy, and the popula-

TABLE I. SOME WARM STENOTHERM SPECIES INTRODUCED BY HUMAN AGENCY INTO TEMPERATE EUROPEAN COASTAL WATERS

Species	*Introduced locality*	*Native locality*
Kamptozoa:		
Loxosomella kefersteinii (Claparède)	*†Swansea, S. Wales (Ryland and Austin, 1960).	E. Mediterranean and Red Sea.
Polyzoa:		
Bugula neritina (Linnaeus)	*†Plymouth, Devon (Marine Biological Association, 1957). *†Shoreham, Sussex (Crisp and Molesworth, 1951). *†Swansea, S. Wales (Ryland, 1960; Naylor, 1965). *†Milford Haven, S. Wales (Ryland, 1960).	Subtropical areas.
Polychaeta:		
Hydroides incrustans Monro	*†Shoreham, Sussex (Monro, 1938). *†Swansea, S. Wales (Naylor, 1965; Gee, 1963).	Unknown (see Gee, 1963).
Mercierella enigmatica Fauvel	*†Introduced to several British and European localities where it thrives best in areas of fluctuating salinity and slightly elevated temperatures (Fauvel, 1922; Monro, 1924; Tebble, 1953, 1956; Naylor, 1959, 1965; Markowski, 1962; Gee, 1963; Rasmussen, 1958).	Probably Indian Ocean (see Gee, 1963).
Nereis succinea Leuckart	*†Possibly introduced by ships to various slightly estuarine localities (Smith, 1963), including a heated dock (Naylor, 1965).	S. Europe, spreading northwards (Smith, 1963).
Mollusca:		
Mercenaria (= *Venus*) *mercenaria* L.	Various European localities (Heppel, 1961) including Southampton Water where it flourishes near heated outfalls (Ansell, 1963a).	N. America.

Crassostrea angulata	Various European localities.	Sub-tropical areas.
Crassostrea virginica	Various European localities.	Eastern N. America.
Crustacea:		
Penilia avirostris Dana	North Sea plankton (Cattley and Harding, 1949).	Sub-tropical and tropical coasts.
Limnoria tripunctata Menzies	†South and West Britain, breeding particularly in heated localities (Eltringham and Hockley, 1958; Jones, 1963; Naylor, 1965).	Widespread in temperate-tropical areas (Menzies, 1957).
Balanus eburneus Gould	From ship fouling in Britain (Bishop, 1951).	Caribbean to Massachusetts, U.S.A. and more recently, Mediterranean and Bay of Biscay.
Balanus amphitrite var *denticulata* Broch	*†Several British localities affected by heated effluents (Crisp and Molesworth, 1951; Naylor, 1965).	Sub-tropical areas.
Processa aequimana Paulson	?†Larvae in North Sea plankton (Rees and Cattley, 1949).	Mediterranean and Red Sea.
Rhithropanopeus harrisi Gould	Several localities in N.W. Europe, including a heated part of Copenhagen Harbour (Wolff, 1954; Rasmussen, 1958).	N. America.
Pachygrapsus transversus Gibbs	Various records in N.W. Europe (Bertelson and Ussing, 1936; Bull, 1962).	Subtropical areas.
Pilumnus perlatus (Poeppig)	Plymouth, Devon (Marine Biological Association, 1957).	S. America.
Neopanope texana sayi (Smith)	*†Swansea, S. Wales (Naylor, 1960).	N. America.
Brachynotus sexdentatus (Risso)	*†Swansea, S. Wales (Naylor, 1957b,c).	Mediterranean.
Callinectes sapidus (Rathbun)	Occasionally in N.W. Europe (Wolff, 1954).	N. & S. America.
Pisces:		
Atherina boyeri (Risso)	*†A possible imported species in a heated dock at Swansea, S. Wales (Bowers and Naylor, 1964).	Mediterranean.

(*Artificially heated localities; †Breeding recorded).

tions are largely recruited from waters flowing northwards from the Gulf of Maine. Off British coasts too, several species regularly transported across the Atlantic by the Gulf Stream and North Atlantic Drift do not appear to have become established. These include the crab *Planes minutus* (Bell, 1853), the isopod *Idotea metallica* Bosc (Naylor, 1957d) and several other species (Hardy, 1956; Naylor, 1957a). Many of these species are, of course, oceanic forms and so not all would be likely replacement species in heated coastal areas.

Of the warm water species which are already known to have appeared in heated localities there is as yet little evidence that they have begun to spread. However, Jones (1963) has reported that *Limnoria tripunctata* is spreading at the expense of *L. quadripunctata* Holthuis in that part of Southampton Water which is affected by heated discharges, and Ansell (1963a) gives some evidence for the acclimatization of *Venus mercenaria* in the same area. In Southampton Water *Venus mercenaria* breed in water where temperatures never exceed 22°C and spawn in the laboratory at 17·8°C (Ansell, 1963a), whereas in their native habitat in North America the minimum temperature for spawning for this species is reported to be 23°C (Loosanoff, 1937) and 20·6°C (Loosanoff and Davis, 1950). Another example which seems to illustrate that speciation may be taking place in warmed localities is seen in the serpulid polychaete *Hydroides*. Monro (1938) first described as *Hydroides incrustans* a form from the artificially warmed Shoreham Harbour Canal which differed from native British *Hydroides norvegica* in several important respects. Gee (1963) showed that though cross-fertilization between the two forms can take place, which might suggest that *H. incrustans* is only a warm-water race, nevertheless interfertility was greatly reduced. Because of this Gee (1963) concluded that it is best to maintain the separation of *H. incrustans* as a separate species, at least until more is known of the geographical distribution of the two forms.

IV. Utilization of Heated Effluents

In addition to descriptions of the use of heated effluents for domestic heating (Klein, 1957) there have also been a few reports of their utilization for more strictly biological purposes. Ritchie (1927), Chadwick *et al.* (1950) and Fox and Corcoran (1957), for instance, have described the thermal control of marine fouling at power stations in Scotland and in Southern California. At those stations it was found that periodic discharge of the hot effluent through alternative intake conduits was sufficient to prevent the attachment of various species of mussel which formed the main source of fouling (see p. 77). Also, pilot experiments on the growth of edible fish kept in cooling ponds of power stations have

been reported by Iles (1963) and Markowski (1965). Carp, rainbow trout and salmon all grew very fast in an estuarine dock used as a cooling pond by a power station, and it is suggested that plaice, flounders, eels and grey mullet might also be profitably introduced (Iles, 1963; see also Shelbourne 1964). The culture of a North American clam *Venus mercenaria* in Britain might also be improved by irrigating the beds with warm-water effluents from industrial cooling systems (Ansell, 1962, 1963b; Ansell *et al.* 1964a,b). Another suggestion (Hynes, 1959) is that cooling ponds, which would normally support only a few surviving native species, could be deliberately stocked with appropriate warm water species to render them attractive or useful, provided proper control and careful investigation were carried out. Finally, it is possible that heated effluents might locally ameliorate the effects of severe winters, which are known to result in a high mortality particularly amongst low latitude species (see Crisp, 1964a). Local commercial fisheries of oysters, cockles and mussels might benefit in this way. However, the possibility that algal blooms might become more prevalent in heated areas (see p. 85) is a disadvantage from the shell-fishery point of view since such blooms usually result in severe mortalities, particularly amongst filter feeding forms.

V. Conclusions

Biological effects which have been observed so far in areas affected by heated discharges include the elimination of some species such as cool water stenothermal forms (see p. 76), together with possible increase in the abundance of remaining eurythermal species (see p. 81), perhaps also accompanied by the introduction of immigrant warm-water stenothermal species. The polewards spread of subtropical species could be facilitated in this way (see p. 72) and there is experimental (see p. 72) and field evidence (see p. 90) that warm-water species may become acclimatized to temperate regions by this means. Some forms too may evolve into warm-water races or even species in heated localities (see p. 90). Apart from the purely biological aspects of heating several economic problems are also apparent in that many fouling and boring species may increase in abundance (see p. 82 and p. 83). Disadvantages such as these may, of course, be offset by beneficial effects, both from the amenity point of view (see p. 90) and by the possible local use of heated effluents to improve the yield of some commercially fished species (see p. 90).

The effects of heated discharges clearly vary according to whether the hot water spills into enclosed areas such as docks or estuaries, or into open areas on the coast. Dissipation of heat is probably greatest

in the open sea but on the other hand there are experimental results (see p. 78) and field observations (see p. 67) that fully marine and sublittoral species are less tolerant of high temperatures than intertidal and estuarine forms. Effects are most striking in marine docks where not only is there considerable retention of heat but many species there are likely to be intolerant of high temperatures and the activity of shipping would increase the chances of accidental introduction of heat tolerant immigrant species. The effects in estuaries are perhaps least striking, particularly if the lack of shipping reduces the chances of faunal replacement by immigrant species, though possible effects upon migratory species such as crustacea and fish should not be ignored (see p. 72). A significant problem in estuaries, too, is the extent to which mixing of water and dissipation of heat is prevented by vertical salinity gradients and the complexities of flushing (see p. 67).

The siting of heated effluents in marine and estuarine localities should therefore be based on a sound ecological approach and future studies would clearly benefit by investigation of faunal and hydrographical characteristics of a region before a heated effluent is discharged. Such investigations concerning a proposed heated discharge from the Bradwell Nuclear Generating Station into the Blackwater Estuary, Essex, have been described by Davis (1963), Key (1963), Rippon (1963) and Parr (1963), others in the Chesapeake Bay area by Mihursky (1962) and Pritchard and Carter (1965), and others at the Hunterston Generating Station are being carried out by Dr. P. R. O. Barnett (see Scottish Marine Biological Association, 1961, 1962, 1963). Conditions in the Thames estuary are particularly well known, where it is possible to predict the effect on temperature of any change which would be made by the introduction of a new generating station (Gameson *et al.*, 1957, Department of Scientific and Industrial Research, 1964). The value of extended observations is also emphasized by the results of Pannell *et al.* (1962) and Raymont and Carrie (1964), who showed that temperatures in deep water adjacent to a power station effluent were still rising slowly several years after the effluent was first discharged.

Finally it is clear that most work so far has been carried out in temperate regions where temperatures normally vary considerably and organisms might correspondingly be expected to be fairly tolerant of raised or lowered temperatures. Tropical and high latitude species, on the other hand, often live in narrow limits of temperature (Kinne, 1963) and of these, tropical species may live at temperatures very near to their death temperatures (Mayer, 1914). Corals, for instance, die at temperatures only 2 or 3 Centigrade degrees above normal (Cairns, 1956). The

lethal temperatures of tropical algae, too, are only slightly higher than those of European species (Biebl, 1962). The effects of heated effluents might therefore be very severe in tropical areas, particularly also since there would be few species normally occurring elsewhere which would be adapted to extreme heating and which could serve as replacement organisms. Arctic species, despite the narrow limits of their environmental temperatures, may live in water as much as 13 to 16 Centigrade degrees below their death temperatures (Mayer, 1914), so heating in high latitudes would be expected to have less severe consequences than in tropical areas.

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ASPECTS OF THE BIOLOGY OF THE SEAWEEDS OF ECONOMIC IMPORTANCE

A. D. Boney

Department of Botany, University College of Wales, Aberystwyth

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I. Introduction

In this review use of the term " seaweed " will be restricted to the macroscopic marine algae found at different levels between the tide marks and in sublittoral habitats. Members of three classes of algae are mainly involved (e.g. the Chlorophyceae, Phaeophyceae and Rhodophyceae), and the representatives grow attached to rocky substrata, are found as epiphytes, and are sometimes " rooted " in the sand;

a few are free-floating. The large scale economics of seaweed utilization have shown alternating periods of expansion and contraction as a particular product has subsequently given way to a cheaper source and means of production. For the brown seaweed industry of the British Isles this state of affairs has been ably summarized recently by Booth (1964a) with the comment that the industry had " twice died and been revived ". The history and development of seaweed utilization has been extensively surveyed over the years (e.g. Sauvageau, 1920; Hoffman, 1939; Delf, 1943) and more recently Chapman (1950), Newton (1951), and Newton (1963). Some further accounts of a similar nature are seen in the work of Brook (1949) and Kirby (1950, 1951a,b); Humm (1951) described the economically important red algae, whilst Sandford (1958) surveyed the wider field of general utilization; the potentialities of marine algae as food were described by Palmer (1961), and Kurogi (1963a) has summarized recent work on the edible seaweeds of Japan. With the bulk of the seaweed industry concerned with organic extractives there are a number of reviews on the chemicals obtainable and their utilization. Thus Tseng (1946) reviewed seaweed polysaccharides, and the manufacture of various algal chemicals has been described by Woodward (1951) and Black *et al.* (1953). Perrin (1955) and Cook (1957) have also given general accounts of chemicals from seaweeds, whilst Mautner (1954) dealt in some detail with the chemical products of brown algae. Schmid (1959, 1960) described the chemical products of red algae and their main sources, whilst a more recent summary of seaweed products and extractives is to be found in the work of Berger (1961); an interesting approach to the biochemical aspects of taxonomy of red algae has been given by Stoloff (1962). Some indication of the extent of the references now available on all aspects of seaweed utilization may be seen in the collected bibliographies published by the Nova Scotia Research Foundation (Nos. 1 and 2, 1953; No. 3, 1955; No. 4, 1958; No. 5, 1960). Many of the practical aspects of seaweed harvesting and processing have been studied by the Institute of Seaweed Research at Inveresk, Scotland.

This brief summary gives in outline some of the data already available, and to present a further review clearly calls for some definition of the terms of reference at the outset. Since many of the publications mentioned above deal in a comprehensive manner with seaweed products and their uses it is not intended to review this aspect in detail. Commercial exploitation of the world's seaweed supplies appears to be based on two conflicting viewpoints (Delf, 1944), the one considering that the available stocks are inexhaustible and the other proposing the

need for conservation and a "farming" of the available stocks. The aim of the present work will be to summarize recent advances in our knowledge of the general biology of the seaweeds of economic importance, and in particular to explore those aspects which are germane to their availability and productivity as crop plants. This will include knowledge of their systematics, life histories, sporing seasons, conditions of growth and effects on the products, and on harvesting times and techniques and subsequent effects on the standing crops. For this purpose the studies carried out within the last two decades have been mainly drawn on as sources of information. It is of interest to note that whilst earlier work stressed the fact that the full potentialities of the seaweed industry were yet to be developed (Chapman, 1950), in later reviews there is clear demonstration of the great expansion in the various aspects of the utilization of marine algae (Newton, 1963).

II. The Seaweeds of Economic Importance

The list of marine algae which follows is not an exhaustive compilation of all seaweeds utilized by man, but will show the main species described in the following pages, together with the authorities for the nomenclature. In subsequent pages only the generic and species names will be given without the authorities. Many of the plants of more restricted use which are confined to certain localities have not been included, since these have been described in a detailed fashion elsewhere, e.g. Zaneveld's (1959) account of the marine algae of south and east Asia. The synonyms of some of the species are given in parenthesis, and the numerical code after each plant indicates the way in which it is utilized, viz., (1) = human food; (2) = animal fodder; (3) = soil dressing; (4) = extractives (organic or inorganic). The many subsidiary uses, medicinal and otherwise, are not included.

List of seaweeds of economic importance

Class CHLOROPHYCEAE

Order ULOTRICHALES

Family **Monostromataceae**

Monostroma crassissimum Iwanoto (sp. nov.) . . . . . (1)
M. grevillei (Thuret) Wittrock . . (1)
M. latissimum (Kutzing) Wittrock . . (1)
M. tubuliforme Iwanoto (sp. nov.) . . (1)
M. nitidum Wittrock . . . . (1)

Family **Ulvaceae**

Ulva lactuca L.	(1)
U. pertusa Kjellm.	(1)
Enteromorpha intestinalis (L.) Link.	(1)

Class PHAEOPHYCEAE

Order LAMINARIALES

Family **Laminariaceae**

Laminaria angustata Kjellman	(1)
L. digitata (Huds.) Lamour.	(2), (3), (4)
L. hyperborea (Gmn.) Foslie (= *L. cloustonii* Edm.)	(2), (3), (4)
L. japonica Aresch.	(1)
L. religiosa Miyabe	(1)
L. saccharina (L.) Lamour.	(2), (3), (4)

Family **Lessoniaceae**

Lessonia variegata J. Ag.	(3)
Macrocystis integrifolia Bory	(2), (3), (4)
M. pyrifera (L.) Ag.	(2), (3), (4)
Nereocystis luetkeana (Mert.) Post. et Rupr.	(2), (3), (4)
Undaria pinnatifida Sur.	(1)
U. undarioides (Yendo) Okam.	(1)

Family **Alariaceae**

Alaria esculenta (L.) Grev.	(1), (2)
Egregia menziesii (Turn.) Aresch.	(4)
Eisenia bicyclis Kjellman	(4)

Order FUCALES

Family **Fucaceae**

Ascophyllum nodosum (L.) Le Jol.	(2), (3), (4)
Fucus serratus L.	(2), (3), (4)
F. vesiculosus L.	(2), (3), (4)
Pelvetia canaliculata (L.) Dcne et Thur.	(2)

Family **Himanthaliaceae**

Himanthalia elongata (L.) S. F. Gray (= *Himanthalia lorea* (L.) Lyngb.)	(3), (4)

Family **Cystoseiraceae**

Hijikia fusiforme (Harvey) Okamura	(1)

Family **Durvilliaceae**

Durvillea antarctica (Cham.) Hariot	(2), (4)

Class RHODOPHYCEAE

Sub-class *BANGIOPHYCIDAE*

Order BANGIALES

Family **Bangiaceae**

Porphyra angusta Okam. et Ueda . . (1)
P. atropurpurea (Olivi) De Toni . . (1)
P. dentata Kjellman . . . . (1)
P. kuniedai Kurogi . . . . (1)
P. leucosticta Thur. in Le Jol . . (1)
P. perforata J. Ag. . . . . (1)
P. pseudolinearis Ueda . . . . (1)
P. purpurea (Roth.) C. Ag. (= *P. umbilicalis* (L.) Kutz. f. *laciniata* (Lightf.) J. Ag.) . . . . . (1)
P. tenera Kjellman . . . . (1)
P. umbilicalis (L.) J. Ag. . . . (1)
P. yezoensis Ueda . . . . (1)

Sub-class *FLORIDEOPHYCIDAE*

Order NEMALIONALES

Family **Gelidiaceae**

Gelidium amansii Lamour. . . . (4)
G. arborescens Gard. . . . . (4)
G. cartilagineum (L.) Gaill . . . (4)
G. nudifrons Gard. . . . . (4)
G. pristoides J. Ag. . . . . (4)
Suhria vittata (L.) J. Ag. . . . (4)
Pterocladia capillacea (Gmel.) Born. et Thur. (= *P. pinnata* (Huds.) Papenf.) (4)

Order CRYPTONEMIALES

Family **Endocladiaceae**

Gloiopeltis furcata (Post. et Rupr.) J. Ag. (4)
G. tenax (Turn.) J. Ag. . . . . (4)

Order GIGARTINALES

Family **Furcellariaceae**

Furcellaria fastigiata (L.) Lamour. . . (4)

Family **Hypneaceae**

Hypnea musciformis (Wulf.) Lamour. . (4)

Family **Gracilariaceae**

Gracilaria verrucosa (Huds.) Papenf. . (4)
G. foliifera (Forskk.) Børg. . . . (4)

Family **Phyllophoraceae**

Phyllophora brodiaei (Turn.) Endl. . . (4)
P. membranifolia (Good. et Woodw.) J. Ag. (4)
P. nervosa Grev. . . . . (4)
Ahnfeltia plicata (Huds.) Fries . . (4)

Family **Gigartinaceae**

Chondrus crispus Stackh. . . . (4)
Gigartina stellata (Stackh.) Batt. . . (4)
Iridophycus cornucopiae (P. et R.) Setchell & Gardner . . . . . (4)

Reference to the following species will also be found in the text:

RHODOPHYCEAE

Gelidium pulchellum (Turn.) Kütz.
G. latifolium (Grev.) Born. et Thur.
Rhodymenia palmata (L.) Grev.

III. Terminology used in Work with Seaweeds of Economic Importance

Many terms have now come into general usage in this type of work, and since these will be used in the following pages they are separately defined here.

A. *Terminology associated with the plants*

Agarophyte, agarphyte, agarweed: terms used to define any plant which can be used as a source of agar (see below) (Tseng, 1946, 1947).

Agaroid: a term used for plants which produce gel-like extracts with properties different from agar, e.g. *Chondrus, Gigartina, Gloiopeltis, Phyllophora.*

Carrageens: Chondrus crispus and *Gigartina stellata,* both sources of the carrageenan extract. *Chondrus crispus* is also called Irish Moss, and *Gigartina* " False Irish Moss ".

Kelps: originally this was used to describe the ash left after burning the larger brown algae (fucoids and *Laminaria* sp.) on the Scottish coast, and which was used as a source of minerals, e.g. potash. It has now come to be used as synonymous with the larger brown algae, in particular the " giant kelps ", *Macrocystis* and *Nereocystis.*

Laver: a term used to describe the edible *Porphyra* sp. At one time it was more commonly used on the Devon and Welsh coasts (" bara lawr " in Welsh), but is now of more widespread use ; thus it is frequently found in Japanese literature. Several anglicized versions of original Japanese names are also in use (Table IX).

B. *Terminology associated with the extractives*

Agar (agar-agar): a term now used to describe the water-soluble gel extracted from agarophytes. Originally a Malayan term for a seaweed which yielded the gel (*Eucheuma*) it then became synonymous with jelly and hence to the general usage mentioned above.

Algin (alginic acid): a cell wall constituent of brown seaweeds. Alkali soluble, and a polymer of mannuronic acid (Mautner, 1954). The source of alginates, important products of the seaweed industry.

Carrageenan: a galactose-sulphate derivative extracted from the Carrageens. The terms *phycocolloids* (Tseng, 1947) and *polysacolloids* (Stoloff, 1962) are both used to describe the polymerized extractives referred to in the earlier paragraphs.

Knowledge of the chemical nature of the extractives mentioned above is now available. Agar has been taken to mean all water-soluble gels extractable from red algae, but in recent work there have been attempts at arriving at a more precise definition, since in their physical properties many of the gel-like extractives differ markedly. Yaphe (1959) has defined it as an extract soluble in hot water and requiring only a 1% solution to set as a gel on cooling. Its main properties are due to agarose and this is a linear polymer of D-galactose and anhydro-L-galactose. It also contains agaropectin, a sulphated polysaccharide about which there is limited knowledge. These compounds are extracted from the agarophytes defined above. A more critical identification is now possible with the isolation by Yaphe (1957) of an agarase from *Pseudomonas atlantica*. This was found to be specific for agar, and only hydrolysed the gel-like extracts from species of *Gelidium*, *Pterocladia pyramidale*, *Gracilaria verrucosa* and *Suhria vittata*.

The use of the term agaroid has already been indicated. By Yaphe's (1959) definition the extractives from *Chondrus*, *Gigartina* and related genera are not true agars. These agaroid extractives (called carrageenans) have been found to consist of two components, the lambda- and kappa- fractions. Yaphe (1959) isolated kappa-carrageenase from *Pseudomonas atlantica* and *P. carrageenovera*, an enzyme specific for hydrolysis of the kappa-fraction. Use of this enzyme also enabled quantitative differences to be shown for the kappa-fraction of different extracts, e.g. the reducing properties of some hydrolyzed extracts were greater than those of others. With two specific enzyme reactions it is thus possible to distinguish between agar and carrageenan in plant extractives.

A further development has been the attempts to link the bio-

chemical nature of the extracted polysaccharides with taxonomic relationships within the red algae. Thus Stoloff and Silva (1957) stated that the agars, carrageenans and gelans were the three principal extractives, and whilst there might be wide variations in the properties within a polysaccharide type, in no instance was there another type of polysaccharide present. Subsequently Stoloff (1962) expanded this work. With the carrageenans it has been found that the kappa-fraction is similar to the agarose of agar, consisting of anhydro-D-galactose and with sulphate esters in the galactose moieties; the lambda-fraction is a linear polymer of D-galactose sulphate. The gelans are similar to kappa-carrageenan, but with a lower sulphate content. These compounds were identified in seventy-one species of red-algae belonging to the orders Gelidiales*, Cryptonemiales and Gigartinales, and the results of this analysis are summarized in Table I. Because of the specific nature of the extractives, Stoloff suggested that a system of classification might be devised using biochemical differences as opposed to those based on reproductive structures and post-fertilization changes. Thus all plants producing agar should be placed in the order Gelidiales; all those which yield carrageenans to be placed in the Gigartinales, and the gelan-producers to be placed in a new order, the Hypneales. As can be seen from Table I, this would imply a radical revision of the present status of some orders in the red algae, and indicates a novel approach to taxonomic problems in the red algae which will repay investigation. In passing, it is worth noting that Schmid (1960) listed fifty-one species of red algae from which agar can be extracted, including two in the genus *Ceramium*. A further sixteen species were mentioned as sources of carrageenan. Since the Ceramiales is one order which appears well established in the red algae (Dixon, 1963a), the significance of changes involved in a biochemical type of classification is clearly evident.

These polysaccharides are of functional significance in the plant as a matrix within which the cells are embedded, and as such play a major role in control of pH, salinity changes in the immediate environment of the cell (Stoloff, 1962) and protection from physical damage through the pounding action of waves. The carrageenans of *Gigartina* and *Chondrus* show a seasonal change in physical properties (Marshall *et al.*, 1949; see also p. 210), and porphyran, the extractive from *Porphyra*, has been found to show different gelling properties with plants from exposed habitats from those growing in shelter (Rees and Conway, 1962). A comprehensive review of the mucilages of red algae has been given by O'Colla (1962).

* See footnote on Table I.

TABLE I. THE TYPE OF EXTRACTIVE OBTAINED FROM VARIOUS GENERA OF RED ALGAE. (Data from Stoloff, 1962)

Order	*Genus*	*Type of Extractive*		
		Agar	*Carrageenan*	*Gelan*
Gelidiales* . .	*Gelidium* (18 species)	+	—	—
	Pterocladia (3 species)	+	—	—
	Suhria (1 species)	+	—	—
	Acanthopeltis (1 species)	+	—	—
	Gelidiella (1 species)	+	—	—
	Yatabella sp.	—	+	—
Cryptonemiales .	*Endocladia* (1 species)	—	+	
	Gloiopeltis (3 species)	—	+	—
	Aeodes (1 species)	—	+	—
Gigartinales . .	*Gracilaria* (8 species)	+	—	—
	Corallopsis (1 species)	+	—	—
	Furcellaria (1 species)	—	—	+
	Agardhiella (1 species)	—	—	+
	Eucheuma (3 species)	—	+	—
	Hypnea (3 species)	—	—	+
	Ahnfeltia (1 species)	+	—	—
	Phyllophora (2 species)	+	—	—
	Gymnogongrus (2 species)	—	+	—
	Chondrus (2 species)	—	+	—
	Gigartina (13 species)	—	+	—
	Iridophycus (3 species)	—	+	—
	Rhodoglossum	—	+	—

* The status of the order Gelidiales has been questioned (Dixon, 1961), and the order has been incorporated in the Nemalionales (Parke and Dixon, 1964).

Algin (alginic acid) is the main constituent of the cell walls of brown algae, located in the middle lamella and in the primary wall (Kreger, 1962). The total alginic acid content of a plant varies with depth of immersion and with the season (p. 201 *et seq.*). Two polymerizing units have been identified, D-mannuronic acid and L-guluronic acid. Fucinic acid has also been reported present in some brown algae. Two other extractives of importance are laminarin and mannitol. Laminarin is of widespread occurrence in brown algae (Quillet, 1958) and is in fact a mixture of polysaccharides. Mannitol, a polyhydroxy alcohol, is a cell-sap constituent found in numerous brown algae, but is now known to occur in other algal classes.

There is at present very little information on the detailed structure of algin from different orders of the brown algae. Quantitative differences between the two polymerizing units have been reported for the orders Dictyotales and Fucales (see Kreger, 1962). The identification of enzymes in marine invertebrates capable of breaking down algin have been reported (Miwa, 1940; Eppley and Lasker, 1959; Galli and Giese, 1959), and the bacterial degradation of algin is also reported (Chesters *et al.*, 1956). An enzyme capable of hydrolyzing laminarin has been identified in the giant chiton *Cryptochiton stelleri* (Middendorff) (Meeuse and Fleugel, 1958). It is thus possible that biochemical differences between brown algal reserves and products may be detectable using specific enzymes, and a biochemical classification be made for some orders of the Phaeophyceae, as already applied to the red algae by Stoloff (1962).

IV. Seaweed Resources

The standing crop of any plant population may be defined as the balance between the rate at which the plants have been growing and the rate at which depopulation occurs. To assess the value of a harvestable crop there is clearly a need for quantitative estimates, and these could also indicate any annual or cyclic changes in the available weed. It is also desirable to know the extent to which regular harvesting affects the standing crop.

A. *Survey methods*

Surveys of both intertidal and sublittoral marine algae are attended by a number of difficulties, in the former due to accessibility, and in the latter because of the tide rips and heavy swells which make boat work a hazardous operation. Despite the difficulties a number of survey methods have been applied with considerable success. Many of the surveyors, however, have indicated their data to be in the nature of

conservative estimates, with sometimes a tendency to underestimate the biomass involved.

1. *Intertidal surveys*

Two principal methods of large-scale survey have been utilized (Baardseth, 1955a), i.e. (1) in which the fresh weight of the plants per unit area (the mean density) is multiplied by the area of the beds and

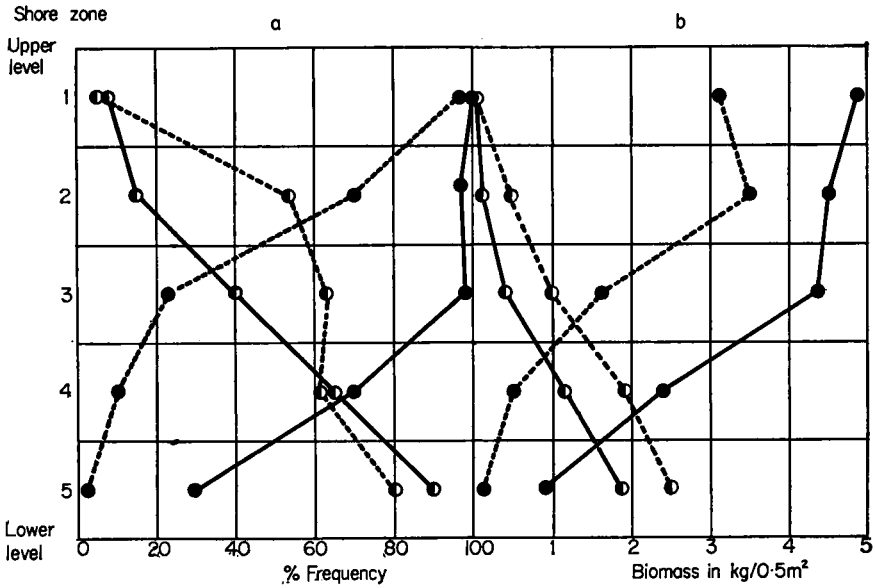

FIG. 1. a—% frequency of *Ascophyllum nodosum* (●) and *Fucus serratus* (◐) in sub-zones passing from the upper part of the shore to the lower; ——— from a shore of even slope; - - - - - - from a shore of irregular configuration. b—density of the two species represented as biomass in kg/0·5 m². Drawn from data in Baardseth (1955a).

(2) in which the mean weight of the plants per standard length (= 1 metre) of shore is multiplied by the overall length of the shoreline. A review of the different methods has also been given by Chapman (1950). Gibb (1950) used the first method in her survey of the fucoid algae of Scottish shores, and the alternative technique was used by Marshall *et al.* (1949) in their survey of the *Gigartina* and *Chondrus* populations of the British Isles. Baardseth (1955a) compared the two methods in a statistical study of the distribution of plants in the *Ascophyllum* zone. If sufficient time was available an assessment of the area of the seaweed beds was considered necessary. Baardseth's observations showed

marked variations of the population density of *Ascophyllum* with substratum. Thus the plant density was much less on stony ground than on rocky shores, and similar features governed its degree of association with *Fucus vesiculosus* and *F. serratus*. The mean density of the *Ascophyllum* plants was somewhat greater in the upper three-fifths than in the lower two-fifths of its zone (Fig. 1). Whilst accurate measurements of plant density are of great value, quick visual estimates by experienced surveyors are sometimes necessary. Grenager (1958) described experimental surveys which were carried out in Norway to expedite population surveys of *Ascophyllum* and *Laminaria* on a much indented region of the coast with numerous islands, inlets and skerries. These surveys were based on experience gained on other shores, and on a knowledge of the principal environmental characters of the shores to be surveyed. In some test surveys the estimated and measured standing crops were compared and were found to differ by relatively small amounts. Thus with sufficient experience and a full knowledge of the ecology of the shore reasonably accurate estimates of the standing crops are possible.

2. *Sublittoral surveys*

Chapman (1944, 1950) described the combined use of echo-sounding apparatus and aerial photography in measurements of the extent of the sublittoral *Laminaria* forests. Use of specially designed grapnels in accurately charted seas was also described. Walker (1950) obtained quantitative data on plant density by the use of a spring-loaded grab which sampled unit areas of the seabed; these methods were combined with accurate navigational measurements. Aerial photography was also used in these studies. The same methods have been applied by other workers in this field, e.g. MacFarlane (1952a). In recent years development of the self-contained breathing apparatus has enabled direct measurements to be made of plant density and vertical distribution (Forster, 1955, 1958; Ernst, 1955, 1958). Kain (1962) has described how divers working in pairs can map the rocky sublittoral profiles, and sublittoral studies on *Macrocystis* using similar techniques have been described (Neushul, 1959; Neushul and Haxo, 1963). Given suitable climatic conditions reasonably accurate assessments can be made of undersea plant populations.

B. *Summary of some data on standing crops obtained by direct measurements*

Much of the available data concerns the brown and red algae used for their extractives and for seaweed meals. The more detailed surveys are

available for places and regions where there are long established industries.

1. *British Isles*

The quantitative data from the British Isles are mainly a result of surveys carried out within the last 25 years, and initiated by the need for home-produced alginates and agar during the 1939–45 war. Information is available on the intertidal fucoid algae (Walker, 1947a, 1952b; Chapman, 1948; Gibb, 1950), and on the sublittoral *Laminaria* forests (Chapman, 1948; Walker, 1947b, 1950, 1952a, 1954a,b,c, 1956, 1957; Walker and Richardson, 1955a,b, 1956, 1957). The *Gigartina* and *Chondrus* resources of the coast of England, Scotland and Wales were reported by Marshall *et al.* (1949). Chapman (1948) and his co-workers based their estimates on both the standing crops of intertidal rockweed and *Laminaria* species and on the quantities of cast weed since this was also considered to be of economic value.

Estimates of the standing crops of fucoid algae on Scottish shores, based on a survey of 80% of the coastline made in 1945–46, have been given by Walker (1947a) (Table II).

TABLE II. STANDING CROPS OF INTERTIDAL FUCOID ALGAE ON SCOTTISH SHORES.* (Data from Walker, 1947a)

Region	*Standing crop tons*	*Acres*	*Density (tons/acre)*
Outer Hebrides . .	125 136	3 500	36
Inner Hebrides . .	8 263	278	30
Orkney Islands . .	38 774	1 813	21
Mainland (W. and N.W.)	8 540	356	24

* Collectively referred to as "rockweed" by Chapman (1948); the sublittoral *Laminaria* spp. were called "bottomweed".

The striking feature which emerged was that over 70% of the available rockweed was located in the Outer Hebrides, and of the representatives present, *Ascophyllum nodosum* predominated, followed by *Fucus vesiculosus*. Walker's estimate of 38 774 tons of rockweed for the Orkney Islands differs from Chapman's (51 740) but is probably the more accurate*. The quantitative surveys carried out by Gibb (1950) showed that rich areas of fucoid algae suitable for harvesting were of restricted occurrence on Scottish shores, amounting to some 87 miles

* Chapman did indicate the probable errors involved with his various survey methods; (see Table 1, p. 367 in Chapman, 1948).

of coastline in a survey of Mull, Skye, Ross-shire and Sutherland. Such harvestable areas were lacking from the north, north-east and east coasts of Scotland. The detailed estimates made on the harvestable areas included also information on the substratum and angle of slope of the shore, and on the relative frequency of the common rockweeds (Table III).

TABLE III. STANDING CROPS OF FUCOID ALGAE ON SOME HARVESTABLE AREAS OF THE SCOTTISH SHORES. (Data summarized from Gibb, 1950)

Locality	*Type of shore*	*Length of coast surveyed (miles)*	*Available Seaweed (tons)*
Sutherland . .	Mainly of gentle slope, some variable; stones and boulders; frequent areas of rock	20·1	2 870
Ross-shire . .	Gentle and moderate slope; mainly stones and small boulders	14·5	2 326
Skye . . .	Gentle and moderate slope, more rarely steep; stones, boulders and areas of rock in approximately equal proportions	37·8	5 191
Mull . . .	Gentle and variable slope, to moderately steep; mainly rock, but with frequent areas of rocks and boulders	14·1	2 328

Within the areas surveyed there was clearly discontinuity in the relative density of the weed cover. In general *Ascophyllum nodosum* predominated, with smaller quantities of *Fucus vesiculosus* and *Ascophyllum nodosum* f. *mackai; Fucus serratus* was of lesser significance. The total area surveyed covered all the Scottish coast with the exception of shores on the south-west, and those of east and north-east of Mull. A further point of interest was that the standing crops with the greatest density were all to be found in sheltered localities, covering distances between 1·5–6·5 miles, and on substrata with moderately gentle to moderately steep angles of slope, and where rocks, boulders and sand and mud tended to predominate over the rocky areas. From Gibb's observations suitable harvesting areas are lacking from the

north, north-east, and east coasts of Scotland. Chapman (1948) gave data for other parts of the coastline, e.g.:

1. South-west (Ayr to Garlieston)	9 100 tons of rockweed
2. North-east (Berriedale to Brora)	2 400 ,, ,, ,,
3. East-north-east (Tarbat Ness to Sutors of Cromarty)	11 700 ,, ,, ,,
4. South-east (Arbroath to Fifeness and Elie)	3 000 ,, ,, ,,

Chapman's data give estimates of the total standing crop of rockweed, whereas Gibb specifically chose areas of harvestable weed. For example, the total rockweed for Mull was estimated by Chapman as 38 400 tons, whereas the harvestable weed was given by Gibb as 2 328 tons. This factor of distribution was also indicated in some further data by Walker (1952b):

Length of coastline surveyed	5 300 miles
Standing crop of fucoid algae	180 000 tons
Length of coastline bearing dense stands of fucoid algae	540 miles
Density of fucoid algae	not less than 100 tons/acre

However, 92% of this quantity of rockweed was located in the Outer Hebrides (70%) and the Orkney Islands (22%). From a commercial standpoint, whilst bulk estimates are an important guide, the potentialities of an area in terms of ease of harvesting are also of great importance.

Estimates of the standing crops of the sublittoral *Laminaria* forests of the Scottish coastline are to be found in the extensive work carried out by Walker and associates (refs. on p. 250). Some preliminary work by Chapman (1948) gave the following results:

1. Girvan (Ayr to Garlieston)	49 000 tons of bottomweed
2. Outer Hebrides (west coast)	670 900 ,, ,, ,,
3. Orkney Islands	576 370 ,, ,, ,,
4. Helmsdale (Berriedale to Brora)	52 370 ,, ,, ,,
5. St. Andrews (Arbroath to Fifeness and Elie)	117 770 ,, ,, ,,

The tabular summary of the standing crops of Laminariaceae (Table IV) indicates also the areas on the Scottish coastline which are harvestable in terms of the quantitative value of the seaweed.

TABLE IV. STANDING CROPS OF LAMINARIACEAE IN AREAS OF SCOTLAND. After Walker (1954a,b,c)* but summarizing work in previous publications by the same author (see p. 250)

Locality	*Standing crop of Laminariaceae (tons)*	*Area (acres)*	*Length of coast (miles)*
1. Shetland	610 000	56 000	700
2. Orkney	1 200 000	56 000	500
3. Outer Hebrides	700 000	41 000	85
4. Enard Bay—Lochalsh	122 000	24 000	160
5. Skye	300 000	18 000	220
6. Tiree and Coll	302 000	20 000	58
7. Mull	20 000	2 300	15
8. Colonsay	20 000	2 000	6
9. Islay	50 000	4 000	11
10. W. Kintyre and Gigha	200 000	11 000	50
11. Arran	56 000	6 800	48
12. E. Kintyre	40 000	2 600	22
13. Girvan	44 000	4 200	21
14. Luce Bay	20 000	1 600	4
15. Dunbar	50 000	3 400	11
16. Crail	22 000	1 300	7
17. Fraserburgh	60 000	18 000	60
18. Tarbat Ness	10 000	1 600	8
19. Helmsdale	22 000	2 900	10
20. Loch Eriboll	20 000	1 300	13

Walker's data are statistically the more significant, and a comparison of the two surveys is of interest:

Locality	*Chapman's data*	*Walker's data*
Mainland (including Skye and Mull)	790 000 tons	930 000 tons
Orkney Islands	576 370 ,,	1 200 000 ,,
Outer Hebrides	670 000 ,,	700 000 ,,

Whilst there is a striking similarity in both estimates for the Outer Hebrides, the other results indicate that more detailed mapping gives appreciably higher values. Walker's results have added value in that information was also given on the relative density of the standing crop with depth. Thus between Dunbar and Fast Castle on the east coast of Scotland the standing crop was estimated at 50 600 tons of *Laminaria* sp. covering 3 400 acres of seabed to a depth of 7 fathoms below L.W.M.S.T., but some 69% of this tonnage was to be found between 0–5 fathoms depth (Walker, 1952a). Of the species present

* See also Walker, F. T. (1957). *J. Cons. int. Explor. Mer.* 22, 288–308.

Laminaria hyperborea predominated, although localized areas containing mainly *L. saccharina* were observed, e.g. off North Shapinsay (Walker, 1954a).

The *Gigartina* and *Chondrus* survey of the British Isles (Marshall *et al.*, 1949) showed that harvestable quantities of seaweed were available on coasts of Devon and Cornwall; on the Gower Peninsular, St. David's Head, south of Cardigan Bay, the Lleyn Peninsula and Anglesey in Wales; Ayrshire, Wigshire, Mull, Skye and the west coast of Scotland, and in the south-east of Scotland and north-east England. In many of these areas *Gigartina* made up between 50 to 90% of the standing crop.

TABLE V. STANDING CROPS OF HARVESTABLE *Gigartina* (WITH SMALLER AMOUNTS OF *Chondrus*) ON SCOTTISH SHORES. (Marshall *et al.*, 1949)

Locality	*Standing crop (ions)*	*Variation in weight of algae (lbs./lin. yd.)*
1. Skye	146	½–60
2. Mull	4·5	1–2
3. Clyde Sea (Loch Long, Loch Goil, Gareloch)	25·4	½–9
4. Clyde Sea (Cowal shore)	1·4	½–3
5. Clyde Sea (Kintyre peninsula)	43·8	½–10
6. Clyde Sea (Wemyss Bay—Ardrossan)	4·85	½–5
7. Clyde Sea (Wright's Isle—Burrow Head)	14·5	—
8. Clyde Sea (Isle of Bute)	38·75	1–24
9. Clyde Sea (Isle of Inchmanock)	4·7	½–5
10. Clyde Sea (Great Cumbrae)	29·35	1½–12
11. Clyde Sea (Wee Cumbrae)	7·6	½–10
12. Clyde Sea (Arran)	18·35	½–18
13. Mainshore coast and inshore islands	11·1	½–5
14. Offshore islands (Islay, Jura, Colonsay and Oronsay)	29.85	½–10

A summary of the data for the Scottish coastline is given in Table V, and shows that the harvestable standing crop was estimated as 360·2 tons; on the north-east coast of England (Coldingham to North Berwick), the crop was estimated as 11½ tons.

These quantitative studies on harvestable standing crops were also responsible for valuable contributions on the relationship between population densities and environmental factors. Whilst the estimates were predominantly designed to determine crops of economic value, the information derived has proved to be of considerable ecological value; some reference has already been made to this, and the data will be further discussed.

2. *North America*

Data on the standing crops of brown seaweeds on the Pacific and Atlantic coasts of North America are available from numerous sources (Tseng, 1947; MacFarlane, 1952a,b, 1956a; Woodward, 1952; Aleem, 1956a,b; Scagel, 1959, 1961). Much of the information concerns the giant kelps, *Macrocystis pyrifera*, *M. integrifolia*, and *Nereocystis luetkeana*. Surveys of the giant kelp populations were stimulated

TABLE VI. ESTIMATED STANDING CROPS OF ECONOMICALLY

Region	*Area (sq. miles)*	Standing crop	
		Macrocystis (integrifolia; pyrifera)	*Nereocystis luetkeana*
Pacific coast			
West Alaska	17·86	—	1 251 200
South-east Alaska	70·78	522 320	6 486 000
Puget Sound	5·0	22 500	375 000
Puget Sound to Point Conception	36·24	748 680	3 349 540
Point Conception to San Diego	97·92	9 000 000	—
San Diego to Cedros Island	91·36	8 500 000	—
British Columbia	—	18 000 (sp. *integrifolia*) (22 500)*	300 000 (370 000)*
Atlantic coast			
Nova Scotia Cape Sable Isle to Chebogue	18·7	—	—
Nova Scotia Digby Neck	3·8	—	—

* Corrected figures suggested by Scagel (1961).

during the years 1914–18 when war conditions led to a search for sources of potash. In many instances pure " stands " of a particular kelp were observed, but mixed beds were quite frequent. The available data are summarized in Table VI. Tseng (1947) considered that many of the estimates made in 1911–13 were too high, in that recorded harvestings were much smaller than the estimated amount. However, the standing crops of harvestable areas of kelp beds are of more importance than gross surveys of a coastline. The quantities of *Macrocystis* harvested between San Diego and Point Conception amounted to 394 874 tons

in 1917, compared with the peak yield for the years 1941–46 of 62 102 tons in 1942 (detailed records are available in Tseng, 1947). Tseng estimated that the productivity of the Pacific coast kelp beds could equal about 3 million tons per annum (Aleem, 1956a,b places the standing crop at 25–40 tons/acre). The figures for British Columbia waters were considered to be too low by Scagel (1961) owing to previous surveys being carried out at unfavourable stages of the tide. He considered

IMPORTANT SEAWEEDS IN NORTH AMERICA

in tons fresh weight					
Macrocystis+ *Nereocystis*	*Alaria fistulosa*	*Alaria*+ *Nereocystis*	*Laminaria*	*Fucus Ascophyllum*	*Authorities*
—	858 500	1 457 300	—	—	Tseng (1947)
—	596 920	—	—	—	Woodward (1952)
—	—	—	—	—	Scagel (1961)
279 180	—	—	—	—	Shrum (1952)
—	—	—	—	—	
—	—	—	—	—	
—	—	—	—	—	
—	—	—	minor quantities	—	
—	—	—	900 000	200 000	MacFarlane (1952a,b; 1956a)
—	—	—	—	80 000	

that readily accessible kelps may approach 750 000–1 000 000 tons annually. Estimates of the standing crops of brown algae for the Atlantic coast of Canada are shown in Table VII. An interesting comparison has been drawn between the standing crops of the rockweed and *Laminaria* spp. on the Scottish coast (see Table VII), and for Southwest Nova Scotia (MacFarlane, 1952a).

The greater productivity of the sea around Nova Scotia has been considered to be due to higher degree of aeration of coastal waters in this part of Canada. There are other indications that environmental

conditions favour rockweed growth. Thus Gibb (1950) observed standing crops of *Ascophyllum nodosum* equal to 16 lb/yd^2, in zones 26 to 50 yd wide in rich areas. MacFarlane (1952c) obtained standing crops of the same species in the region of 37 lb/yd^2, in zones some 50 yd wide. Similarly, whilst the plants on Scottish shores were from 1 to 6 ft in length, those in Nova Scotia were frequently 6 to 7 ft long, and rarely less than 3 ft. Considerable data on the economically important seaweeds of Nova Scotia and Prince Edward Island are available (MacFarlane, 1952b,c; 1956a,b; 1958; 1964a).

TABLE VII. COMPARISON OF STANDING CROPS FOR THE SCOTTISH COAST AND THE COAST OF SOUTH-WEST NOVA SCOTIA. (Data from MacFarlane, 1952a, and Walker, 1947a, 1950)

	Scotland		*Nova Scotia*	
Type of seaweed	*Length (miles) or area (acres) of coast*	*Standing crop (tons fresh weight)*	*Length (miles) or area (acres) of coast*	*Standing crop (tons fresh weight)*
Rockweed (mainly *Ascophyllum nodosum*) .	540 miles	180 000	325 miles	200 000
Kelps (*Laminaria* spp.) . .	38 000 acres (Orkneys and Outer Hebrides)	1 200 000	12 000 acres	900 000

In North America the red algae of economic importance consist of species of *Gelidium* (mainly *G. cartilagineum*), *Gracilaria* (*G. verrucosa*, *G. foliifera*), *Gigartina stellata* and *Chondrus crispus*.

There appear to be few estimates of standing crops, although much information on the quantities of material harvested is available (p. 130). MacFarlane (1952a) stated that in South-west Nova Scotia harvestable areas of *Chondrus crispus* could be found with a standing crop of approximately 1½ lb/ft^2 of rock. *Chondrus* belts some 30 ft wide were available in the region of L.W.O.S.T. A notable feature is that whilst 95% of the standing crop of harvestable red seaweeds on Scottish shores consists of *Gigartina*, the predominant plant in the Maritime Provinces of Canada is *Chondrus crispus*. There are indications of large standing crops of *Chondrus* in other parts of North America. Thus extensive beds have been reported at depths of 40 ft off the coasts of Maine and Massachusetts.

3. *U.S.S.R.*

Data on the standing crops of brown and red algae in the Russian seas have been supplied by Zenkevitch (1963). Thus, for the Murman coast of the Barents Sea standing crops of *Laminaria* sp. are estimated at between 500–600 000 tons fresh weight, and for the White Sea up to 800 000 tons. The density of the *Laminaria* cover appears greatest down to 10 m (32·8 ft). The Sea of Japan is rich in economically important brown seaweeds (*Laminaria japonica, L. dentigera, Alaria crassifolia*), whereas certain of the water masses are poor in their algal populations (Kara Sea, Laptev Sea, Chutotsk Sea, Sea of Azov, Caspian Sea, Aral Sea). In the Baltic Sea there is a progressive impoverishment of the flora with lowering of salinity. Estimates of the rockweed populations have also been given. On the west Murman coast of the Barents Sea the average standing crops of *Ascophyllum nodosum* and *Fucus vesiculosus* were 16 and 9·4 kg/m^2 respectively, whereas for the White Sea the quantities varied between 8–9 and 15–16 kg/m^2 for *Ascophyllum* and 2–4 and 8–9 kg/m^2 for *Fucus vesiculosus*.

The most important of the red algae is the species of *Phyllophora* forming the " Zernov (Zernova) *Phyllophora* Sea " in the Black Sea. This plant mass is thought to have originated from detached fragments of littoral plants, and to have been maintained by vegetative growth. Zenkevitch (1963) places the standing crop at 5–6 000 000 tons fresh weight, with densities varying between 1·7–13 kg/m^3. The predominant species, according to Zenkevitch, is *Phyllophora rubens* var. *nervosa*. Vinogradov (1962) gives a similar estimate for the standing crop, and states that the area of seabed covered is 10 000–11 000 km^2, and that three species (*Phyllophora nervosa, P. brodiaei* and *P. membranifolia*) are present. The " field " is situated in the north-west part of the Black Sea, and according to Vinogradov there are changes in its boundaries due to strong currents near the sea bed. The water temperature in the algal mass was 4–5°C higher than that of the rest of the sea. According to Zenkevitch, the *Phyllophora* beds lie at depths between 30–60 m. Yakubova (quoted by Caspers, 1957) placed the depths at 40–65 m, and considered that they were displaced southward compared with the original plotting by Zernov. Smaller quantities of *Phyllophora* (29 kg/m^2) are reported at depths of 6–22 m off the Murman coast of the White Sea. Harvestable areas of *Gloiopeltis capillaris* occur in the littoral of the Sea of Japan.

4. *Australia and New Zealand*

The standing crop of *Macrocystis pyrifera* on the east coast of Tasmania has been estimated as 355 000 tons fresh weight, covering an

TABLE VIII. SUMMARY OF REPORTS ON SEAWEED RESOURCES FROM VARIOUS COUNTRIES

Region surveyed	*Seaweeds of economic value*	*Authority*
1. Zanzibar	*Eucheuma striata*	Anderson (1952)
2. Spain	*Fucus* sp.	Aravio-Torre (1952)
	Laminaria iberica	Belton (1952)
	Gelidium sp.	
3. New Zealand	*Ecklonia* sp.	Chapman (1950)
	Macrocystis pyrifera	
	Gigartina sp.	
	Pterocladia capillacea	
	P. lucida	
4. South China	*Sargassum* sp.	Chiu (1956)
	Laminaria japonica	
	Gloiopeltis furcata	
	Digenia simplex	
5. Hong Kong	*Ulva lactuca*	Chiu (1958)
	Sargassum sp.	
	Porphyra suborbiculata	
6. Polish Baltic Sea	*Furcellaria fastigiata*	Czäpke (1960, 1964)
7. Cuba	14 genera of Chlorophyceae	Diaz-Piferer and Lopez (1959)
	30 genera of Phaeophyceae	
8. Greece	*Porphyra atropurpurea*	Diannelidis (1949)
	Gelidium latifolium	
	Gelidium crinale	
	Pterocladia capillacea	
	Gracilaria verrucosa	
	G. compressa	
	G. dura	
	Hypnea musciformis	
9. Chile	*Macrocystis pyrifera*	Etcheverry (1952, 1960)
	Durvillea antarctica	
	Lessonia nigrescens	Llaña (1948)
	Gelidium filicinum	
	G. ligulatum	
10. Australia	*Macrocystis pyrifera*	Wood (1952)
	Ecklonia radiata	
	Gracilaria verrucosa	
	Eucheuma sp.	
11. Eire	*Laminaria hyperborea*	Flood (1952)
	L. digitata	
	Chondrus crispus	
12. Norway	*Ascophyllum nodosum*	Grenager (1958)
	Laminaria digitata	
13. Iceland	*Ascophyllum nodosum*	Hallsson (1964)
	Laminaria digitata	

Further data relevant to this Table may be seen in " Some notes on the development of statistical systems covering the seaweed industries of the world." *Proc. 4 Int. Seaweed Sym.* Appendix 1, 453–63 (1964).

TABLE VIII (continued)

Region surveyed	*Seaweeds of economic value*	*Authority*
14. Ceylon . . .	*Sargassum* sp. *Turbinaria* sp. *Gelidium* sp. *Gigartina* sp. *Gracilaria* sp.	Holsinger (1952)
15. South Africa . .	*Macrocystis* sp. *Laminaria pallida* *Ecklonia maxima* *Gelidium pristoides* *Gigartina radula* *Gracilaria verrucosa* *Hypnea spicifera*	Isaac and Molteno (1952, 1953)
16. Netherlands . .	*Fucus* sp. *Ascophyllum nodosum*	Koster (1952)
17. Sweden. . .	*Fucus* sp. *Ascophyllum nodosum* *Laminaria* sp. *Chondrus crispus* *Furcellaria fastigiata*	Levring (1952)
18. Denmark . .	*Furcellaria fastigiata*	Sund and Petersen (1952, 1964)
19. Egypt . . .	*Pterocladia capillacea*	Mohamed and Halim (1952)
20. Patagonia . .	*Macrocystis pyrifera*	Nicola and Pecora (1952)
21. Argentina . .	*Macrocystis pyrifera* *Corallina* sp. *Gymnogongrus* sp. *Gigartina* sp.	Nicola and Pecora (1952)
22. Travancore-Cochin	*Ulva* sp. *Sargassum* sp. *Gracilaria* sp.	Pillay and Varier (1952)
23. Spanish West Africa	*Sacchorhiza polyschides* *Laminaria ochroleuca* *Gelidium sesquipedale* *G. attenuatum* *G. spinulosum*	Primo (1952)
24. Canary Islands .	*Porphyra* sp. *Gelidium* sp.	Rodriquez (1952)
25. Philippines . .	*Enteromorpha intestinalis* *Caulerpa racemosa* *Sargassum* sp. *Turbinaria* sp. *Porphyra* sp. *Gelidium* sp. *Gracilaria verrucosa* *Hypnea musciformis*	Velasquez (1952) Sulit, Navarro and San Juan (1953)

area of 30 000 acres (Thompson, 1952; Womersley, 1952, 1959; Cribb, 1954). *Ecklonia radiata* is of potential economic value on the eastern coast of Australia, together with the red alga *Gracilaria verrucosa* (Wood, 1952). In New Zealand the standing crop of *Macrocystis* in the Cook and Foveaux Straits has been estimated at 78 000 tons fresh weight (Chapman, 1952). Thivy (1952) states the Australian standing crop of *Gracilaria verrucosa* to equal 62 500 tons fresh weight.

5. *India*

Gelidium micropterum appears to be the seaweed of most importance, and in the Mandaparam-Kilakarai region occurs in quantities of about 3 lb fresh weight per m^2 of reef. The total " agarophyte " standing crop is estimated at 3 000 tons on Indian shores. *Sargassum* and *Turbinaria* are the more important brown algae (Thivy, 1952). The same author estimates that the total agarophyte standing crop for Korea stood at 1 103 tons fresh weight, and for Japan 700 000 tons.

Whilst the standing crops are not known for all parts of the world where there are harvestable seaweeds, there is now much information on the types of marine algae which may be exploited. The relevant data are summarized in Table VIII, together with the authorities for the information. This Table gives some indication of the extent to which the potentialities and use of economic seaweeds is now being studied.

C. *Summary of data concerning the quantities of harvested seaweed*

Whilst estimates of standing crops are essential for knowledge of the exploitable seaweed beds, useful information can also be obtained from data on the quantities of seaweed harvested in previous years. Results of this nature may be biased by the requirements of a particular industry, but long-term records may indicate the possible effects of regular cropping on the harvestable material.

The Danish use of *Furcellaria fastigiata* forma *aegagrophila* may be taken as an illustration. Over a 15 yr period some 112 900 tons of fresh weed have been harvested (Sund and Bjerre-Petersen, 1952, 1964), from which 4 926 tons of extractive have been obtained. A graphical summary of the quantities of seaweed collected over the years is shown in Fig. 2, and shows the marked acceleration in the amounts harvested over recent years. In common with the increased quantities harvested, a more efficient utilization has also been observed; an agar production of 2·5% per weight of raw weed in the early years has been increased to 4%. The quantities collected reflect the increased demand for the

extractive furcellaran (Schachat and Glicksman, 1959). Harvesting is restricted to a single locality in the Central Kattegat off the north coast of the Djursland Peninsula. To date there appears to be no marked change in the standing crop, although it would seem that only in recent years have heavy demands been made.

Data concerning the quantities of *Chondrus crispus* collected on the shores of the Maritime Provinces of Canada over a period of 21 years have been recently given by MacFarlane (1964). The quantities

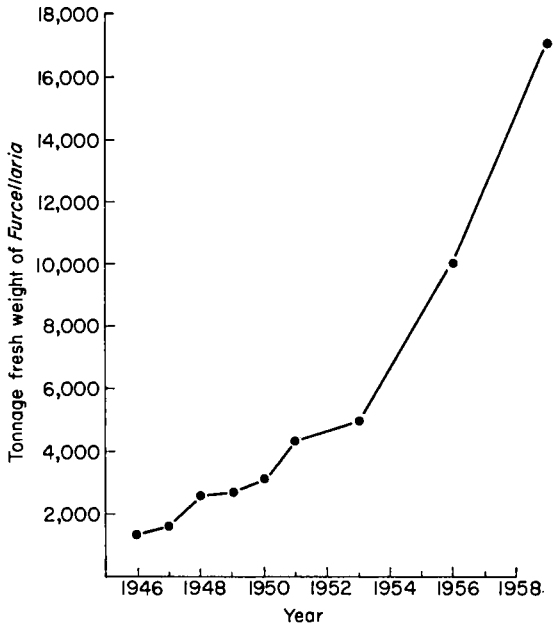

FIG. 2. Annual tonnage (fresh weight) of *Furcellaria fastigiata* harvested in Denmark. Drawn from data in Sund and Bjerre-Petersen (1952, 1964). (Reproduced with the permission of the Danmarks Fiskeri and the Pergamon Press.)

of dried seaweed were recorded, and the data are summarized in Fig. 3a and b. The peak year (1953) for Nova Scotia represents a fresh weight of plants which approximates 10 750 tons. The subsequent fall-off probably represents a carry-over from the heavy croppings of previous years. The data for Prince Edward Island are of additional interest in that a large proportion of the harvested material is in the form of cast weed. The bulk of the harvesting in Nova Scotia is restricted to the south-west of the Province where environmental conditions result in plants of large size and good market quality. *Chondrus* harvests for Newfoundland equalled nearly 25 tons of seaweed (1941–1943). A

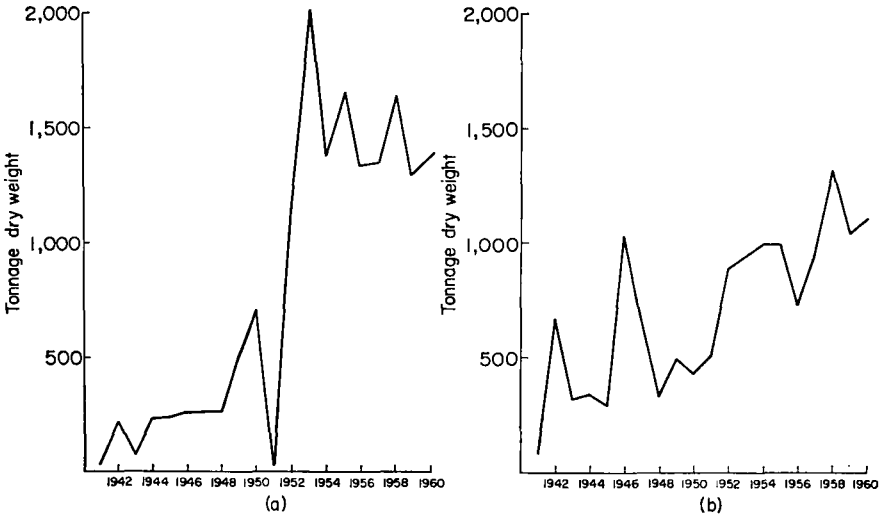

FIG. 3. Annual *Chondrus* harvests from the Maritime Provinces of Canada. a, Nova Scotia*; b, Prince Edward Island. Drawn from data in MacFarlane (1952, 1964a). (Reproduced with the permission of the author and the Pergamon Press.)

detailed account of the methods used in the seaweed industry of the Maritime Provinces has been given by Newton (1963).

Feldmann (1952) reported that in France 101 000 tons fresh weight of *Laminaria* sp. were utilized in 1950–1951, and mainly used in the

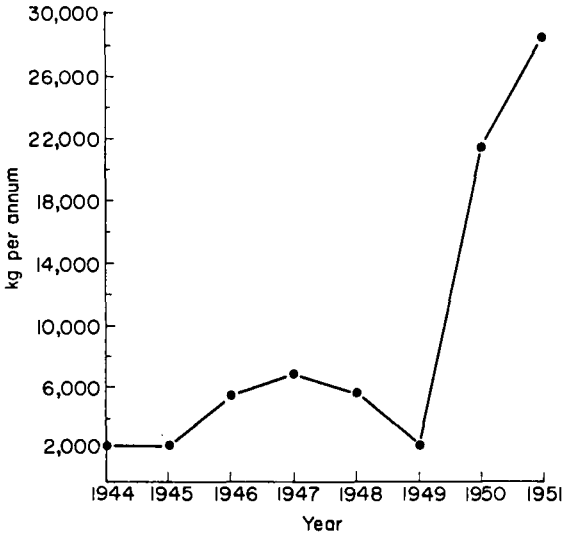

FIG. 4. Agar production from agarophytes in Spain, 1944–1951. Drawn from data in Aravio-Torre (1952).

* Quantities in 1961, 1962 and 1964 were 1 840, 2 256 and 1 842 tons dry weight (MacFarlane, personal communication).

production of alginic acid; considerable quantities of intertidal fucoid algae were also collected. About 4 400 tons fresh weight of *Chondrus* and *Gigartina* were harvested. The increased utilization of red algae in Spain was shown by data for the annual output of agar (Fig. 4) (Aravio-Torre, 1952). Brown algae have also been collected for alginic acid (Val and Pineda, 1949).

Records of kelp production for the California coast over the years

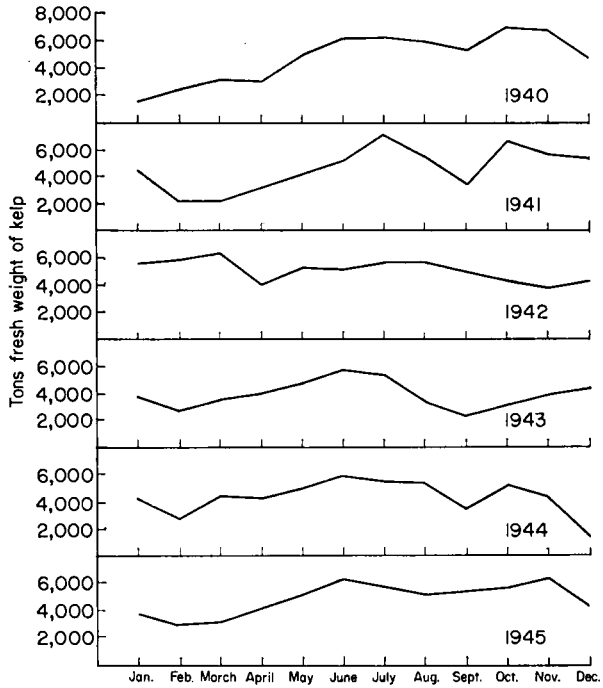

FIG. 5. Kelp production from California for the years 1940–1945. Drawn from data in Tseng (1947).

1940–1945 have been given by Tseng (1947), and are summarized in Fig. 5. There are general indications that the greater quantities are harvested during the summer months, but there is a good deal of variation from year to year. The mean annual harvests for 1940–1945 were about one tenth of those obtained during 1917 and 1918. For the Atlantic coast, Tseng estimated that 4 000 tons fresh weight of kelp (mainly *Laminaria* sp.) were harvested in 1944. Estimates of *Gelidium* production in California and Baja California, Mexico, indicated that in southern California some 240 tons wet weight of *Gelidium* were

TABLE IX. SUMMARY OF DATA ON SEAWEED PRODUCTION FOR JAPAN

Genus	*Japanese name*	*Use*	*Mean annual yields (tons)* 1936 (*Fresh weight*) (from Chapman, 1950)	1940–49 (*Dry weight*) (from Suto, 1952)	1955–60 (*Fresh weight*) (from Kurogi, 1963)
1. *Monostroma* sp. (+ *Enteromorpha*)	Awo-nori (Aonori)	Food, fertilizer	—	1 000	9 000
2. *Laminaria* sp.	Kombu	Food, mucilage material	293 284	20 000	142 200
3. *Undaria* sp.	Wakame	Food	44 601	7 000	50 000
4. *Ecklonia* sp.	Kajime	Mucilage material	—	12 000	—
5. *Sargassum* sp.	Hijiki	Food	—	2 200	—
6. *Porphyra* sp.	Asakusa-nori (Amanori; Nori)	Food	31 539	2 400	78 000
7. *Gelidium* sp.	Tengusa	Medicinal uses	12 000	2 200	14 800
8. *Gloiopeltis* sp.	Funori	Mucilage material	4 975	700	—
9. *Iridophycus* sp.	Ginnanso	Mucilage material	—	1 200	—
10. *Chondrus* sp.	Tsunomata	Mucilage material	—	400	—

harvested in 1944, whilst the quantities collected at Baja, California, were ten times this amount (Tseng, 1947). On the east coast, *Gracilaria* has been obtained in quantities of 1 500 tons fresh weight in 5 months of collecting at Beaufort, North Carolina. From information on standing crops it seems likely that there is still considerable scope for development.

Data on seaweed utilization in Japan are summarized in Table IX. In addition to the information listed in the Table, Kurogi (1963a) reports that a further 63 000 tons fresh weight of seaweed was used annually between 1955 and 1960. The figure of 78 000 tons fresh weight of *Porphyra* harvested annually is equivalent to 2 117 537 800 " sheets " of dried laver weed sold for human consumption. This contrasts with the 213 tons of *Porphyra* sold in South Wales during 1954 (Hampson, 1957). Scagel states that the Japanese " *Porphyra* " industry employs some 300 000 individuals, and that the areas under cultivation in Tokyo Bay approximated some 10 000 acres (Scagel, 1961). Allowing for the annual turnover in the Japanese industry, it is interesting to see reports that *Gracilaria verrucosa* is being imported from Africa for the extractives contained (Tagawa, Takeyama and Kojima, 1961).

V. Life Histories and Seasonal Ecology of the Seaweeds of Economic Importance

There have been a number of reviews in recent years on the ways of classifying the different life histories seen in the algae and on the nomenclature to be used (Drew, 1955; Chapman and Chapman, 1961; Dixon, 1963a,b). Since a relatively small number of algae are under discussion here it is not proposed to deal any further with this point, and in any case a good deal of confusion surrounds the basic features of such a classification at the present time. Some of the more recent work on the various genera will be separately considered.

A. *Chlorophyceae*

Three genera of algae are principally concerned, *Monostroma*, *Enteromorpha* and *Ulva*. Their main use is as food, but two of the genera, *Enteromorpha* and *Ulva*, are of further economic significance because they are mainly responsible for ship-fouling. This last topic warrants a separate review, and will not be dealt with in detail here.

Monostroma plants are utilized as food in Japan, and are cultivated on " hibi "* in specific areas of the shallow seas. The origin of *Monostroma* cultivation appears to lie in the plants growing as contaminants on the *Porphyra* " hibi " (p. 165). Certain areas of the culture grounds

* Artificial substrata for cultivation, see page 165.

are called " taneba ", and these are where the fixed spores and germlings appear in appreciable quantities in early autumn. The precise location of the taneba shifts with the years, and the spore distribution appears to be linked with the direction of the main surface current of a flood tide (Segi and Kida, 1960). Iwanoto (1960) described four species of *Monostroma* which occurred in Tokyo Bay either as contaminants on *Porphyra*, or as plants for cultivation. *Monostroma tubuliforme* favours regions with lowered salinity, and grows from autumn to spring; *M. crassissimum* and *M. latissimum* appear at about the same time, whereas *M. grevillei* grows over a much shorter period between December and March. The four species show distinct morphological characteristics. *M. nitidum* is collected for food in China. Life history studies on *Monostroma* have been carried out by Yamada and Tatewaki (1959) and by Kornmann (1962). As with the related genera, motile zygotes and zoospores are the main means of dispersal.

Ulva plants are more frequently used as food than *Enteromorpha*, and the species *Ulva lactuca* and *U. pertusa* are the two plants mainly used. Life histories have been studied in both instances, and an isomorphic alternation of diploid and haploid plants is observed, the diploid plants liberating quadriflagellate zoospores which give rise to gametophytes on germination. The gametophytes produce biflagellate gametes of different strain. The two genera appear in considerable numbers in intertidal habitats, and are not cultivated on special " hibi " like *Monostroma*. The conditions which govern zooid discharge are of interest, and the several factors involved here have been recently summarized by Lersten and Voth (1960). Christie and Evans (1962) in studies on the periodicity of zoospore and gamete release by *Enteromorpha intestinalis* in the British Isles, observed that maximum emission was obtained 3–5 days prior to the highest tide of each lunar month. Conditions of light, temperature and salinity were also important. Lersten and Voth (1960) emphasized the light dependence of zooid discharge in *Enteromorpha*. Data of this nature would be of importance in planning times of spore settlement should cultivation be envisaged.

Føyn (1955) investigated " southern " and " northern " plants of the ubiquitous *Ulva lactuca*, enquiring into their morphological features and the environmental conditions governing growth, and making crossing experiments between the gametes. From his observations Føyn concluded that the " southern " forms of the *Ulva lactuca* in fact constituted a distinct species which was called *U. thureti*. This species was described as occurring on Mediterranean shores, and on the Atlantic shores of Spain, Portugal and France.

Studies by Provasoli (1958) on the growth of *Ulva* plants in bacteria-free culture have shown that growth of the normal foliaceous thallus is not readily obtainable from the filamentous germlings. " Islands " of dark green cells were observed in the bleached germlings, and these cells produced fresh germlings on transference to new culture medium. Under culture conditions plant hormones were found to be necessary for normal morphogenesis. Such compounds are available in appreciable quantities in coastal waters with a high bacterial contamination, and may influence the growth of all types of seaweeds in areas under cultivation.

B. *Phaeophyceae*

1. *Productivity of benthic intertidal marine algae*

Some quantitative data on this aspect of the biology of the larger marine algae has become available in recent years, and will be described in the relevant sections under discussion. The importance of continuous remineralization of decomposition products of the littoral algae has been stressed in some recent papers (Hoffmann and Reinhardt, 1952; Hoffmann, 1963). Some of the commoner marine algae have been found to remineralize 30% of their organic phosphorus content within 12 hours. Kuznetzov (1956) estimated that for the shores of the Barents and White Seas the annual production of plant organic matter per metre of shore was equal to 180 kg. For this he estimated that 877·5 gm of nitrogen would be required, and this quantity was contained in 15 953 m^3 of sea water at the time of maximum nitrogen content. The quantity of sea water " available " for 1 metre of shore was estimated at 60 m^3, however. In laboratory experiments with *Fucus vesiculosus* 0·52 mg nitrate-N was found to be necessary per 9 gm of plant per day, and 0·63 mg of this would enable an algal growth rate of 8 mg/g/day. In fact under field conditions the growth rates were found to be 7·2 mg/g/day. Blinks (1955) estimated that the productivity per unit area of the littoral zone equalled 100 times that in the open sea. Conover (1958) has reported on a study of the standing crops of benthic marine algae.

2. *Fucus*

Fucus vesiculosus and *F. serratus* are the two species utilized in greatest quantities. The most detailed study of the seasonal ecology of the two species was that carried out by Knight and Parke (1950), and it is intended to summarize here only the data relevant to the problem of economic utilization.

In the British Isles *Fucus vesiculosus* is found zoned in the mid-

shore region, competing with *Ascophyllum* in some localities, and replacing it in others where there is exposure to more severe wave action. Irrespective of the time of settlement of the oospore, growth in length is a relatively uniform process. Mean growth rates (linear extension) were found to equal 0·45–0·48 cm/week on the Devon and Manx coasts. On sheltered shores in Scotland the rate was 0·68 cm/week, and on shores exposed to severe wave action 0·31 cm/week. Slower growth rates than this have been observed on the Norwegian coast (Printz, 1926). Very long plants appear to develop where there is a marked lowering of salinity.

The morphological rhythm of frond development over the seasons shows a clear-cut pattern. Successive dichotomies of the apical cells results in a division of the plant into sections, called " internodes ". The internode will further extend in length after branching. The dichotomy/length relationship is available for different parts of the geographical range of the plant; for instance plants in the Isle of Man showed a greater degree of forking (i.e. more dichotomies per length) than those on the Devon coast, and on Scottish shores exposed to severe wave action it was considerably greater. The division of the apical cell appears not to be a true dichotomy, since the growth potential of one arm of the branching is greater than the other, and in fact the growth appears to be more monopodial than dichotomous. Usually the arm with the lower growth potential forms reproductive branches. As the plant ages so the basal region consists mainly of the midrib portion with spur-like branch remnants. Vesicle formation is observed in the spring, and with young plants a minimum length must be reached before vesicles are formed (10–14 cm). The degree of vesiculation varies with habitat; vesicles are lacking on plants exposed to very severe wave action. In Norway vesicle formation and branch growth commences earlier on southern shores than in more northern latitudes, but (as for *Ascophyllum*, p. 141) the summer growth of plants in the north rapidly catches up with and passes that of plants in the south, with the result that conceptacle development is initiated in early autumn in the north, whilst in the south this phase is not reached until a further six weeks have passed. Further development in the northern plants is suppressed during the winter months, with the result that the conceptacles of the southern plants mature and release their oogonia earlier in the following year than those of plants in the north (Printz, 1959a).

Fucus serratus grows in a lower zone on the shore than *Fucus vesiculosus*. Growth in length on the Devon coast shows a mean value of 0·49 cm/week. On the coast of the Isle of Man the rate varied

between 0·59–0·84 cm/week, and in Scotland between 0·57–0·93 cm/week. The length/dichotomy relationship for *F. serratus* differs over the geographical range as for *F. vesiculosus*, but in the reverse order. The greater number of dichotomies per length are observed on the Devon coast, and the smallest number on Scottish shores. A similar inequality of growth potential was observed, with one arm of the fork greater than the other, but with increasing age of the plant this inequality gradually diminishes. This length/dichotomy relationship is important with regard to growth after harvesting.

In both species the various phases of reproduction are of a protracted nature. In *Fucus serratus* initiation of receptacles commences in May in Devon, and this continues until July. Release of gametes commences at the end of July, and continues through until early autumn, and then wanes. The sterile period lasts through the winter months. The phasing is later in the Isle of Man, with initiation of receptacles commencing in late summer, release of gametes in late autumn, and the sterile period lasting from March to July. The times of fruiting have been observed to be more protracted on other shores. In *Fucus vesiculosus* receptacle initiation commences in December, but release of gametes is not observed until May on the Devon coast and is preceded by one month in the Isle of Man. In this last locality initiation of new receptacles commences almost as soon as the fruiting of the same year is completed. Completion of fruiting in both species is followed by a wholesale defoliation of the spent fertile branches. The maximum weight of the plants is reached during the fruiting period.

Plants of both species rarely survive for more than 3 years on the shore, and the depopulation rate of marked plants may be as much as 83 to 90% at the end of the 3rd year. A particularly high mortality rate was observed with very young plants in the earliest stages of germination, mainly due to the browsing action of molluscs (p. 223). Studies were also made on growth after cutting and on repopulation of cleared areas. Repopulation studies with *Fucus vesiculosus* on areas cleared of plants showed that after long periods the plant population was only slightly less than the original population before clearance. When the fronds are removed by cutting a marked proliferation of new branches is observed in *F. vesiculosus*. This is due to a loss of apical control, and Burrows (1956) observed this same sort of proliferation from cut plants of *Fucus spiralis*, with new branches always appearing from the midrib region. Knight and Parke (1950) observed that proliferation only occurred in cut fronds which had borne an apical cell, and that with increasing age of the frond the amount of proliferated frondage decreased markedly. Sauvageau (1920) observed that the

weight of proliferation on young plants could be as much as to loosen the holdfast, and so cause loss of the plants. Two harvests a year are carried out on the French coast. In experiments on the Isle of Man where fronds of *F. vesiculosus* were cut to 30 cm and 15 cm from the base, plants with fronds cut 30 cm from the base had a similar fresh weight after 13 months regrowth to that of uncut control plants, whereas those cut to within 15 cm from the base equalled about half the fresh weight over the same time interval.

Culture studies on regenerative growth of *Fucus vesiculosus* have been described by Moss (1964a,b). At any time of the year the apical segments of young thalli show a more rapid growth rate. The relative failure of cut ends of the thalli to regenerate a new apical meristem is an indication of the apical dominance phenomenon. Other parts of the plant which lacked a functional meristem prior to their introduction into culture solution produced new branches, each bearing an apical meristem. In practice, new branches are produced in greater numbers from the middle regions of young thalli, and in much smaller numbers from older plants. The more marked regenerative growth is observed during April and May, and indicates a seasonal pattern of regenerative growth. Such a feature will be important in re-growth of plants after harvesting. *Fucus serratus* plants do not show the marked degree of proliferation demonstrated by *F. vesiculosus*, but the results of experiments in which regrowth was measured after various degrees of frond truncation showed a similar trend to those described above. Repopulation of cleared areas after 3 years showed an almost complete recuperation of the standing-crop. Knight and Parke (1950) suggested that cropping of *F. vesiculosus* and *F. serratus* every 2 years would give the best results. This strikes an even balance between young 1st-year plants which are not large enough and 3rd-year plants in which the frondage is large, but the rate of depopulation due to natural causes is steadily rising. Since the plants are at their maximum vigour in spring prior to the onset of fruiting this would appear to be the best time for harvesting, and certainly before the controlled defoliation which follows fruiting although it would be important to leave some fertile branches to allow repopulation by sporelings. Removal of fronds can be of advantage in encouraging the growth of young plants and sporelings which would be shaded or crowded out by the dense cover of older plants.

In more recent years a considerable amount of work has been carried out on the taxonomic relationships between *Fucus* species (Burrows and Lodge, 1951; Powell, 1957a,b; 1963; Evans, 1962) and a more detailed knowledge of the specific limits is now available. Burrows and

Lodge (1951) observed that whilst hybrid forms between the different species of *Fucus* can be obtained under experimental conditions, e.g. in laboratory cultures, or on " cleared strips "(p. 223), they are rarely found in closed shore communities, possibly due to a factor of competition, or to separate times of fruiting. In more open communities hybrid populations have been recognized (Burrows and Lodge, 1951).

3. *Ascophyllum*

Ascophyllum nodosum is a fucoid alga which has become an important component of seaweed meals in many parts of the world. It grows astride the middle of the foreshore, in a zone which roughly extends from high level neap tides to low level of neap tides. Thus methods of harvesting do not entail extensive underwater cropping. Seaweed meals are being produced in ever-increasing quantities, so that knowledge of the life history and seasonal behaviour of the plants is of considerable importance. *Ascophyllum* plants are also harvested as manure. It is noticeable that controlled harvesting has been instituted in Canada (MacFarlane, 1964a). *Ascophyllum* plants seem to grow best on shores which are sheltered from more severe wave action, and the type of substratum is an important factor here. David (1943) made an extensive study of the autecology of *Ascophyllum* on the coast of Wales, together with similar observations on other shores in the British Isles. The strap-shaped *Ascophyllum* frond bears a terminal meristem lying in an apical groove, and has numerous short lateral branches arising from narrow slits in the frond margin which also contain meristematic cells. Regenerative growth of cut branches is clearly important. Thus, when fronds were severed below the fertile regions no regenerative growth was observed, whereas if cut in the fertile regions of the frond new branches developed from lateral pits which normally developed receptacles. Regenerative growth was possible in branches up to 5 years old, whereas in much older plants this power was lost. Where lateral meristems are lacking, as in very young plants, regenerative growth is not observed. Branching systems are monopodial, dichotomous or trichotomous, with plants in the mid-part of the shore zone showing predominantly monopodial growth. Baardseth (1955b) observed that if fronds were cut within 4–15 cm of the base in regions which did not contain lateral meristems no regenerative growth would be observed. Frond damage of this nature can also result from natural causes, such as mollusc browsing, fungal parasites, overloading with epiphytes (particularly *Polysiphonia lanosa*). If, however, short basal " shoots " are present which bear apical meristems then growth can continue, and these basal shoots

arise from between the holdfast tissue and actively growing branches. Hence if frondage is cut at a sufficient height from the ground regenerative growth from the remaining stumps will be possible, and also from the basal shoots. It is also evident from observations made by Baardseth on cut fronds that growth from lateral pits becomes noticeable 1 month after cutting as well as initiation of the base shoots, but that after 5 months the new growths are not very long. The base and stump shoots combined contribute to the new frondage. The base shoots are initially long and slender, probably because of the reduced light intensity in the holdfast region.

During the annual life of the plant, David (1943) observed that new apical growth was initiated in October, but that afterwards little elongation was noticeable for as long as the autumnal fruiting receptacles were produced, suggesting a shunting of metabolic products accompanied by a decline in light and temperature. The first signs of apical growth appeared on plants in the upper regions of the shore, and then moved progressively down shore. Vesicle initiation was observed in the following March, with one vesicle produced per year. Marked apical growth of the frond was observed between April and October, and lateral receptacular pits were laid down on either side of the growing branch. With increasing age of the branch only those receptacular pits in the youngest (distal) part of the frond produced receptacles. The meristematic cells in the receptacular pits maintained their viability for a number of years, but rarely more than 5 years. On the coast of Wales, David observed vesicle production at the start of the 2nd year of growth, whilst Printz (1926) stated that in Norway vesicle formation is not observed until the 3rd year. There is evidence that a new branch must reach a certain (minimum) fresh weight before vesicle formation is observed. The period of rapid apical growth mentioned above is also the one in which the most marked regeneration of new branches from cut fronds is to be observed (Baardseth, 1955b). David (1943) observed that the angle of the slope of the substratum influenced plant size, and that longer frondage was observed with plants growing on flat substrata. Other factors affecting the size of the plants included the degree of infestation by the red algae *Polysiphonia lanosa*, which caused considerable stunting of growth if present in appreciable quantities on the frond.

Printz (1950) measured both growth and dry weights of *Ascophyllum* branches from Norwegian plants. In 1st-year branches growth and dry weight increased throughout spring and early summer, and then speeded up appreciably by autumn. Thence the dry weight values decreased. In the 2nd year there was again an increase in spring and

early summer, but a rapid fall in value later in the summer due to maturation of fruiting receptacles formed in the course of the 2nd year. In autumn the dry weights started to pick up again, but fell off in December. With short-day conditions and sea temperatures remaining fairly high this was probably due to an excess of respiration over assimilation. Increase in dry weight was a cumulative process with growing branches.

The production of fertile receptacles and release of gametes is of importance not only because of the recruitment of young plants, but also because of the metabolic changes involved. The effects on the growth rate of branches has been mentioned above. In the British Isles the first indications of receptacle formation are to be seen in May and June, and conceptacle formation is first observed in the autumn (David, 1943). With approaching maturity of the conceptacles the receptacles increase in volume, and the most marked increase in size is from February to May. After May dehiscence of the receptacles and discharge of their contents is observed. There is evidence that on southern shores of the British Isles the phasing of this process is timed a little earlier in the year than on more northern shores. Similarly David showed that dehiscence and release of gametes occurs earlier with plants growing near the upper limits of the *Ascophyllum* zone. Studies in the fruiting of *Ascophyllum* on the coast of Norway have been reported by Printz (1956a, 1959a), with plants selected from localities at different latitudes. Maturation of the receptacles occurs during April in Southern Norway, whereas on northern shores this is later by 40–45 days. However, whilst various phases of the growth of *Ascophyllum* plants in Northern Norway lag noticeably behind those of plants on southern shores during winter, spring and early summer, this gap is reduced considerably during the rest of the summer when the northern plants show a marked increase in the rates of growth, and in some instances may overtake the southern plants. The retardation due to lowered temperatures and much shortened day length in the north during the winter is then largely made up. Whilst the period of fruiting may be a protracted one, reports on the growth of very young *Ascophyllum* plants are sparse, and David (1943) found very few specimens in the *Ascophyllum* zone (Fig. 6).

Harvesting of *Ascophyllum* must clearly be linked with both the growth and reproductive phases in the life history, but since there are indications that sporeling growth is not very prolific, both longevity of the plants and frond growth after cutting need to be assessed. Estimates of the age of the plants are made easier by the annual production of vesicles. David (1943) estimated the mean age of plants in the middle

of the zone to be 8 years (ranging from 5 to 15 years), whereas plants from the top of the zone showed a mean age of 4–5 years, and those from the lower limit 6–7 years. The plants thus survive on the shore for much longer periods than species of *Fucus* (p. 137) and *Laminaria* (p. 155). Printz (1950) found plants 10–13 years old on the Norwegian coast, and a specimen 19 years old was observed.

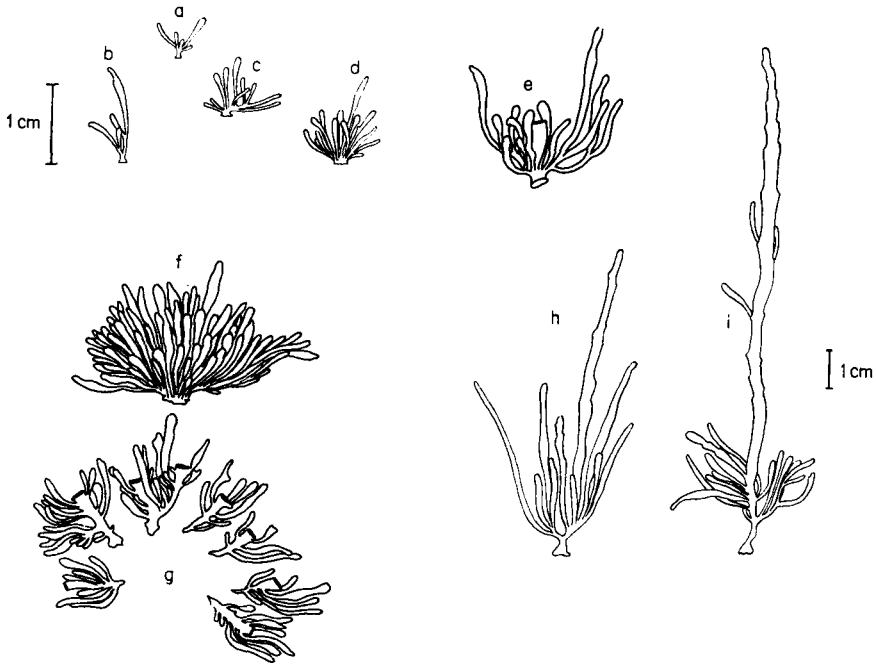

FIG. 6. Germlings of *Ascophyllum nodosum* collected from the top of the *Ascophyllum* zone on the College Rocks, Aberystwyth. Material collected in the early winter months. a—d, very young germlings; e, young plant showing effects of browsing damage on main axis; f, form of young plant in which successive main axes have been browsed by molluscs; g, the same plant dissected apart; h, i, young plants showing development of main axis without browsing damage, and with basal lateral branches.

Baardseth (1955b) made a particular study on regrowth of *Ascophyllum* after harvesting. New growth was obtained from stump shoots and base shoots, the stump shoots developing laterals from pre-existent meristems. The meristematic origin of the base shoots from the holdfast region is at present uncertain. Few of the stump shoots were found to bear fruiting receptacles after 8 months regrowth. Growth in length of a stump shoot is illustrated in Fig. 7. Cutting of the weed is

usually carried out in the spring, and new shoots appear and grow a few centimetres in length in the course of the 1st year. A feature noted by Baardseth was the failure of base shoots to produce receptacles, whereas the stump shoots eventually give rise to fertile branches. Whilst stump shoots will produce vesicles annually, the base shoots may take up to 18 months before first vesicle formation. In the first 12 months after cutting, therefore, stump shoots with and without vesicles and base shoots in the same condition will be observed together with small laterals on the stump shoots. Successive development

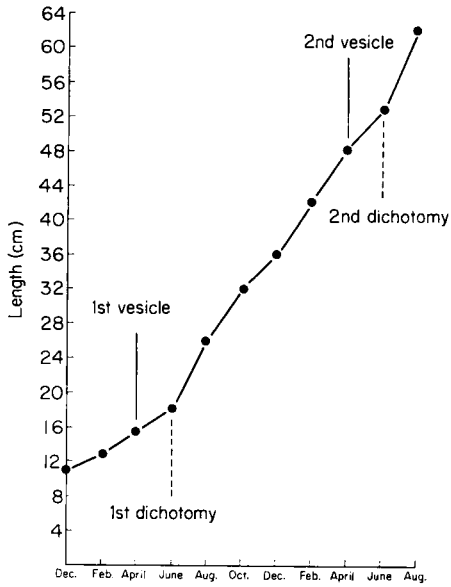

FIG. 7. Growth in length of a stump shoot of *Ascophyllum nodosum;* measurements commence approximately 8 months after cutting. Drawn from data in Baardseth (1955).

in the course of the next year will result in further vesicle formation on those branches which produced them in the 1st year after cutting, growth of laterals on the stump shoots and first vesicle formation on those branches which produced none in the year immediately after cutting.

Baardseth stated that, as long as the fronds were cut at a height of 5–15 cm from the holdfast, adequate resources for regenerative growth would then be left.* From estimates made on the shores of Galway, an interval of 5–6 years was considered to be sufficient for complete regrowth of the cut frondage, and the powers of recuperation shown by the plant after regular harvesting seem to be quite considerable

* Lengths of 20–25 cm have been recommended in Nova Scotia, and 5% of the plants to be left untouched. Personal communication from Miss C. I. MacFarlane.)

on the west coast of Ireland. Harvesting in spring is important because discharge of gametes and casting of the receptacles follows soon afterwards, and this would account for a 25 to 30% fall in the yield on a dry weight basis. Printz (1959b) observed that on the Norwegian coast regeneration did not take place when fronds were cut to within 1 cm of the holdfast and both stumps and holdfasts had rotted away in many instances. Stump lengths of 5 cm were also inadequate, but with lengths of 25 cm growth was sufficient to allow almost complete recovery over the course of 2 years. With older plants or branches this regenerative growth may take a more protracted time, possibly because of a lack of active growing points. This slower pace of regeneration may also be a result of a reduced action on the part of growth substances; in the lateral pits of fronds 8–10 years old there is a marked decline in the activity of the meristematic tissue. Since the long-fronded plants are those preferentially harvested, the stumps which remain will not recuperate as well as younger branches. Printz suggested that pulling up the plants instead of cutting would be a safer way of harvesting the frondage, and would ensure more adequate recuperative growth, since the older fronds would tend to be removed, and younger branches could then continue growth and development.

Attention has been drawn in recent years to the serious effects of uncontrolled harvesting on shore fucoid algae (de Virville, 1952; Printz, 1959b). This is particularly applicable to *Ascophyllum*, Printz observed, not only to obtain recuperative growth, but also recolonization of areas of the shore cleared of *Ascophyllum*. Similar observations have come from many sources where areas of the shore have been cleared of their *Ascophyllum* cover (e.g. Knight and Parke, 1950; Lodge, 1948; Southward, 1958; Boney, unpublished observations), and lack of recolonization by *Ascophyllum* has been observed for periods of 8–11 years. On regions of shore densely populated with other fucoid algae, *Fucus spiralis* and *Fucus vesiculosus* have been observed to recolonize the region cleared. Whilst there is a prodigious output of gametes by *Ascophyllum*, this occupies a relatively shorter period of the year (2–3 months) whereas species of *Fucus* often produce gametes over a 6-monthly period (p. 137). Oospore settlements in *Ascophyllum* occur during the summer months, at a time when rather harsh environmental factors are operative. The stability of the *Ascophyllum* cover on sheltered shores is thus mainly due to the relative longevity of the plants, and this can be radically altered by too severe a cropping. The question remains as to the part played by the production of gametes and oospores. Printz (1959b) suggested that by classifying a population into age groups, an estimate could then be made of the years during which

augmentation of the stocks from sporelings would have taken place. The suggestion was also made that such favourable years may be long spaced. Whilst younger plants are to be found at the top of the *Ascophyllum* zone, it is possible that these are in a state of protracted juvenility—a condition noted by Knight and Parke (1950) for high-level *Fucus vesiculosus*. There is the possibility that favourable periods for sporeling growth and subsequent recruitment of young plants might only occur over 10–15 year intervals. The competitive factor of other fucoid algae on areas cleared of *Ascophyllum* has been noted above. The time taken for re-establishment of the *Ascophyllum* cover—the climax in suitable habitats, is as yet unknown but will clearly take many years. Indiscriminate and uncontrolled harvesting can therefore present a major problem over the years, and certainly with *Ascophyllum* some measure of conservation would appear necessary, and this applies to areas where the population seems less severely affected by regular cropping, e.g. Galway, West Ireland (Baardseth, 1955b).

A detailed study of the free-living forms of *Ascophyllum*, particularly *A. nodosum* ecad *mackai*, was carried out by Gibb (1957). This form arises entirely from broken pieces of the attached plant which are carried into land-locked seas sheltered from wave-action, where there are marked salinity variations due to fresh-water streams, and where there is a flat substratum of sand or of thin mud overlying pebbles or stones. These conditions are found in the sheltered sea lochs on the coast of Scotland. Development of the ecad can be followed through a number of stages, of which a marked increase of branching is the first indication, first seen in the receptacles and then observed on the main branches. Low salinities seem to govern this condition, and midshore plants show a much greater degree of apical branching than do those at higher levels. Marked increase in lateral branching follows alternate periods of immersion in fresh and salt water. Frond injury will also stimulate lateral branch formation in the ecad. Continued growth in water of low salinity results in a thinner, more flexible frond. Bending and twisting of the frond also occurs—a condition commonly observed in salt-marsh fucoids. The habit of vesicle formation may also be lost under these conditions, and prolific receptacle formation observed. The release of gametes commences in April and continues over a more protracted period than that observed with the attached plants, but oospores develop into the attached form of plant. In general the standing crop is maintained by vegetative growth of the ecad, although certain diseased conditions appear to have caused appreciable reductions (e.g. "mud" decay; "freshwater" decay; "spotted" decay; "sun" decay). "Beach" forms and "turf"

forms of the ecad have been described. The potentialities for commercial exploitation of this free-living form of *Ascophyllum* have not been fully explored, and more information on the speed of recuperation after harvesting is necessary.

4. *Hijikia fusiforme*

This is a member of the order Fucales which is utilized as a source of food in Japan. The plants are compact and cone-like, and built on a radial plan. The plants show an annual growth of erect fronds which grow from prostrate " rhizome "-like branches. The young buds appear in the autumn, and growth and formation of new rhizome tissue proceeds throughout the winter. Receptacle development proceeds throughout the winter, and the mature conceptacles shed the eggs and spermatozoids in the spring. This is followed by defoliation of the spent receptacles and some of the erect frondage during the summer months, but the rhizomes are perennial structures. This cycle of events has been watched over a period of 5 years, and the erect frondage (or " bush ") can reach a length of 100 cm, and about a kilogram in weight (Suto, 1951b). Yendo (1907) described different forms of the plant from the northern and southern seas of Japan.

The plants grow on rocks near low water mark, and reach maximum development in April. Thereafter there is marked defoliation in the following summer months. Receptacle development commences in the winter months, but fertile plants are rarely seen before late spring (May). Yendo (1907) stated that fertile plants were rare in some parts of Japan because of the severe defoliation and depopulation seen in the summer months. If plants survive the summer they may subsequently reach lengths of 6 to 8 ft in sheltered habitats. Suto (1951a) reported a periodic shedding of the eggs at 7–8 day intervals in May with sea temperatures in the range of 19°–20°C, and this continued until July. The majority of eggs are released at flood tide, and fertilization takes place whilst the eggs are attached to the parent plant. Initial development of the embryo is accomplished within 24 h of fertilization, at which time the mucilaginous coat dissolves and the embryo is shed into the water. Fixation is then rapidly achieved. According to Chapman (1950), plants can be harvested from January until May, but the best crops are obtained in January and February when the fronds are young and tender.

5. *Sargassum*

Sargassum species are conspicuous elements of the flora of southern seas, but their morphological complexity and uncertain specific limits

are well known. Whilst occurring in considerable quantities as free-floating representatives, there are few reports of extensive commercial exploitation, although numerous species are used as sources of food in parts of Asia (Chapman, 1950). Studies on the biology of *Sargassum* in Japanese waters have also been concerned with the importance of the free-floating species as nurseries for young fish.

6. *Durvillea*

Durvillea antarctica plants reach a considerable size (fronds up to 10 m in length), and in many ways they resemble the larger members of the Laminariales. This alga is a source of food in some parts of South America, and in New Zealand is used as manure (Chapman, 1950).

7. *Macrocystis*

The bulk of studies on the biology of these giant kelps have been carried out on *Macrocystis pyrifera* (L.) Ag. This work has revolved around both the seasonal life, reproduction and physiology of the plants, and in many cases has been aimed at obtaining a more complete knowledge of full economic potential of the species. The other species *M. integrifolia* has been less studied. Womersley (1952, 1954), has recently described the taxonomy of *Macrocystis*.

Numerous accounts of the anatomy and morphology of *Macrocystis* are available (see summary in Cribb (1954)) and an account of the more recent work will be given here. The young sporophyte grows as a stipitate blade which splits into two equal halves; the secondary blades so derived then split unequally, and the outer of these last two products develops a thickened margin at the base on the outer side and this develops into a stipe which by its elongation separates the many newly formed blades. Each blade bears a basal pyriform pneumatocyst, so that the whole stipe then floats along the surface. The terminal blade continues this splitting process throughout its active life (Fig. 8). Once the active growth phase is over, deterioration sets in so that the stipe eventually breaks away from just above the holdfast region. The number of blades formed wil lvary with the length of the stipe—thus a stipe of length 250 cm may bear up to sixty blades. The overall length of the stipe can be appreciably greater than this, and plants of length 65 ft and over have been described. The holdfast system of dichotomously branched haptera develops at the same rate as the stipe and blades, but with age only the outermost haptera survive; hence the life of the attached plant will depend on the efficiency of the outermost haptera. However, in most instances a firm anchorage is obtained.

Methods of harvesting *Macrocystis* fronds have usually been by the

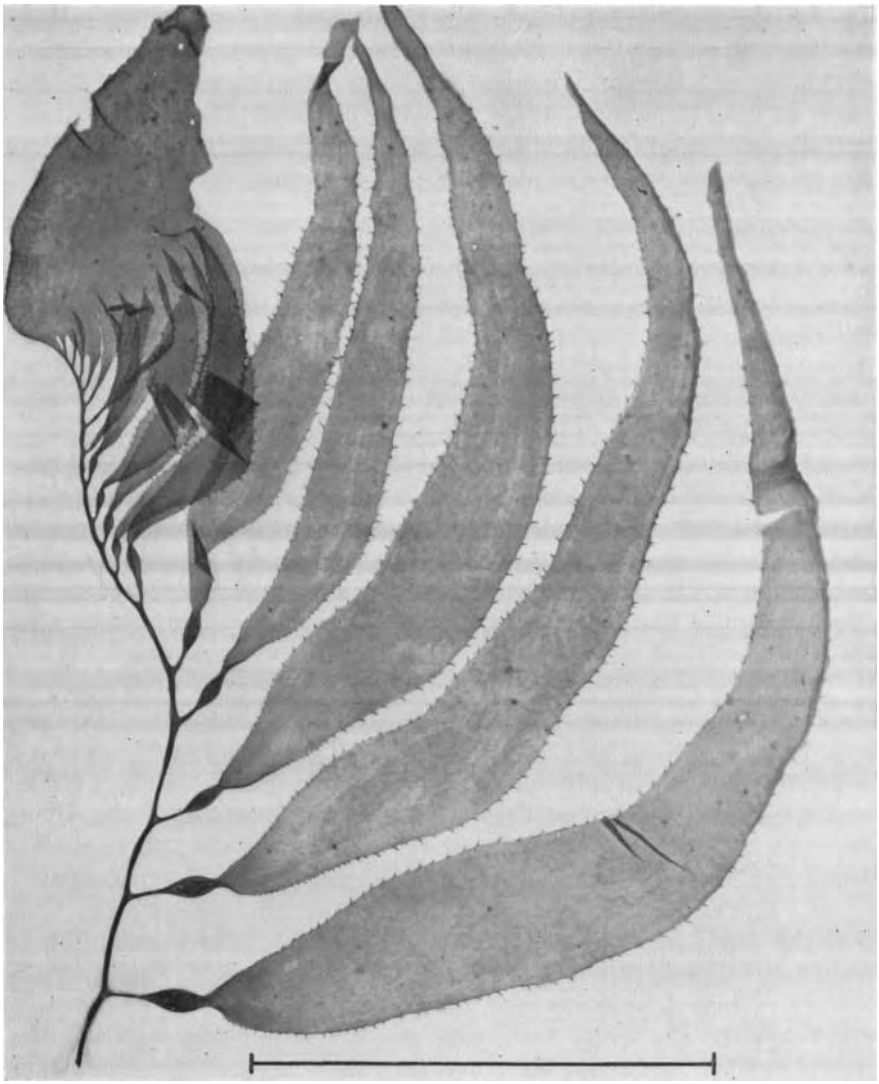

FIG. 8. Apical region of frond of *Macrocystis pyrifera* showing mode of origin of the blades, and young blades with basal pneumatocyst and fringed margin. Scale line = 15 cm.

mechanical cutting of the branches floating on or near the surface (Tseng, 1947). If harvested at suitable intervals there is no evidence of any excessive fall in the standing crop; Cribb (1954) suggested that in Tasmania the stipes cut 3 ft below the surface at 4-monthly intervals would enable growth of the plants to make good the loss through

harvesting. This is possible because of the extensive growth observed, and the ability of the plant to replace the lost surface canopy (Clendenning, 1964). There can also be a prolific growth of new frondage from the holdfast region, these new branches growing upwards as bundles of stipes entwined together in a helical pattern, and so able to withstand the buffeting of the sea despite their slender nature. Intercalary growth enables extension growth of the stipe, and the activity of a primary meristem at the blade tip acts as a source of new blades. In deeper water the internode lengths show a considerable increase in length. It has been stated that young fronds growing from the holdfast region are unable to maintain an adequate rate of growth by their own assimilation, and that some of the assimilatory products of the surface blades are translocated in the medulla. Evidence for this has been obtained by observing the effect on growth of the young fronds of exfoliation of the blades near the surface; more conclusive evidence would be obtained by use of radioactive compounds (Sargent and Lantrip, 1952; Clendenning, 1964). Metabolic activity is at a fairly high rate in blades with a high percentage of meristoderm, and a marked gradient of activity is to be observed. At the apex of the frond the activity is relatively low in the young fronds, and at a maximum between the 80 to 100th blade from the tip. Thence the rate falls off with increasing senescence of the blades near the base.

Assimilatory activity in a giant kelp frond has been likened to an advancing wave, with the " crest " 2–3 m back from the frond tip (Clendenning, 1964).

In California the average frond life is estimated to be 4–7 months, although North (1961) found that some fronds survived only 2–3 months, and with growth in deeper water lengths of 100 ft can be reached in the course of a year. A rapid regeneration of new frondage after harvesting has been observed, and growth rates of up to 18 in per day have been recorded. Observations on the rates of growth of *Macrocystis* in Tasmania were made by Cribb (1954). In sheltered fringing beds the age of the stipes was estimated to be between 7–10 months, and new blade production occurred on approximately every 2nd day. Rapid elongation of the stipe may be seen in the larger number of blades being initiated in the apical 5–10 cm. Detached apices with stipes 50 cm or more continued to produce new blades, although shorter lengths of stipe failed to do so. Removal of the terminal blade inhibited continued growth of newly initiated blades. The bulk of the meristematic activity of the blade is in the basal region immediately above the pneumatocyst. Some 2–3 months elapse between blade initiation and maturity. With advancing age the apical

region starts to decay and eventually growth of new tissue fails to compensate for this process of apical breakdown. With general decay of the blade and pneumatocyst the stipe is left bare in this region; and on long stipes only a small proportion near the apex will ultimately bear blades and pneumatocysts. On Tasmanian coasts decay of the blade usually sets in 4 months after its date of initiation.

Quantitative data on the growth of young *Macrocystis* plants is also available (Neushul and Haxo, 1963) from growth measurements made in the sea by carefully transplanting the plants to submerged rafts and under controlled laboratory conditions. When grown in the sea at a depth of 15 m the plants doubled in area every 20–30 days, and in length every 16–20 days during the autumn and winter months. " Growth saturation " under laboratory conditions was measured at 1 345 lux. Pressure effects were also found to be important, since implosion of pneumatocysts would cause fronds to sink in the water away from more adequate illumination. Growth studies are also available for *Macrocystis* in New Zealand (Moore, 1943; Rapson *et al.*, 1943), and for *Macrocystis integrifolia* in British Columbia (Scagel, 1948).

The large plants described above represent the diploid sporophytic generation—e.g. for *Macrocystis integrifolia*, 2n = 32 (Walker, 1952)—and bear superficial sporangia in sori on specific blades (the sporophylls) in the basal regions of the plant. Observations from a number of different regions indicate that fertile sporophylls are to be found at all times of the year—e.g. Papenfuss (1942) for S. Africa, Brandt (1923) and Neushul (1963) for California, and Cribb (1954) for Tasmania. Brandt observed that zoospores germinated in early winter showed the most rapid growth. Neushul described the various forms of sporophyll and their productivity. In Southern California a conspicuous ring of blue pigment surrounds the base of a fertile sporophyll. In many instances up to 96% of the surface area of the sporophyll was found to bear sporogenous tissue. Spore patches were observed to form and release their contents in about 2 weeks under controlled conditions.

The filamentous gametophytes have been described by Brandt (1923), Delf and Levyns (1926) and Papenfuss (1942). Neushul (1963) obtained growth of gametophytes, fertilization and young sporophytes 1–2 cm in length some 35–45 days after spore release from the sporophytes under a light intensity 1 345 lux at 15°C. Gametophytes were observed to grow at 237 lux—a much lower value than that required for growth of the young sporophytes. Clendenning (1964) noted that production of surface frondage from the sexual phase was a protracted process, and a full " canopy " of fronds could take up to 2 years to

establish. Gametophytes will normally grow under very shaded conditions, and this is indicated in the very low light requirements recorded above. Growth of the basal sporophylls is thought to be partially dependent on translocation of nutrients from the surface frondage. Neushul estimated that the minimum time for completion of the sexual cycle in the sea would be 14 months, of which growth and maturation of the sporophyte would take the most protracted time. Growth of the gametophytes appears not to have been fully observed in nature. Cribb (1954) failed to obtain gametophytes on plates of glass and bakelite immersed in the sea near fruiting plants in Tasmanian seas.

There is evidence that considerable damage is done to the kelp frondage by browsing animals. North (1958) described how, after oil pollution had killed most of the fauna, the sporophyte population increased nearly tenthousandfold. Haxo and Neushul (1958) described the grazing effects of " kelp-fish " and North (1964) commented on the importance of sea urchins as kelp grazers, together with the gammarid *Amphithoë humeralis* (Stimpson) and *Idothea resecata* (Stimpson). Sporophytes transplanted from another locality appeared to suffer more from animal depredation than the local population. *Macrocystis* growth near sewage outfalls was found to be considerably less than elsewhere, and this was found to be due to water turbidity not enabling the growth of the kelps to keep pace with the browsing action of attendant herbivores. Suggestions have been made that transplants of *Macrocystis* to Scottish shores might be attempted. North's experiments show that this type of work is attended by numerous difficulties. Thus North (1964) observed that strains of *Macrocystis* from South California were sensitive to sea temperatures a little more than 20°C for several weeks, whilst plants from 600 km farther south were able to survive exposure to 24°C.

Whilst the gametophyte phase poses many questions of ecological interest, the great productivity of the surface frondage of the sporophyte is evidently well able to cope with regular harvesting.

8. *Laminaria*

The *Laminaria* sporophytes are macroscopic structures harvested either for their food value, or for the extractives they contain. These large plants alternate in their life history with microscopic filamentous gametophytes from which the young sporophytes develop after fertilization of the oosphere within the oogonium. Hence a knowledge of the growth and survival of the gametophytes is an essential preliminary to studies on the seasonal life of the larger plants.

Growth and environmental tolerances of the gametophytes. Studies on the growth and environmental tolerances of several species of *Laminaria* are available. Since the earlier work of Ikari (1921); Ueda (1929); Schreiber (1930); Harries (1932) and Carter (1935) more recent reports are available from Parke (1948); Walker and Smith (1948); Tseng, Sun and Wu (1955a, 1957); Nishibayashi and Inoh (1956); Burrows (1961); Hasegawa (1962); Sundene (1958, 1962) and Kain (1963).

The initial development of the gametophytes of *Laminaria* and related species appears to be much the same. Zoospores liberated from superficial zoosporangia on the blade of the sporophyte settle on a substrate, round-off and put out a germ-tube into which the cell contents pass, and from which the filamentous gametophytes are formed. Equal numbers of male and female gametophytes develop; the male plants being invariably filamentous, whereas the female plants are either single celled, or may be filamentous. Filamentous female gametophytes tend to be more resistant, and are perennial.

The zoospores are planktonic during the time immediately prior to settlement, and Kain (1963) made observations on the time for which the zoospores remained motile. At 17°C the settlement rate was much faster than at 10°C, but it is possible for spores to remain non-motile for several days in suspension, and to commence development whilst still planktonic. Spores were found to be capable of surviving fairly protracted periods in the dark at 5°, 10° and 17°C.

The greater importance of temperature, light and salinity. Gametophytes of *L. religiosa* are viable between 2°–17°C, but produce gametes between 6°–12°C; gametophytes of *L. saccharina* grow well in the temperature range 5°–10°C, and those of both *L. saccharina* and *L. hyperborea* were observed to produce gametes when the temperature was suddenly lowered to 2°–4°C (Schreiber, 1930), whereas Parke (1948) obtained gametes from gametophytes of *L. saccharina* at 19°C. Gametophytes of *L. digitata* were grown by Sundene (1958) at temperatures between 5°–13°C and Kain (1963) observed survival and growth of gametophytes of *L. hyperborea* between 5°–19°C, and survival at 20°C and above. Ripe oogonia were not obtained above 19°C. Tseng *et al.* (1957) found 10°C as the optimum temperature for growth of the gametophytes of *L. japonica*. Wang and Hsüeh (1959) observed that *Laminaria* plants kept through the summer in a culture room at low temperature took some 2–3 months between release of spores and appearance of sporophytes 1–2 cm in length.

A number of studies have been made on the influence of light on gametophyte development and fertility, although in many instances

insufficient data are available on the precise conditions utilized. Thus Harries (1932) found that 10 h daily illumination at 1 000 lux was sub-optimal for gametophytes of *L. saccharina* and Hasegawa (1962) found 1 400 lux sub-optimal for *L. angustata*, and growth at 5 500–6 000 lux at 10°C was greater than in natural light. Gametophytes of *L. angustata* matured in 10 days under these conditions. Sundene (1962) maintained gametophytes of *L. digitata* at 5°C and in feeble daylight for indefinite periods without them becoming fertile. Kain (1963) made a detailed study of light effects on gametophyte growth in *L. hyperborea*, in particular keeping a close watch on the irradiance necessary for gametophyte development (at 10°C). Results showed that gametophyte development was possible at 26 lux (irradiance = 2·6 μg cal/cm^2 sec) using 12 h/day illumination at 10°C, and that the minimum continuous irradiance was 2 μg cal/cm^2 sec (= 20 lux). The saturating irradiance for growth was considered to be approximately between 33–40 μg cal/cm^2 (330–400 lux). The results indicate that growth of the gametophytes is possible at very low levels of light intensity.

Kain also commented on many earlier references to two types of female gametophyte, the few celled, early fertile types and the long filaments which are perennial. High temperatures, enriched media, or enriched media with low light intensity have been causative factors. A more critical examination by Kain has shown that gametophytes of *L. hyperborea* in the British Isles are produced during autumn and winter, at a time when there is reduction in available light, lowering of sea temperature and greater turbidity of the sea. Results show that spores can survive quite long periods at low light intensity or in the dark, and that respiratory activity is reduced to a minimum to prevent excessive use of reserves. Under natural conditions this means an adequate survival during the winter months, and fairly rapid growth when conditions improve in the spring. The perennial filamentous female plants were obtained at higher temperatures in unchanged media and low light intensities. Sundene (1962) observed that *Laminaria* gametophytes could be cut into fragments, and could still regenerate cells and be fertile on transference to fresh media.

Various studies have been reported on the nutritional factors influencing growth of gametophytes (Harries, 1932; Carter, 1935). Walker and Smith (1948) observed that best development of gametophytes of *L. hyperborea* was obtained in natural sea water enriched with nitrate, phosphate and bicarbonate, and that addition of uranium nitrate significantly increased spore development with enhanced rates of nuclear division, although sporelings died very soon after. Addition

of *Ascophyllum nodosum* fronds similarly had a harmful effect, suggesting production by *Ascophyllum* of substances toxic to the *Laminaria* sporelings.

9. *Taxonomic observations on Laminaria species*

More recent works on the taxonomy of *Laminaria* species have tended to show overlapping of the specific characters of some entities. Parke (1948) pointed out that growth forms of *L. saccharina* were obtainable which could be fitted to the description of other distinct species (e.g. *L. hieroglyphica*, *L. faroensis*, *L. longicruris*). The need was shown for more data on chromosome numbers, and for transplant experiments. Sundene (1962) has shown how the forms of *L. digitata* can be modified by environmental conditions. Burrows (1964) has reviewed the more significant diagnostic characters of *Laminaria* species, (e.g. shape of base of blade; blade texture and condition of margin; presence or absence of mucilage ducts), together with appearance of the meristoderm cells in surface view (Wilce, 1959). In a comparative study of European forms of *L. saccharina* cultured at 5°C and 10°C and of plants of similar forms from Nova Scotia which failed to show all of the main characters of *L. saccharina*, the two forms were found to be quite distinct in shape and size, even to size of cells of the gametophytes. However, continued growth of plants of *L. saccharina* at the lower temperature resulted in marked differences in its specific characters (e.g. loss of mucilage ducts). Evidence has been produced for a marked plasticity of form, which poses a number of questions with regard to interspecific relationships. Evans (1964) has reported on the chromosome numbers and condition in *Laminaria* species.

Laminaria saccharina. The results of a 4-year study on *L. saccharina* in the British Isles were reported by Parke (1948). Gametophytes were observed to be fertile throughout the year, but the young sporophytes produced can be found in one of four types of habitat depending on the season. Thus near low water mark, where the shore will be continually covered by the sea, sporophytes are found throughout the year, continuous crops being produced by either protracted fruiting of the gametophytes, or by continuous production of gametophytes. In intertidal habitats, where the shore is uncovered by the sea at low water of spring tides, sporophyte production ceases during May and June. In habitats several metres below E.L.W.S.T., sporophyte development ceases between November and February due to a marked reduction in light intensity. Sporophytes are not observed in habitats

6–12 m below E.L.W.S.T. where the sea bed is of soft mud. The seasonal cycle of sporophyte production shows a shore migration, being found at higher levels during winter, early spring and late summer; and at low levels during spring, early summer and early autumn. Winter sporophytes rarely survive, and depopulation rates can be 100% in exposed areas, whereas sporophytes produced in the spring show a higher survival rate and grow more vigorously. Plants which survive the initial settlement and growth rarely live for more than 3 years. Summer and autumn sporophytes rarely survive beyond the first winter, whilst the spring sporophytes are more firmly attached and are more robust and can survive the winter storms.

Growth of the plants show alternating periods of fast and slow growth. The period of fast growth lasts from January until mid-summer, with most rapid growth from March to June. Slow growth is from July to December, with the slowest rate from September to the end of the year. Early growth of the sporelings is variable in rate depending on the habitat and time of year; during the spring the growth rate is more rapid than in late autumn.

Growth of the lamina shows annual cycles of fast and slow growth, the fast growth period extending from January to July, with the growth rate gradually increasing from January to March and remaining fairly constant then until June. The rate slows down from June to July, and this continues until the minimum rate is reached in October or November, and thereafter it increases again. In the second year of the plant's life the increase in frond length during the rapid growth phase reaches its maximum compared with the first and third years. Depending on where the sporophytes commence growth, so the maximum growth phase will also vary. With spring germinated sporophytes this will be at 15 months old, whereas with sporophytes produced in the following summer this phase is reached after 21 months. Increase in frond length is greater the lower down the shore that the plant grows. In exposed intertidal habitats the plants show very little further growth after the second period of rapid growth, whereas plants immersed in rock pools at the same levels will survive well into the third year of growth. Increases of 6° latitude generally result in a higher rate of frond growth at the northerly limits.

Primary growth of the frond from the basal meristem follows the course of rapid and slow growth. Secondary growth of tissues above this region occurs by cell division and elongation of cells already formed. Secondary growth continues throughout the year in the lower part of the frond, but lags behind the rapid growth phase by 1–2 months. Complete regeneration of frond tissue is only possible in plants under

12 months in age, and the younger the plant the more readily it recovers after cutting (or tearing) of the fronds. This is probably because of the more intensive meristematic activity of the base of the frond. Regenerative growth is possible as long as some 15–20 cm of the frond is left,—complete removal results in a rotting away of the stipe. Change in frond width during the growth phase is reflected in the shape of the base of the lamina, being fusiform in rapid growth, changing to cuneate and to sub-cordate with successive slowing down of the growth rate. Frond width is also greatest during the second rapid growth phase. A regular defoliation of lamina tissue from the apex is observed, shown more regularly by plants in sublittoral habitats than in intertidal habitats where environmental conditions may supervene. In sublittoral habitats tissue formed in the first 6 months of the year is cast in the second half, and the tissue formed between July and December is cast in the next 6 months.

Stipe and holdfast growth is also rhythmic in pattern. Growth in length and thickness of the stipe is more rapid between January and June than from July to December. As the rate of stipe growth increases in the apical region the formation of a new series of haptera is observed at the base of the stipe. Increase in girth of the stipe is accompanied by formation of a secondary meristem, and the first activity of this is seen at the base of the stipe. The seasonal change in stipe growth may be seen in the alternate zones of lighter and darker tissue, the darker (small-celled) tissue being formed during the periods of slow growth.

Reproductive tissues develop in the plants 8–12 months old, and plants which start developing in the fast growth period mature more rapidly than those which commence growth later in the year. For the sporangium development to commence, the distal tissue of the frond must be at least 6 months old. Once initiated, sporangia mature in 14–21 days and may continue to liberate zoospores for 2–3 months. Ripe sporangia number some 1 000 000/cm^2 at first, falling to 500 000/cm^2 of frond surface after 14 days and the greater number of zoospores are released during the first few weeks of reproductive activity. Thirty-two zoospores are produced per sporangium, and estimates show that 1 cm^2 of frond surface can produce 64 000 000 spores over a 3-month period. Despite this vast potential, the time over which spore production can take place will depend upon the degree of survival of the plant, or of the frond tissue, in a particular habitat. Maximum production of reproductive tissue is observed in a 2nd-year plant. During the year, the main period of formation of reproductive tissue is observed between October and March, particularly with frond tissue developed from April to September. Whilst there will be variations in the time

of onset of fruiting due to the age of frond tissue and environmental factors, it appears that actual initiation of the reproductive tissue is controlled by some agency within the plant, but whether this is of a hormonal nature is not known. *L. saccharina* plants on the British coasts commence fruiting in their 3rd year, and sporangium production commences in July, attaining a maximum in early August, and is completed by the end of the same month. On the Norwegian coast fruiting plants are found from the middle of May to the end of September. Tikhovskaya (1940) observed maximum growth of *L. saccharina* in the Barents Sea in April, showing a sharp drop in June and a slight increase in October. This fall in growth rate occurs at a time when the nitrate and phosphate content of the sea is at its lowest.

Annual fluctuation in weight of the sporophytes of *L. saccharina* have been described by Kireeva and Schapova (1933, 1938) and Tikhovskaya (1940) from the Russian coast, and by Parke (1948) for south-west England. On Russian shores maximum weight is reached in August, and minimum weight in March, whereas on the British coast maximum weight is reached in June or July, and minimal values in December or January. Kuznetzov (1956) observed that *L. saccharina* grew faster than *L. digitata* in sheltered localities bordering the Barents Sea, but that *L. digitata* was the more abundant in exposed localities. For that region growth of *L. digitata* blade is observed up to July, whereas stipe and haptera grow throughout the summer, and since the haptera attain maximum development in autumn, the plants will be more able to withstand severe autumnal gales.

More recent work on *Laminaria digitata* in Norway (Sundene, 1964) has shown that growth of the new blade is an overall process, but with more extensive cell production from the basal meristem over the year, and with considerable growth above this region between January and June. Between spring and summer there is a steady slowing down of blade growth above the basal region, and new growth commences again in September. For south and west Norway the rapid growth period is from February to April, and slow-growth is observed between late summer and autumn. During the phase of rapid growth the blade elongates at the rate of 0·03 cm/day. By contrast, in north Norway the maximum growth period is from March to May, it then slows rather abruptly, declining through summer and autumn and finally ceases in mid-winter. The growth rhythms of plants from south and north Norway are fundamentally distinct; on southern shores the growth is slowed down by increased sea temperatures, whereas on northern shores the rate falls with onset of winter darkness.

Depopulation studies on sporophytes of *Laminaria digitata* and

L. longicuris carried out by MacFarlane (1952a) showed that of 200 marked plants, fifteen per cent of *L. digitata* and three and one quarter per cent of *L. longicuris* survived a 9-month period (October–July).

Laminaria hyperborea. The growth of plants of *L. hyperborea* follows a similar course to that of *L. saccharina*, showing annual phases of fast and slow growth, and primary and secondary growth in stipe and frond. In analyses of several populations (Kain, 1963) plants up to 13 years old have been found, but the bulk of the population usually lies between 1–4 years. The density of the sublittoral population appears to be fairly constant down to the lower limit of density of the population, although this lower limit will vary with the type of habitat. Larger plants of 6 years or more of age are more selectively destroyed by some environmental factor. The period of fast growth commences in winter, and initial growth of the frond takes place at the expense of reserves stored in the stipe. These reserves are then built-up again when there are more suitable light conditions. Fronds and stipes have similar fresh weights in April. This increase in fresh weight will result mainly from assimilatory activity. Observations made within the limited latitude range of the British Isles showed that conditions for the growth of *Laminaria* plants were more favourable in the northerly latitudes (Scotland), and large plants of an older age grouping were to be found there.

Transplantation experiments with Laminaria. Different forms of *Laminaria digitata* are known in Norway, (e.g. f. *stenophylla*, f. *genuina*, and f. *typica*), and these have been found interfertile (Sundene, 1958). Hybrids between these various forms and " true " forms were successfully reared in culture up to 1–2 cm in length and then " transplanted " by careful attachment to floating rafts in the sea. Change in environmental conditions had marked effects on the form of frond. Thus the fronds of plants in sheltered habitats retain an elongate, undivided habit, whereas those exposed to severe wave action become broader and much divided.

Transplantation experiments showed that the most favourable time was in early autumn when sea temperatures were below 15°C, and plants placed in the sea at this time attained a greater length than those transferred during the winter months. The summer months were observed to be the most critical during which loss of plants was greatest with noticeable decrease in length of the fronds in many instances. Growth of the fronds was also markedly reduced during the summer months. Growth rates then picked up again during the autumn and

winter, and by January the production of new blade tissue outpaced the rate of defoliation of the old. General observations indicated that the plants are 3 years old before sporangia are produced.

Laminaria japonica. Various aspects of the seasonal life of *Laminaria japonica* in relation to environmental factors have been reported by Tseng *et al.* (1955a, 1957). In cultivation areas in north China the main growth season extends from November to June and settlement of spores on " collectors " for purposes of cultivation is possible over much the same period. During the main growth season blades 3–4 m in length, 25–30 cm wide and 2–3 mm in thickness may be obtained, but this varies along the coastline depending on the quantities of available nutrients. When surface temperatures of the sea remain below 20°C two to three weeks elapse between liberation of spores and development of very young sporophytes. Frequently there is intensive competition on the " collectors " with smaller algae (e.g. *Ectocarpus* sp.) which grow vigorously at 20°C. Thus if the very young sporophytes are kept at 10°C under controlled conditions and then placed in the open sea where the temperatures fall below 20°C, this spatial competition with smaller algae is not observed. The sporophytes, now several millimetres in size, are able to compete successfully with other algae. As a result, the " haitai " yields are almost doubled. Improved growth of the plants was also obtained by cultivating young sporophytes in racks in the near vicinity of earthenware vessels containing $NaNO_3$, Na_3PO_4, NH_4NO_3 and apatite powder. With the gradual outflow of nutrients into the sea a marked enhancement of growth was observed over a 94-day period. The mean dry weight of thalli grown in this way was 32·2 g, having shown a daily increase in fresh weight of 353·2 mg. By contrast, control material showed a mean dry weight of 7·9 g, and a daily incremental change of fresh weight of 89 mg. It was estimated that 1 kg of " haitai " required 1·01 kg of " nitrogen " fertilizer. These methods certainly offer scope for further exploitation of fertilization techniques.

The factor of competition with other algae referred to above is also known in Japanese waters, where large-scale removal of *Phyllospadix* is practised in order to encourage the spread of *Laminaria* plants. *L. japonica* is harvested between July and October (Chapman, 1950).

Laminaria angustata. Two types of sporophyte have been observed, the ovate-bladed winter type which remains immersed, and the lanceolate-bladed spring sporophyte which is intertidal. Certain aspects of the two forms are distinguishable at the earliest stages of

development (Hasegawa, 1962). The depopulation rates of young stages of the immersed forms is greater during summer and autumn. Annual growth of the blade shows one period of fast and one period of slow growth during each annual cycle. Growth in frond width is more noticeable during the second and third years, and reaches its maximum between May and August in the third year of the plant's life. The phases of fast and slow growth vary with the age of the plant. Thus the fast growth period in the 1st year is from March to July, and is most rapid in April and May. In the 2nd year this phase of rapid growth lasts from January to July, and in the 3rd year from December until the following August. In most years the spring and early summer are the main periods of rapid growth, and the slowest growth takes place between August and October. The plants reach their maximum weight in the rapid growth phase in the 3rd year of development. Plants of *L. angustata* survive for some three years in their shore habitats, and the annual growth of the blade results in the previous year's blade gradually wearing away at the new blade tip. Each year's growth of the plant is accompanied by the production of new whorls of haptera at the base of the stipe.

Sporangia of *L. angustata* appear on the blade early in October in the 1st year of growth, continuing until March of the next year. During the 2nd year, sporangia appear in July, the peak period of spore production being in October, and tailing-off into January. In the 3rd-year plant the sporangia appear in June, and continue to be fertile until January. Harvesting of the 3rd-year plants will allow ample spore productivity in the first 2 years of growth, and enable adequate establishment of gametophytes. Sporophyte depopulation is at a greater rate during the spring of the first year of growth, viz. 96·6%, although this falls to 77·5% after 15 months. A seasonal variation in frond weight is also observed with *L. angustata*, with maximum weight in June and minimum weight between November and April.

10. *Undaria*

Recent data on the commercially important *Undaria* plants have described two species, *Undaria undarioides* and *Undaria pinnatifida*. Segi and Kida (1957) studied the effects of certain environmental factors on the growth of gametophytes of *Undaria undarioides*, obtaining better growth at 2 200–3 300 lux than in " full " sunlight (4 500 lux). Very slow growth was obtained at 400 lux, and although gametophytes were successfully germinated in the dark, they were less viable than those obtained in the light. The gametophytes of *Undaria* would appear to show a much higher degree of tolerance to light than those of

Laminaria (p. 153). The newly attached zoospores, gametophytes and the young sporophytes of *U. pinnatifida* survive protracted periods of exposure to air of 100% R.H., whereas at 70% R.H. zoospores are killed within 30 min and the young gametophytes and sporophytes in 1–3 h. At higher temperatures the period of tolerance is reduced (Saito, 1960a). The form range of *Undaria pinnatifida* has also been studied (Saito, 1960b; Kato and Nakahisa, 1962). Of the three principal forms described (f. *typica*, f. *distans*, f. *narutensis*), biometrical measurements of the main diagnostic characters and growth of the sporophytes under identical conditions from gametophytes collected from a number of widely separated localities showed that the different characters (frond and stipe length, frond shape, relationship between blade and sporophyll, condition of sporophyll surface) were mainly hereditary, and were not changed under conditions of culture or in changed habitats.

The periodicity of zoospore shedding has also been studied with *Undaria* sporophylls (Suto, 1952). Release of spores commences when the sea temperature reaches 14°C (i.e. during spring). Spores are released in vast quantities at 2-hourly intervals between 9 a.m. and 6 p.m., and on every 4–5 days enormous numbers are released. Light or dark periods seem to have little effect on spore release. Previous drying of the sporophylls prior to immersion hastens the onset of zoospore release, but has no effect on the numbers obtained.

Undaria plants grow best in sublittoral habitats (depth 20 to 40 ft) where there is a strong current action. The blades are harvested between February and June by means of long-handled rakes (Chapman, 1950).

11. *Alaria*

Although used to a smaller extent than the other genera so far described, detailed studies on some aspects of the biology of certain *Alaria* species have been reported in recent years: Sundene (1961) described the results of some interesting experimental work carried out with *Alaria esculenta* in Norway. For example, in transplant experiments where plants were moved during autumn from the west coast to Oslofjord, whilst the test plants reattached themselves successfully to the new substrata, they died and disappeared in late summer and early autumn. And whilst the transplanted material became fertile in the winter months and produced young plants in the vicinity, none of the young plants survived the summer. Experimental studies on the male and female gametophytes showed that they were able to survive long periods in culture medium at low levels of light intensity, and with

enhanced growth in increased illumination could be fragmented, and the separate fragments still be fertile. The young sporophytes then obtained were cultured under variable conditions of salinity and temperature. In water of salinities 25 and 30°/$_{oo}$, satisfactory growth was obtained at temperatures up to 14°C, but poorer development was observed at lower salinities (15 and 20°/$_{oo}$). At higher temperatures (17°C) the plants kept in media of low salinity died, and those at 25 and 30°/$_{oo}$ were in a poor condition. All the young sporophytes were killed at 20°C.

Sundene considered that summer temperature was the principal factor governing distribution of *Alaria*. In fact, distribution was found to be almost limited to coasts where the mean surface temperatures remained at 16°C or below.

12. *Nereocystis luetkeana*

This species is another of the giant kelps found on the western coast of North America, frequently growing mixed with *Macrocystis*, (as on the coast of British Columbia (Scagel, 1961)). The plant consists of a long flexible stipe which terminates in a vesicle, and this vesicle bears numerous lanceolate blades. The plant grows in 5–8 fathoms of water, usually where tidal action is swift (Fritsch, 1945). Growth of the plant is annual, so that only one harvest per year is possible. In this case, however, it would be important to delay harvesting until fruiting had been sufficient to allow some recuperation of the standing crop (Scagel, 1961). The sporangia are borne in elongated sori on fertile blades, and the entire sorus degenerates and falls out when fruiting is completed. There appears to be little information on the gametophytic phases in the life history. The heavy loss of frond tissue after fruiting indicates that this is a serious feature to be considered with regard to harvesting. A number of alternative uses other than as a source of algin have been suggested for *Nereocystis* (Chapman, 1950; Scagel, 1961; Newton, 1950, 1963).

13. *Egregia*

This genus, which is related to *Alaria*, grows in extensive stands in the region of the sublittoral fringe and just below on the western coasts of North America. The plant consists of somewhat flattened stipes and fronds which are fringed by numerous short laterals, many of which have an assimilatory function. Plants can reach lengths of 30 to 40 ft, and could be a potential source of harvestable weed. However, few attempts appear to have been made to exploit this plant on a commercial basis.

14. *Ecklonia*

This genus resembles *Nereocystis* and is found principally in the Southern Hemisphere. The long stalk terminates in a small air bladder, this in turn bearing a primary blade from the base of which numerous sporophylls are produced (Fritsch, 1945).

Other large members of the Laminariales used for a number of purposes include *Lessonia variegata*, *Eisenia bicyclis*, and *Pelagophycus porra* (Chapman, 1950). The prodigious spore output of the Laminariales is also of significance as food for invertebrate larvae in the general turnover of organic matter in the sea (Scagel, 1959).

C. *Rhodophyceae*

1. *Porphyra*

The several species of *Porphyra* used as food have been enumerated (p. 109). The starting point for present-day knowledge of the *Porphyra* life history may be seen in Drew's work (Drew, 1949, 1954a, b), which first showed the presence of both a shell-boring *Conchocelis*-phase and the leafy thallus during the life history. The shell-boring phase was obtained by Drew when the " carpospores " of the leafy thallus of *Porphyra purpurea* (= *P. umbilicalis* f. *laciniata*) were germinated on sterilized shell fragments. The use of this substratum (as found in the vicinity of the leafy *Porphyra* plant on the shore) followed unsuccessful attempts to obtain the continued growth of the filaments on glass slides. Drew's studies showed that the filaments of the *Conchocelis*-phase produced " fertile cell rows ", and that the cell contents of these fertile cell rows rounded-off, forming spore-like bodies; the intervening walls of the cell rows then broke down forming a tube which could pass to the surface of the shell (Drew and Richards, 1953). Small, much-branched " plantlets " were obtained under culture conditions, but these were not observed to form spores. Neither spore release nor subsequent germination was observed by Drew, who was also unable to obtain the leafy-thallus from the shell-boring phase. A *Conchocelis*-phase has been shown to be part of the life-history of a number of *Porphyra* species (e.g. Tseng and Chang, 1955a, b; Kurogi 1953a, b, for *Porphyra tenera;* Graves, 1955, for *Porphyra capensis;* Miura, 1961, for *Porphyra tenuipedalis;* Joly and Yamaguishi, 1963, for *Porphyra atropurpurea*). Demonstration of the full cycle from leafy thallus to shell-boring phase and back to the leafy plant again has been reported under culture conditions by Tseng and Chang (1955b), Hollenberg (1958), Kornmann (1960), and Iwasaki (1961). Whilst Graves (1955) was able to obtain the large plants of *Porphyra capensis*

in vessels in which its *Conchocelis*-phase had been cultured, the direct link between the two was not clearly demonstrated.

At present the critical problem in *Porphyra* life-histories is seen in the lack of any convincing data on the sexual process, and the stage at which meiosis takes place. The data on this have been summarized by Drew (1956) and Dixon (1963b), and each has stressed the absence of any reliable information. Drew (1956) described two types of spore which were formed in the *Porphyra* life-history; monospores are formed by a simple rounding-off of the contents of a vegetative cell, and smaller spores formed by successive divisions of the contents of a mother cell. Prior to the formation of the last type of spore many claims have been made that fusion took place between the contents of the mother cell and those of the spermatium, and that a reduction division then preceded formation of the " carpospores ". This term has been used with reservations by a number of workers in the field. For one species for which detailed cytological studies are available (*P. purpurea*) there is no evidence of a reduction division. Krishnamurthy (1959) obtained the same chromosome number in the cells of the leafy thallus, in the cells producing spermatia, in the spore mother cells and the newly released and germinating spores, and in the *Conchocelis*-phase. Magne (1952) recorded only mitotic divisions in the nuclei of the " carpospore "-producing cells of *Porphyra linearis*, but did indicate that a fertilization process was to be observed. This lack of any convincing data on the sexual fusion in *Porphyra* raises a problem in descriptive work, since it is claimed that a fusion of spermatia and the contents of mother cells does occur in the cultivated species of *Porphyra* in Japan and China. A number of features of this fusion process have been described, except the fusion of gamete nuclei.* Thus in the following account the terms " carpogonia " and " carpospore " are used with the reservations outlined above. As pointed out by Dixon (1963b), no clear explanation of the mass of conflicting data is possible without a considerable amount of further critical work on the problem.

The significance of Drew's observations on the *Conchocelis*-phase in the *Porphyra* life-history will be indicated in the following pages. Its importance to the Japanese seaweed industry has been immense, and a tangible reminder of this is the granite memorial to the late Dr. K. M. Drew erected by fishermen at Uzuchi, near Tokyo.

More recent reviews on the *Porphyra* life-history have been supplied by Conway (1964a, b, c), with particular emphasis on the species growing in the British Isles. In this work also the question of gametic

* Fertilization in *Porphyra yezoensis* has been described recently (Yabu, H. and Tokida, J. (1963) *Bull. Fac. Fish. Hokkaido Univ.* **14**, 131–6).

fusion has been considered, and the lack of any concrete evidence re-emphasized. The need for a re-examination of " spore terminology " was also stressed. Thus the large spores produced by the *Porphyra* thallus were called α-spores, and the smaller ones β-spores. Observations on *Porphyra umbilicalis* have shown that spore release and germination are optimal in May and June, and again in October and early November. The vigour of spore production was found to vary appreciably with habitat.

Germination and growth of the α-spores were best obtained at low temperatures (5°–7°C), and in a light regime of 80–100 lux for 8 h/day. Culture at 1 100 lux for 12 h/day at 20°C resulted in bleaching and deterioration of the cells. The growing *Conchocelis*-phase produced terminal monosporangia, and the liberated spores then gave rise to more *Conchocelis* filaments on germination. Conchospore-bearing branches were observed in filaments which were kept for several months at higher temperatures and increased day length. It seems likely that the monospores and conchospores are different structures; and whilst the conchospores can give rise to young plants similar to those of the leafy thalli, under certain environmental conditions they produce again the *Conchocelis*-phase, in common with the liberated monospores. Dwarf plants have also been observed which are intolerant of raised temperatures, increased day lengths and higher light intensities. The northern plants of *Porphyra umbilicalis* show adaptations to a wide range of environmental conditions. The α-spores are produced asexually. The β-spores appear functionless, and in the absence of any adequate cytological data the occurrence of a gametic fusion must be a matter of conjecture.

2. *Techniques used in the large-scale cultivation of Porphyra*

In the more traditional methods bundles of twigs were planted in suitable inshore areas to serve as substrata for spore settlement. In addition the cultivation of plants in intertidal habitats was practised, notably by the " weeding-out " of competitors. Modern techniques include much which was of older foundation, and in intertidal areas new substrata for spore settlements are obtained by concreting the rock face, placing boulders or concrete structures in the sea, and by blowing-up rocks in suitable habitats (Kurogi, 1963a). Methods of inshore cultivation still include the use of " hibi " of bamboo brush, and of " blinds " of split bamboo bound together. Alternatively nets of mesh size 15 cm × 15 cm made from synthetic plastic fibre, or from coconut palm or hemp palm are used. The " hibi " are spread in the sea at suitable times of the year (see p. 167 *et seq.*). The " hibi " are usually spread at

about mean sea level, so that there will be daily periods of exposure to air. This exposure time will vary from month to month, (e.g. daytime exposure is longer from March to April than from October to December, whereas nightime exposure is long from November to March and short during May and June). At the time of year when " hibi " are spread (September) they are exposed to the air for daily periods of 4–4½ h in the day and 6 h at night; during the main harvesting period (December–January) the daytime exposure is 2 h and the nightly period 8½ h. At the end of the season of cultivation the " hibi " are exposed for 8½ h by day and for 2 h at night (Kurogi, 1961). Studies on the metabolism of *Porphyra tenera* when immersed in water of different salinities, at different temperatures and after drying have been reported (Ogata, 1963; Ogata and Matsui, 1963). Changes in respiration rate were observed with increased temperatures, and lowering of the salinity resulted in an increase in the rate. Measurable respiration was observed with plants left uncovered by the sea.

3. *Cultivated Porphyra species in Japan and China*

Kurogi (1961) described five species of *Porphyra* which are cultivated in Japan, viz.:

Porphyra tenera, P. kuniedai, P. angusta, P. yezoensis, and *P. pseudolinearis.*

Numerous studies have been carried out on the *Conchocelis*-phases of these five species, and this work will be considered separately (see p. 177 *et seq.*).

Porphyra tenera. The type material of this species was described by Kjellman in 1897 from samples of the dried laver " sheets " used as food (Segi, 1959). Two types of frond were described, those of a linear form and those which were rounded. A type specimen based on a commercial product is more than usually confusing, and no doubt accounts for the variability of form attributed to the species by several authors. *Porphyra tenera* appears to be the most commonly cultivated laver in Japan and China. Detailed accounts of this species and its cultivation in Japan have been given by Kurogi (1961, 1963a, b); it is a plant characteristic of the " inner " seas, (e.g. those almost surrounded by land), and shows a relatively short annual growth period.

Frond shape varies from linear to oval, with a tendency to become much broader with age, and to widen appreciably when grown in shallow seas rich in nutrients. The apex of the frond is generally obtuse and the base cuneate, rounded, and sometimes cordate. Plants which appear in the early part of the growth period are noticeably broader than those which develop later. Frond lengths range up to 25 cm, and

plants 1 m in length have been recorded from the calm waters of the " inner " seas. The fronds tend to grow paler with increasing age except in regions which are particularly rich in nutrients. The plants are monoecious, but those bearing only male organs are relatively common. The male organs are borne in pale-coloured areas of the fronds, situated either at the margins or scattered between the darker areas of the frond which bear the " carpogonia ". The spermatangia bear 32–64 (sometimes 128) spermatia, and 4–8 " carpospores " are produced per " carpogonium ". Examples in which the spermatangia and " carpogonia " are restricted to different areas of the frond have been described. Where both organs are borne on the same frond the spermatangia mature and release their contents before the " carpogonia " are fully developed. Whilst the "carpogonia" are identical with vegetative cells in surface view they appear spindular in cross section, and Kurogi (1961) stated that a thread-like connection was to be seen between a " carpogonium " and an attached spermatium. As the growth season terminates so a number of the " carpogonia " apparently abort, but in inshore waters rich in nutrients cells are found in the " carpogonial " region of the frond which produce spores, and these in turn give rise to leafy thalli on germination.

In Matsushima Bay the best settlements are obtained when " hibi " are spread from mid-September to early November. The young " buds " which develop are at first filamentous (up to 250 μ in length) thence broadening by longitudinal cell division. " Hibi " spread after mid-November fail to show young plants. The young " buds " on the " hibi " liberate monospores (" neutral spores ") at an early stage in development, and these monospores also settle on the " hibi " and their growth and development leads to dense settlements of young plants (equal to 2 000–3 000 plants per 10 cm of bamboo thread). Production of monospores continues into mid-November, and the broader form of thallus observed with plants growing in inshore waters is a result of the protracted spore production which truncates the thallus apex. Small plants 1 cm in length appear after 1 month, and after 2 months lengths of 25 cm are recorded with frond width 7·5–10 cm. The plants start to reach a harvestable size in late November and early December, and a dense growth of plants on the " hibi " is then seen until March. A rapid decline in the population is seen during April and May.

" Carpogonia " and spermatangia appear on the thalli 3–5 cm in length early in November, and production of " carpospores " continues until May. The " carpospores " produce the shell-boring *Conchocelis*-phase on germination and the filaments of this stage in the life-history

continue to grow throughout the summer and it liberates its monospores (or "conchospores") from mid-September to early November,—the time of spreading the "hibi". The copious release of spores in the sea was known long before the association of the *Conchocelis*- phase with the *Porphyra* plants had been demonstrated. Various interpretations as to the origins of these spores have been given. Thus Kusakabe (1929) suggested that *Porphyra* plants from the previous spring sowing of "carpospores" were the sources, and Ueda (1929a) stated that this autumnal "flush" of spores came from *Porphyra* plants which were able to survive through the summer months.

Sea temperatures fall from 25°C to 15°C during the early part of the growth season in the Matsushima district of Japan. It is in this same temperature range that both maturation of spores of the *Conchocelis*-phase and their liberation are observed (Kurogi and Hirano, 1956a, b). Whilst dense spore settlements are obtained when "hibi" are spread from mid-September to early October in North Japan, in South Japan the time of spore settlement is mainly observed in October (Kurogi, 1961). Even during this time monospore production and settlement have been observed to show periodicity. "Hibi" spread on the 2nd, 3rd and 4th days following the 1st or 15th days of a lunar month apparently show the most dense spore settlements. Dense spore settlements are also obtained after heavy seas, but a marked fall in the spore population is observed after heavy rain.

The period of growth of *Porphyra tenera* in Japan is a relatively short season which coincides with reduction in day length and a fall in sea temperature. Since the *Conchocelis*-phase grows throughout the summer months it is evident that day length and temperature must influence this phase in the life history. Iwasaki (1956) has given experimental verification of this. Young germlings (0·5 cm in length) obtained from monospores of the *Conchocelis*-phase were grown at controlled temperature (14°–16°C) in a light regime of 13 h daily illumination, using an incandescent light source (400–500 ft candles). Control plants were illuminated for 8 h daily. Thalli of the narrow-fronded *Porphyra* developed in the control material whereas the long-day samples produced plants of irregular shape which became pale in colour; after 40 days these plants produced spores, (considered to be functionally the same as "carpospores"), which on germination again formed the *Conchocelis*-phase. The 13 h daily illumination corresponds with that obtained in late April in localities where the plant is cultivated in Japan; at this time also degeneration of the fronds proceeds at a rapid pace. By contrast the *Conchocelis*-phase grows best under long-day conditions.

A somewhat different interpretation of the life-history has been given by Tseng and Chang (1955a, b) for *P. tenera* cultivated in China. It is also claimed that a sexual fusion does occur in the life cycle. The cell constituting the " carpogonium " was said to send out a filamentous projection, subsequently withdrawn, to which the spermatia adhere. Passage of the contents of the spermatium into the " carpogonium " was described but actual fusion of the cell nuclei was not observed. Tseng and Chang stated that meiosis occurred in the carposporangium, so that haploid " carpospores " would then be produced. Two peaks of " carpospore " production were described, the first starting in late November and continuing through December, and the second extending through April and May, corresponding to the initial and final stages of growth of the leafy thalli in the culture " grounds ". The leafy thallus reproduces asexually by means of monospores when sea temperatures lie between 15°–17°C; the production of sexual cells is observed when sea temperatures remain below 15°C. Apparently both monosporangia and " carposporangia " have been found on occasions on the same thallus due to a particular combination of environmental factors. The *Conchocelis*-phase grows throughout the summer, but is reported to commence spore liberation in early summer (sea temperatures 15°–20°C). The *Porphyra* plants formed as a result of the germination of the early summer spores are of a dwarf form, whereas the monospores produced by the *Conchocelis*-phase during autumn grow into the normal leafy thalli. The summer dwarf plants have smaller cells and produce monospores which throughout the summer months give rise to dwarf plants. Whilst dwarf plants are produced through the early part of the autumn, they later grow into normal thalli. These " summer dwarfs " do not seem to have been described in Japan, and Tseng and Chang (1955a) point out that these dwarf plants are not an obligatory phase in the life-history. The plants from both regions appear to grow best during the autumn when there is a fall in sea temperature, and when there is the shortest growth period (1 month) in order to obtain a plant of harvestable size.

Various factors known to influence the early development of *Porphyra* have also been investigated, (Hasegawa *et al.*, 1952; Iwasaki and Matsudaira, 1958; Matsumoto, 1959; Iwasaki, 1961). Healthy growth of the thalli was obtained in media enriched with nitrate and phosphate, trace elements, and with organic nutrients such as Vitamin B_{12}, thiamine and biotin. Young " buds " appeared to require iron enrichment of the medium. Incandescent light allowed growth under culture conditions, although natural sunlight was preferable; fluorescent light proved harmful to the young plants. A light regime of 8–10 h

daily illumination was the most satisfactory; continuous illumination inhibited growth. Frond growth was also affected by water movement, as well as temperature. Spore attachment was most effectively obtained with a current velocity of 7 cm/sec, whilst growth of the frond was equally satisfactory over a current velocity range of 7–25 cm/sec. Variations in water temperature, degree of illumination and water movement were all found to affect the colour, toughness and taste of the laver frond. Plants grown in Tokyo Bay are predominantly of the long-fronded type (Suto and Fujiyama, 1952).

Porphyra angusta. This species resembles *P. tenera* in having a relatively short growth season in the year, but is a species more characteristic of the " outer " seas in Japan. The fronds are elongate and elliptical, tapering towards the base or at both ends. Their length may reach 45 cm with a width of 8 cm; the margin is undulate. According to Kurogi (1961) the younger fronds are narrow and they broaden with age. The plants are dioecious, with female plants reddish-brown in colour and rounded at the apex. Male plants taper towards the base and apex, and are pale yellow-brown in colour. Both spermatangia and " carpogonia " are borne on the upper marginal part of the frond. Monoecious plants (male or female) are found on occasions. Sexual organs first appear on plants 4–5 cm in length. Whilst the carpogonia are indistinguishable from vegetative cells in surface view, in transverse section they appear spindular. A *Conchocelis*-phase is obtained on germination of the " carpospores ".

The young " buds " which first appear on the " hibi " attain a filament length of 10–20 cells before commencing longitudinal division with a subsequent broadening of the frond. The early production of monospores by the young buds is observed after 10 days growth, and this leads to some truncation of the apex of the juvenile plant. Spreading of the " hibi " between late September and early October results in heavy spore settlements. Young plants reach a length of 1 cm after 4 weeks' growth, and 2–4 cm after 3 months. Monospore production and spore settlement from the young buds is observed from September until the end of November, but only with young plants under 1 mm in length. Once this length is exceeded monospore production ceases. Formation of spermatangia and " carpogonia " commences in late October and they continue to be formed until the end of the growth season. The *Conchocelis*-phase obtained by germination of the " carpospores " grows through the summer, and liberation of the monospores from this phase commences early in the autumn. The time of harvesting the plants is much the same as with *P. tenera.*

Porphyra yezoensis. Frond shape varies from elliptical to oval or rounded, with sometimes a cuneate, cordate or even umbilicate basal region; the margin is undulate and sometimes deeply divided. The basal part of the frond is blue-green, and the upper reddish-brown. Kurogi (1961) has outlined in some detail the seasonal life of this representative. The young fronds are often elongated and elliptical, tapering at both ends, and becoming more rounded in outline with increasing age. The fully grown plant can reach a length of 30–50 cm, and in general only monoecious plants are found. Whilst this is typically a plant of the " outer " seas (Kurogi, 1963a) and so found in similar habitats to those of *P. angusta,* the growth period of *P. yezoensis* is more protracted.

Very young plants appear on " hibi " which are spread in late September. Where there is an abundance of *P. tenera* the two plants appear together on " hibi " spread before the end of October, but after this the young buds of *P. yezoensis* appear exclusively, thus indicating that the period of monospore production by the *Conchocelis*-phase of *P. yezoensis* is more protracted; in fact this extends into December. Asexual reproduction by the young buds appears to be delayed somewhat until after the development of a multiseriate thallus 1–7 cm in length. Once this process has started, however, it seems to continue for the greater part of the growing season. With this plant asexual reproduction is the only form of multiplication seen in early autumn, whilst later in autumn and during the winter monosporangia, " carpogonia " and spermatangia are observed. Some plants show only asexual organs, and others only " carpogonia " and spermatangia. During spring and summer only the " sexual " organs are borne on the newly developed plants. There is thus some degree of overlapping in the types of spore produced during autumn, winter and spring, but asexual reproduction is not observed during the summer months. Plants persist on the " hibi " until July, so differing markedly from the two species already described.

For this species, as with the others so far described, a sexual fusion is considered to occur in the life-history. Organs associated with this process first appear in October, frequently on thalli which are still producing monospores. Areas of the thallus which bear spermatangia are pale-coloured, and this colour change is first observed in the apical region, subsequently spreading to the margins. " Carpogonia " are formed in darker coloured parts of the frond below that bearing the spermatangia, and as the spermatia are released and the " male area " of the frond sloughs away so the " carpogonial " areas come in turn to what is now the upper margin of the frond. Thence vertical

"stripes" of pale coloured cells come to lie between the darker areas, so producing the effect which is considered a specific character, (resembling also *Porphyra leucosticta*). During autumn and winter the following sequence is frequently observed: the plants commence the reproductive phase bearing only monosporangia, then monosporangia and spermatangia, then monosporangia, spermatangia and "carpogonia", and finally only sexual organs. Various transitional stages are to be observed between these main phases. "Carpospores" produce the *Conchocelis*-phase on germination, but towards the end of the growth season certain "abortive" cells are observed amongst the "carpogonia", and these cells are also seen to produce spores. With such a protracted period of asexual reproduction, very young plants are found on the "hibi" throughout the greater part of the autumn, winter and spring. Liberation of spores from the *Conchocelis*-phase apparently continues for as long as the sea temperature remains above 10°C. In Matsushima Bay it was observed that the times of the phasing of the reproductive stages depended on the position of the "hibi", (e.g. whether they were situated near the open sea, or close inshore).

The similarity between this species and *Porphyra leucosticta* was emphasized by Kurogi, who, nevertheless, left the problem largely unresolved; several forms previously attributed to *Porphyra tenera* by workers in the past should in fact be incorporated in *P. yezoensis*. In natural habitats the plants of this species are usually found as epiphytes, showing some degree of seasonal migration over the course of the year. In these shore habitats the plant appears in greater numbers between March and May, but in the cultivated areas they appear on the "hibi" from December onwards. The period for harvesting can extend from December until May.

Porphyra kuniedai. The cultivated laver described as *P. kuniedai* by Kurogi (1961) has previously been described as forms of *P. tenera* and *P. yezoensis;* Kurogi has emphasized that there is a close similarity between this species and *P. leucosticta* f. *mediterranea* described by Hamel (1924). This species shows a longer growth season than any of the lavers so far described, and may persist for the greater part of the year. In common with *P. tenera* it is a component of the "inner" seas of Japan (Kurogi, 1963a).

The plants are rounded or reniform in shape, with a rounded, cordate or umbilicate basal region; the margins may be lobed and undulate and the frond deeply cleft. Frond length can reach up to 10 cm, and the plants are reddish-brown in colour. The plants are monoecious, and the male areas on the frond show a similar sequence of

development to those of *P. yezoensis*. It is also possible to find admixture of monosporangia and sexual organs on the same frond with this species, but not so frequently as with *P. yezoensis*.

During the initial stages of frond growth monosporangia are found on plants between 0·1–5·6 cm in length, and the monospores produced germinate to form new thalli. The main season of monospore production appears to last from September to November, and whilst a few plants bearing monosporangia are to be found throughout the winter and into the spring, the " carpospores " are the main reproductive cells through the winter months. Plants bearing monosporangia, spermatangia and " carpogonia " are found on the " hibi " during November. Thus there is an upsurge in the number of plants bearing the sexual organs after the early production of monospores. An unusual feature, however, is that during the following spring and summer there is a second " wave " of monospore production at a time when " carpospore " formation has ceased. This may be due to the persistence of the monosporangium-bearing plants throughout the winter (possibly in small numbers), but may also be a result of the somewhat earlier maturation of the sporangia in the *Conchocelis*-phase. There is, in fact, some evidence that the shell-boring phase commences to liberate spores in June, to be followed by the prodigious output of the autumn months. Whilst the laver fronds of this species may be found on the " hibi " during early summer, they usually disappear in July, and very few are found after this. Some variation in the seasonal life is observed with habitat. The plant is harvested from December until May.

Porphyra pseudolinearis. This species has the shortest growth period on the " hibi " and in consequence there is a much shorter harvesting time. The young plants appear on the " hibi " in mid-September, but monospore production is not observed, and spermatangia and " carpogonia " are first seen in early October. Release of carpospores is observed from October until mid-March. The " carpospores " produce the *Conchocelis*-phase when germinated on suitable substrata, and the filaments grow throughout the summer liberating monospores in September and October. With this species harvesting is limited to the months of November and December.

The seasonal life of the cultivated *Porphyra* species described above is also reflected in the changes in population which are observed when " hibi " are spread at different times of the year, particularly in areas where there is some abundance of the different species. Thus, on nets spread in the first half of September the dominant plants will consist of *P. linearis* and *P. kunieda*, and the *P. tenera* and *P. yezoensis* will

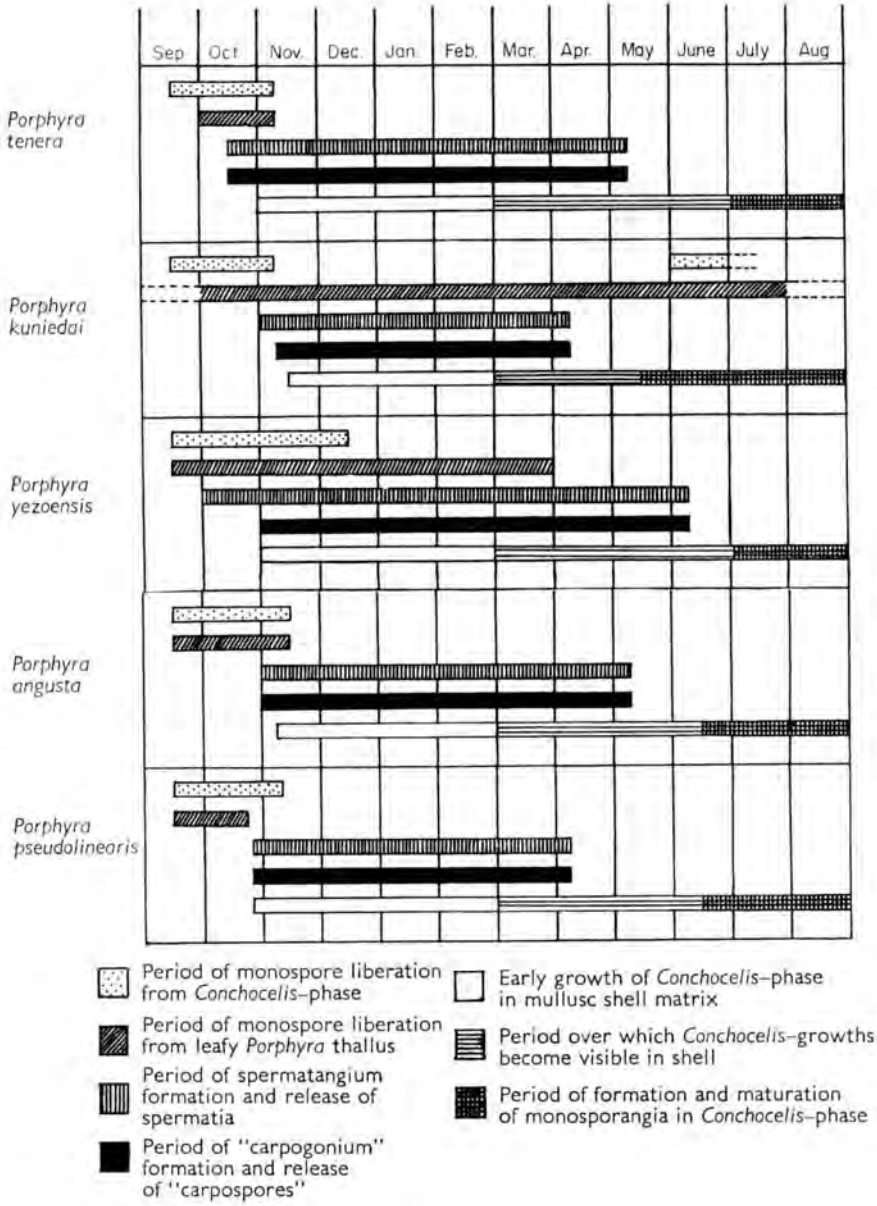

Fig. 9. Summary of seasonal fruiting behaviour of *Porphyra* species cultivated in Japan (drawn from data in Kurogi, 1961). For details of the " peaks " and " troughs " of activity in each case refer to text, or to diagrammatic summaries in Kurogi's paper.

occur to a lesser extent. On " hibi " spread during late September and early October, *P. tenera* and *P. angusta* are the dominant species, with some *P. kuniedai*, *P. pseudolinearis* and *P. yezoensis*. " Hibi " spread from mid-October to early November become dominated by *P. kuniedai* and *P. yezoensis*, with some *P. tenera* mixed. *P. yezoensis* tends to dominate on " hibi " spread from November onwards, mixed with some *P. kuniedai*, whereas *P. kuniedai* only is found on nets placed in the sea during spring and summer. Whilst certain of the species have a somewhat restricted distribution in the bays where seaweed cultivation is practised, successful transplants have been made.

Some experiments carried out by Suto (1963) are relevant to the problems of the *Porphyra* life-history outlined above, and of importance to practical seaweed cultivation. In these experiments some interspecific crossings were attempted with cultivated lavers in order to obtain new lavers suitable for commercial purposes. Five species were involved, the dioecious *P. pseudolinearis*, *P. umbilicalis* and *P. angusta*, and the monoecious *P. tenera* and *P. yezoensis*. The formation of the *Conchocelis*-phase was taken as evidence that fertilization had been achieved (see p. 164); according to Suto the contents of an unfertilized " carpogonium " either produced spores which again produced leafy thalli on germination, or the cell contents degenerated. To fully evaluate the effects of an attempted " cross " between two species, the full cycle of the life-history was observed. It was claimed that successful crossings were obtained between monoecious species and between dioecious representatives, but that high mortality rates were obtained with attempted cross-breeding between monoecious and dioecious plants. Experiments of this nature involving non-motile reproductive cells are very difficult to perform, although Sundene (1959) has successfully cross-fertilized different " strains " of *Antithamnion plumula*. However, full evaluation of these experiments must await demonstration of the cytological changes involved in a fusion of gametes with *Porphyra*.

4. *Species of Porphyra utilized in the British Isles*

Three species of *Porphyra* are utilized in the British Isles:

Porphyra umbilicalis, *P. purpurea* and *P. leucosticta*.

The *Conchocelis*-phase is produced by all three species, although that of *P. umbilicalis* is less readily found. The features of the *P. purpurea* life-history which require more adequate verification have been indicated (Conway, 1964a, b, c).

Whilst in the British Isles the *Porphyra* species which are utilized

have mainly a restricted area of sale in Wales and south-west England, the plants are harvested on the coast of Wales, and from the shores of Scotland, Ireland, Cornwall and north England. More than 50% of the *Porphyra* sold in 1954 came from shores in Scotland (Hampson, 1957). Of the three species, *P. umbilicalis* in particular is found throughout the year on shores exposed to severe wave action in Scotland, whereas *P. purpurea* is found from early spring onwards and tends to disappear during the storms of late winter (Conway, 1964a). Whilst the plants can be collected all the year round, there is a fall in demand during the summer months. The time of maximum sale of the " laverbread " is in fact the period of much slower growth by the plants, and when a reduction of the standing crops results from the action of gales and heavy seas. The *Porphyra* harvesters work a type of rotation on the shores or on regions of the shore, and the best plants are found hanging from the underside of boulders with the free ends immersed in water. Prolific growths are observed where fresh water runs into the sea and lowers the salinity of the sea water, but this may also be due to increased nutrients brought down by land drainage.

5. *Species of Porphyra utilized in the United States*

The principal species utilized as food on the Pacific coast of the United States is *Porphyra perforata.* Here the lanceolate fronds can attain a length of 5 ft, but are usually harvested when 2 to 3 ft long (Tseng, 1947). On shores where the laver grows the rocks are burned with driftwood in the fall, so clearing the substratum of other plants and leading to an enhanced growth of the *Porphyra* plants the following spring. When Hollenberg (1958) germinated the " carpospores " of this species on glass slides he obtained filamentous plants which might well be the form of plant occurring in nature, since the shell-boring phase of this species does not occur on the California coast of the United States. In experiments carried out with the filaments under conditions of culture these filaments developed sporangia, and the spores produced young *Porphyra* plants on germination. Whilst the shell-boring phase is not known on the coast of California, it has been reported from the Washington coast (Jao, 1937).

Porphyra species utilized in other parts of the world include *P. suborbiculata* which is collected from the shores of Hong Kong during December, January and February, (Chiu, 1958), and in the Philippines a *Porphyra* species was under test as a source of feeding stuff (Sulit *et al.*, 1953).

6. *Studies on the growth, viability and spore output of the Conchocelis-phases of Porphyra*

The initial penetration and growth of the *Conchocelis*-phase of *Porphyra purpurea* was described by Drew (1954a) for various calcareous substrata, including shell fragments and egg shells. Yamasaki (1954b) observed that once the carpospore was attached to the shell surface penetration into the calcareous substance took place after 2–3 days, the spore membrane remaining on the shell surface. Saito (1956a) noted that a lowering of salinity tended to inhibit carpospore germination, but subsequent growth of the filaments was less affected. After this initial penetration " protonema "-like branches develop. Ogata (1959a, 1960a, 1961) studied in detail the early growth of *Conchocelis* filaments of *P. tenera* in the shell matrix. The early " protonema "-like branches were observed to form " tap-root "-like filaments which then grew vertically down into the calcareous matrix. These deep-penetrating filaments then produced laterals which were seen to grow upwards at a slight angle until they come to lie just below the shell surface, then branching extensively and forming a dense network of filaments. At first the growth of the main filament is a slow process with shell penetration at the rate of 350μ in the first 30 days. The time taken for the filamentous mass to develop immediately below the shell surface has some practical significance, since it is an obvious sign of the degree of development of this phase for material growing in the culture tanks (p. 182).

This development of the filamentous mass just below the shell surface precedes the formation of sporangia. There is some evidence that the pattern of the filamentous growth varies with the type of shell material. Thus when grown in crystals of calcite the filaments tended to grow along the cleavage patterns of the crystals, but the growth forms of the filaments in calcite, aragonite and limestone were much the same as those found in mollusc shells. The filaments have also been successfully grown in cement mortar (Ogata, 1961), and any calcareous substratum appears to be suitable, e.g. barnacle shells, coralline red algae, and calcareous worm tubes (Drew, 1954a). These observations indicate that a range of artificial calcareous substrata might be used for large-scale culture processes. Observations by Ogata (1960b) indicate that gravity has very little influence on the early directional growth of the filaments. Experience has shown that the shells of a particular appearance (e.g. those with " rough-touch ") are those in which a particularly successful establishment of the *Conchocelis*-phase is obtained (Ogata, 1959b). These shells are characterized by numerous small papillate projections on the surface which presumably favour initial spore

attachment. Taneka (1957) noted that a denser growth of *Conchocelis*-filaments occurred on the hinge side of the oyster shell.

Information is also available on the conditions which influence growth and development of the filaments within the shell matrix. Despite the earlier statement that growth of the filaments appeared not to be affected by lowering of salinity, a number of workers have produced evidence that changes in salinity can influence both elongation and branching of the filaments (Saito, 1956b; Yamasaki *et al.*, 1957; Ogata, 1960b, 1961). Thus the growth rate of the filaments was halved when the salinity was reduced by the same amount; whilst the " tap-root " filaments were less affected, their growth was inhibited by any further fall in salinity. Tetrazolium salts have been used as indicators of the viability of filaments in the shells under test conditions (Ogata, 1956a,b).

Direct exposure to sunlight and severe desiccation rapidly killed the *Conchocelis* filaments within the shells (Saito, 1956), and growth was totally inhibited when shells were buried in the sand. Takeuchi *et al.* (1956b) found that, together with exposure to direct insolation, subjecting the shells to air of low humidity and to high temperatures in shallow water also rapidly killed the filaments. Observations on the pigmentation and metabolism of the *Conchocelis* filaments under different environmental conditions have also been made (Sano, 1960). Ogata (1960b) found that the compensation point for the filaments lay between light intensities of 5–50 lux. An earlier observation by Ogata (1960a) indicated that vertical growth of the filaments ceased at 5 lux, and was most rapid at 2000 lux. The general features of the metabolism of the *Conchocelis* filaments were identical with those of *Porphyra*. When the filaments were grown on glass slides development was enhanced by the addition of HCO_3^- to the culture medium, whereas addition of calcium had little effect; variation in pH was ineffectual. Little significant effect on growth of the filaments within the shell was observed after addition of bicarbonate, phosphate, nitrate or ammonium salts to the culture medium (Ogata, 1961). The pH of the cell contents was found to lie between values of 5 and 6, and passage of the filaments through the calcareous matrices of the shell could be brought about by the secretion of extracellular products of this pH. When carpospores are germinated on the shell in a medium rich in bicarbonate the developing filaments tend to grow on the shell surface, indicating that availability of CO_2 could be a factor conditioning growth within the shell. An interesting observation by Ogata (1961) showed that filaments continued to grow within the matrix even when the shells were immersed in liquid paraffin.

Maturation of the *Conchocelis* filaments can be seen both by the growth of the cells and the formation of sporangia and spores. Sporangium formation is slowed down in water of lowered salinity. The output of monospores was also appreciably diminished (Yamasaki *et al.*, 1957). The effects of light on growth and maturation of the *Conchocelis*-phase have also been studied (Kurogi, 1959; Kurogi and Sato, 1962a,b; Kurogi *et al.*, 1962; Iwasaki, 1961; Iwasaki and Matsudaira, 1963).

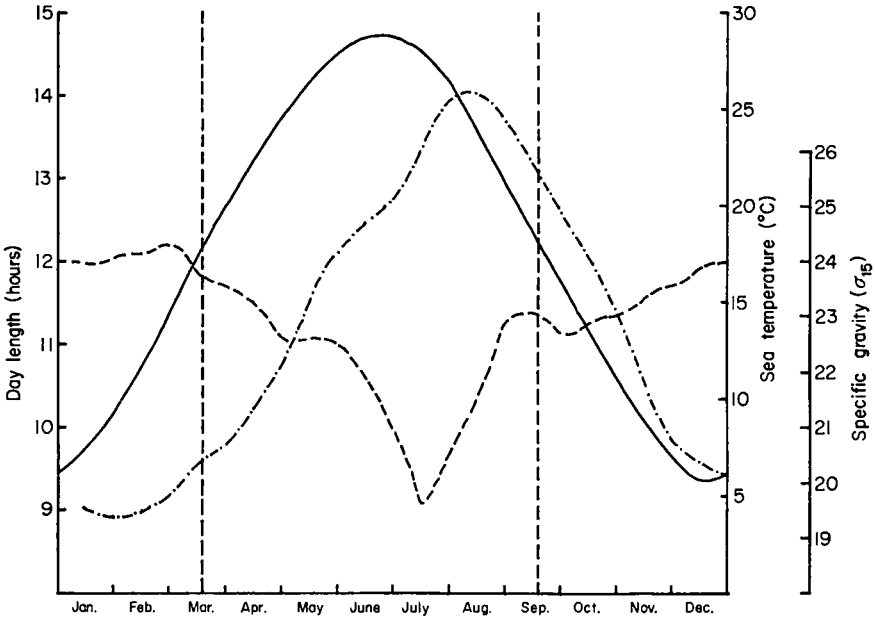

FIG. 10. Summary of general environmental conditions over the year at Matsushima Bay, Japan. Drawn from data in Kurogi (1960), and Iwasaki (1961).

——— Day length; –·–·–·– Sea temperature; - - - - - - Specific gravity of sea water.

The vertical broken lines indicate the period of growth and maturation of the *Conchocelis*-phase (mid-March to mid-September).

The observation by Drew (1956) that *Conchocelis* is usually found in quantity by dredging at 32 m depth is borne out by the low value of the compensation point mentioned above. Recent work has been concerned with photoperiodism and its effects on the shell-boring phase, and this work has a particular significance for the studies on seasonal behaviour of the cultivated species described earlier. Experiments in which studies were made on the filaments growing within the shell showed that dark periods of 10–12 h in each day resulted in dense filaments with

dense pigmentation. Long periods in darkness (20–23 h) resulted in a more stunted growth and greenish colour of the filaments. Actual growth was best observed under long-day conditions, whilst the rates of growth varied with the different light intensities used. Formation of monosporangia commenced only when the filaments were kept under short-day conditions, and in cultures kept in the dark for 14–23 h/day monosporangia were obtained in large numbers at all levels of filaments growth within the shell. Shortening of the dark period at this time, (e.g. to 9–12 h) resulted in monosporangia being formed only on the innermost branches, but any further reduction in the dark period markedly reduced sporangium formation. The general results quoted above appear to be true of the five species of *Porphyra* cultivated in Japan. These experimental results show some measure of agreement with what is known about the seasonal life of the cultivated lavers in Japan, since the main period of growth of the *Conchocelis*-phase is in the summer, but that formation of sporangia and release of spores is mainly observed under short-day conditions (Fig. 10). For example, Taneka (1959) studied the condition of the *Conchocelis* filaments in mollusc shells kept on the seabed over the course of a year and verified much of the experimental data summarized above; growth of the filaments was also observed, however, between November and March, indicating that some development of the filaments follows the earliest settlements of " carpospores " on shell surface. Iwasaki (1961) and Iwasaki and Matsudaira (1963) studied the growth of the free-living filaments of *Porphyra tenera* obtained by germination of the " carpospores ". Their results are a general confirmation of those already quoted above, with growth of the filaments under long-day conditions; and growth of the leafy *Porphyra* thallus and formation of the monosporangia on the filaments under short-days. Release of spores from the monosporangia appears to be affected by photoperiodism (p. 168). When the free-living *Conchocelis* filaments were kept under a long-day regime for a protracted period under controlled temperature conditions three types of plant " body " were obtained, (a) inflated spherical cells, (b) " strawberry "-like structures, and (c) " special " sporangia. The first two structures were obtained at temperatures greater than 18°C. The inflated spherical structures produced single spores which formed *Conchocelis* filaments on germination, and the spore-like bodies produced by the " strawberry " structures also produced similar filaments. The " special " sporangia showed a different type of behaviour. Two types of plantlet were obtained, depending on whether the spores were grown under long- or short-day conditions. It was also observed that fragments of the *Conchocelis* filaments could reproduce by vegetative

means. These observations are relevant to some earlier work by Drew (1954a,b, 1958) who demonstrated that shells containing the *Conchocelis* filaments could " infect " other (sterilized) shells introduced into the medium without physical contact between the two. It seems likely that the cultural conditions used by Drew favoured the growth of structures similar to those described by Iwasaki and Matsudaira. Iwasaki and Matsudaira have also drawn attention to the marked plasticity of the stages of *Porphyra tenera* under a wide range of environmental conditions (see also Conway, 1964a,b,c, and p. 165). Many of the conflicting data obtained by the study of *Porphyra* under culture conditions is probably due to similar properties exhibited by other species.

The importance of sea temperature and the onset or waning of these different phases in the life-history has already been described. Studies have also been made on the temperature conditions governing formation of the sporangia on the *Conchocelis* filaments and release of the spores (Kurogi, 1961 ; Kurogi and Hirano, 1956b ; Tseng and Chang, 1955b, 1956). Monosporangium formation by *P. tenera* was observed in a temperature range of 15°–27°C, whilst for *P. kuniedai* the temperature limits were 15°–25°C. Tseng and Chang (1955b, 1956), stated that whilst sporangium formation was possible in a temperature range of 15°–30°C, a period of lower temperatures (15°–20°C) was necessary before spore formation was observed in the sporangium.

Spore release by the Conchocelis-phase. The diurnal periodicity in spore release by the shell-boring phase of *P. tenera* has been investigated by Suto *et al.* (1954), Yamasaki (1954b), and Tseng and Chang (1956). In general, spore discharge commences in the morning in the first few hours after sunrise and quickly reaches its peak (7–10 a.m. according to Suto ; 8–10 a.m. according to Tseng and Chang). Over 50% of the total spores liberated during the day are released during the first few hours ; in the later part of the day this productivity falls to 5 to 9% of the overall daily spore output. Yamasaki stated that whilst the shedding of spores commences at sunrise and continues apace for the next 2 or 3 h on sunny days, on days with heavy cloud the copious output is delayed for 2–3 h. Light reactions are evidently a necessary preliminary to spore release from the sporangia. However Saito (1956a) considered that increased sea temperature enhanced the rate of spore emission along with changes in the level of illumination.

The temperature conditions governing spore emission have also been studied. Release of spores, which is preceded by dissolution of the sporangium wall, is most abundant at temperatures between 12°–20°C, but is markedly reduced at temperatures of 9°C and 24°C (Fig. 11)

(Suto *et al.*, 1954). A total inhibition of spore release is obtained at 27°C. Tseng and Chang (1956) also found that spore emission was inhibited at temperatures between 26°–30°C, but was able to proceed at 25°C. Lowering of salinity was also observed to inhibit the rate of spore release (Yamasaki *et al.*, 1957).

Evidence that a periodicity of spore release accompanies tidal activity has also been obtained, and this is particularly relevant to the time of spreading the " hibi ". Takeuchi *et al.* (1956a) observed that with daily measurements of spore release from shells kept in the sea, the monospores were shed on every day between September and December, but that during the period of the spring tides between 200–1000 spores were obtained per cm^2 of shell,

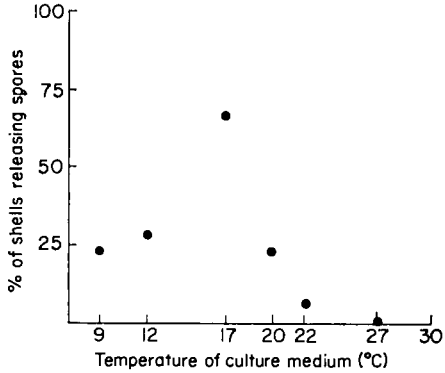

FIG. 11. The effects of temperature on the release of spores by the *Conchocelis*-phase of *Porphyra tenera*. Drawn from data in Suto *et al.* (1954).

whereas on other days the overall productivity was 10 spores per cm^2. Saito (1955), reporting on observations extending over 4 years, found that from direct counts of the spores in the sea considerable increase in spore numbers followed severe vertical disturbances of the water. Arasaki *et al.* (1956) made similar observations to those of Saito, and also found that increases in spore numbers in the sea were paralleled by a similar productivity from shells kept in culture tanks on land.

It is evident that whilst there are numerous aspects of the life-history of the cultivated *Porphyra* species which remain to be fully elucidated, a great deal of information has already been obtained which is of value in the cultivation of this crop plant. Kurogi (1963a) has described how, after the " seeding " of oyster shells with " carpospores " from fruiting thalli of *Porphyra tenera*, the shells are then suspended in concrete " culture " tanks on land which are filled with sea water.

Subsequently, when spore release is imminent from the " cultured " filaments, two methods of " seeding " the " hibi " are practised. In the first of these the nets are suspended in the culture tanks and the sea water then agitated. In the second the shells are suspended in vinyl bags below the " hibi " in the culture grounds, or are fixed in split bamboo rods. The information recorded above on the times of maximum spore release are also of value in determining the best times for spreading the " hibi " during the day. The methods of land-based culture can be successfully carried out on a relatively small scale by individual fishermen, and will undoubtedly lead to a more efficient industry than one solely dependent on spore release in nature. Taneka (1959), for example, suspended 150 " infected " oyster shells in a linear series on steel wire and then suspended a number of these wires in the sea. A copious spore release was obtained from the suspended shells in early autumn. Useful information has also come from a study of the polarity of spores released from the *Conchocelis*-phase (Nakazawa, 1958). A predetermined polarity was demonstrated by means of vital stains, and the " reductive " region of the spore was found to be the one which formed the attaching surface. This implies that secretion of an adhesive substance is only found on one part of the spore surface. It was also reported that some amoeboid movement was exhibited by the newly settled spore. This type of information could be relevant to the type of artificial substratum chosen as the " hibi ".

7. *Gelidium*

Gelidium and the related genus *Pterocladia* are the two genera principally used as sources of agar. Despite the common occurrence of species of *Gelidium* in many parts of the world, and the fact that several species are utilized as sources of agar, comparatively little is known about many aspects of its biology. The polymorphic nature of the genus, showing not only variations in growth form in different habitats, but also in the same habitat at different seasons, is probably one reason for the lack of critical data about several of the species. Stoloff (1962) lists eighteen species of *Gelidium* which can be used as sources of agar, but few of these appear to be exploited on a large scale. The species principally used are *Gelidium amansii* and *G. cartilagineum;* and used to a much smaller extent *G. arborescens* and *G. nudifrons.*

Other species which could be used as sources of agar, and which appear to grow in sufficient quantity, are listed in the survey of potential resources (Table VIII, p. 126).

The most detailed study of the seasonal life of the species of

Gelidium is that by Dixon (1958a,b) for *Gelidium pulchellum* and *G. latifolium*, both species being commonly found in the British Isles. They are relatively small plants (erect branches 2–10 cm in length), and are not used extensively as agarophytes, but in many ways their biology is relevant to the larger species which are regularly harvested as sources of agar.

Growth of the fronds is effected by a single apical cell which is lenticular in shape, and this cuts off a segment cell on its inner face. This then gives rise to a cell which produces in turn axial cells and the pericentral cells, and these give rise to lateral branches of limited growth which clothe the main axis and give the appearance of a cortex and medulla in the maturer parts of the frond. Due to the elongation of the medullary cells and the formation of secondary pit-connections the main axis takes on a multiaxial appearance when seen in transverse section. Downgrowing hyphae are formed from the outermost cells of the medullary region, and these undergo rapid development. These hyphae probably have a supporting function, and their position varies with both the age of the frond and the part of the plant concerned. The possession of lateral branches of unlimited lateral growth is one reason for the variability in frond pattern seen over the seasons, and for the profuse branching growth typical of *Gelidium*. Branches are initiated by the transformation of a cortical cell. With *G. pulchellum* this change in character of the cortical cell usually takes place at a certain distance from the main apical cell, and a lateral branch similar to the main axis then develops. With *G. latifolium*, whilst certain of the erect branches behave in much the same way, in others a more flattened form is found with a number of apical cells (3–8) dispersed over the margin of the apical meristem. The rates of division of the apical cells at the extremity of the " meristematic area " are the same, whilst apical cells on the flanks divide at a much slower rate, with the result that a branch primordium grows out at right angles to the main axis. Depending on the time of year and the number of functional apical cells, the lateral branches may either be as broad as the main axis, or of a cylindrical nature. Under favourable environmental conditions the lateral branch initials arise in great profusion in close proximity to the apical cell, tending to obscure it by their development, and resulting in a marked change in the appearance of the frond. Transitional forms between these two extremes are also frequently observed. Adventitious lateral branches can also develop, either as a result of frond injury, or because of certain environmental changes.

The erect fronds grow from prostrate creeping branches fixed to the substratum by small attachment discs, and each prostrate branch has a

single apical meristematic cell. In a number of field observations, Dixon showed that several markedly different forms of erect frond, which in some instances were characteristic of other species, could all be traced to a common prostrate axis. Both the attaching branches and the erect fronds are perennial structures, the prostrate branches in particular showing a marked persistence in many shore sites. Whilst the state of the erect fronds will vary to some extent with season, experiments have shown that wholesale removal of the branches causes no diminution in the vigour of the plant, nor of the number of branch primordia produced in the following year. Thus whilst the extreme polymorphism of this genus will continue to present appreciable difficulties in the taxonomic field, the vigour of its growth and branch proliferation is an exceedingly valuable feature for plants used as agarophytes.

The reproductive behaviour of the two species was also studied by Dixon (1958b). In both cases sexual plants and tetrasporophytes are known, and the plants bearing tetrasporangia appear to be more common. Male plants of *G. pulchellum* were not, in fact, collected in the British Isles, although this could be a result of their small size and lack of readily detectable characters. Observations on the seasonal periodicity of fruiting by the species in the British Isles have shown that although tetrasporangia are initiated in June and July, actual maturation and release of the spores is a protracted process which may run on into the early part of the succeeding year. Male plants of *G. latifolium* have been found during the autumn. Germlings have not been found in the British material so far examined, and all young axes were traced back to a perennial attaching structure.

The vigorous growth of frondage observed for the plants in the British Isles seems to be paralleled by the species used as a source of agar on the western coasts of the United States, i.e. *Gelidium cartilagineum*. This plant grows in sublittoral habitats as far north as Washington and is harvested in quantity at depths down to 40 to 50 ft on the coast of California (Tseng, 1947). The branching of the erect fronds is repeatedly pinnate, giving a fern-like appearance (Fig. 12), and the branches can reach lengths of 4 to 5 ft, with a number of fronds arising from a prostrate attaching branch. Harvesting is usually effected by diving and hand-picking, although raking from boats has also been used. Tseng remarked on the frequent association between dense beds of the agarophyte and a species of the goldfish *Hypsypops* which might serve as a " fisherman's sign ". These dense beds always tend to occur on the seaward edges of rocky reefs where there is fairly intensive wave action, and also where harvesting can be rather a hazardous operation. Times of collecting the seaweed are therefore more condi-

FIG. 12. *Gelidium cartilagineum* (Scale line = 15 cm].

tioned by climatic factors than by the growth phase of the plant. Johnstone and Sweeney (1944), in an investigation designed as a study of the possible need for conservation of the agarophyte, found that tetrasporangium-bearing plants predominated throughout the year, and that there was little evidence of any seasonal periodicity in fruiting behaviour. Nor was any variation noted with the relative proportions

of the different types of fruiting plant with depth. The smaller number of plants bearing sex organs were thought to be due either to a failure of the tetraspores to germinate, or to death of the young germlings. Vegetative growth by the tetrasporophytes was most vigorous, and three principal periods of branch growth and regeneration were observed during the course of the year, in February, July and October. Each of these times appear to follow periods of intense surf activity which probably cause large numbers of damaged fronds, and so stimulate regenerative growth. Production of new erect fronds from the attaching branches shows no clear seasonal periodicity, although there were indications of greater vegetative activity in March and early July. The conclusion was drawn that, in the absence of any distinct seasonal periodicity in fruiting behaviour, and because of the vigorous regenerative growth, no specific season need be chosen for harvesting. However, Cooper and Johnstone (1944) reported that there was a seasonal variation in the quantities of agar extracted per dry weight of plant, and that this was at a maximum in May, June and July, the months with maximum photosynthetic activity.

The regeneration properties of *Gelidium amansii*, the principal source of agar in Japan, were studied many years ago by Okamura (1911), who scattered small fragments of frondage along the shore, and found that in many instances these small fragments quickly became attached to pieces of stone and regenerated new vegetative apices and attachment organs. More recent work on the propagation of this species by Yamasaki and Osuga (1960) studied the succession of plants on freshly set stones in the culture grounds. After 2 years the plants bearing carposporophytes outnumbered those bearing tetrasporangia by a ratio which approached 2 : 1, but after 4 years this ratio was almost exactly reversed, and became much the same as that found with plants growing on neighbouring regions of the shore which were left untouched. The best time for harvesting *G. amansii* appeared to be between August and October, probably coinciding with the period of maximum photosynthetic activity.

Spore productivity is an important aspect of the seasonal life of the seaweeds. Dixon (1958b) was unable to obtain spore germination for *Gelidium pulchellum* and *G. latifolium*. Various reports are available about the early development of *Gelidium* sp., and certain of the observations are relevant to the growth of the adult plants. Early developmental stages have been described by Chemin (1937), Katada (1949, 1955), Suto (1950), Yamasaki (1960) and Boillot (1963). All observers agree that the early stages of germination follow the release of the spore contents into an indirect germ tube. The first division of the

sporeling is parallel with, or at an oblique plane to the substratum and to the longitudinal axis of the germ tube, with the result that upper and lower cells are formed. The cells which subsequently develop from the uppermost cell become the first part of the creeping stem of the erect frond. Branching takes place at an early stage in germling development, so that two axes may arise, an early indication of the vigorous branching shown by the adult plant. Prostrate branches also arise at a very early stage in development. Changes in the salinity of the medium appear to have little serious effect on the early growth of the germlings, although some influence is recorded on the primary rhizoids. Early vertical growth of the germling is soon followed by the production of secondary shoots from the main axis, and these give rise to creeping stolons. Young plants of *G. amansii* show a slow growth during the summer, autumn and winter, but develop much more rapidly in the following spring, and secondary shoots are rapidly formed from the basal portion of the frond.

8. *Pterocladia*

A taxonomic study of the European species *Pterocladia capillacea* has been recently reported by Dixon (1960). This species is utilized as a source of agar in many parts of the world, so that all aspects of its biology are of interest. In the British Isles it shows a southern distribution, and appears to be sterile in the northern parts of its range. As with the related *Gelidium*, form range is extremely variable, and is the result of a complex interplay of both seasonal and environmental factors. Observations on specimens from the British Isles, for example, have shown that the spring initiation of new axes results in cylindrical branches and these rapidly lengthen during spring and summer. Linear growth slows down appreciably during the autumn and ceases during the winter. A steady broadening of the fronds proceeds throughout the year, however, and this is particularly noticeable during the winter months. Because of the perennial nature of the erect fronds the lower laterals are discarded, so that the plants assume a dendroid appearance (Dixon, 1963c). In *Pterocladia*, as with *Gelidium pulchellum*, lateral branches of unlimited growth are always initiated at some distance behind the apical cells of the main axis (Dixon, 1958b), so that a regular dispositioning of laterals is observed.

The erect fronds arise from the prostrate creeping axis in a series of dense tufts, a factor which will facilitate hand picking. Some commercial usage of *Pterocladia* was reported from the British Isles during the Second World War (Dixon, 1958b). The plant is fertile in the southern parts of its range, and the fronds are much larger, so that

a more successful utilization is possible, e.g. in New Zealand (Fig. 13). Frond injury, such as may occur as a result of the browsing activity of animals, results in new apical cells being formed from existing cells oi the cortex, and these bring about the growth of new branches. The structural features of the other *Pterocladia* species have been reported by Fan (1961).

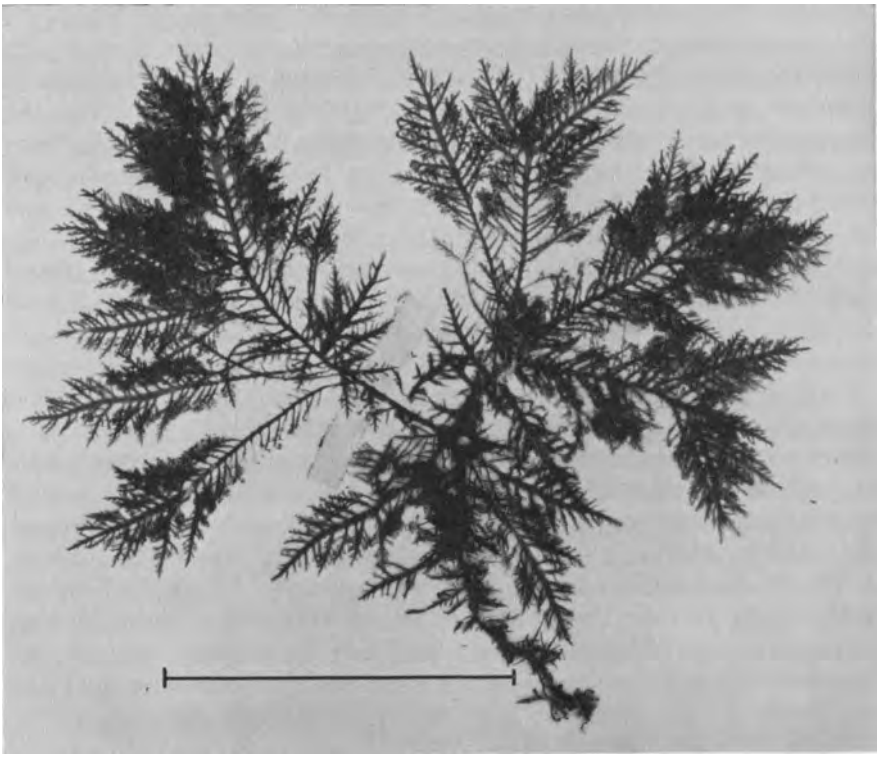

FIG. 13. *Pterocladia lucida;* drift specimen from New Zealand. (Scale line = 15 cm).

9. *Suhria*

Suhria vittata is a potential agarophyte growing on the coast of South Africa. The plants have a ribbon-like form of frond with a fringed margin and a distinct midrib. It usually grows as an epiphyte, so that the attaching system is a small discoid structure. The basic features of thallus construction are the same as those described for *Gelidium* and *Pterocladia*, with medullary and cortical regions to the frond containing downgrowing hyphae. The profuse growth of these

hyphae in the cortex and medulla of central regions of the frond gives the midrib, a structure seen also in some species of the two related genera, e.g. *Gelidium pristoides*, *G. japonicum* and *Pterocladia lucida* (Fan, 1961). Having similar modes of thallus construction to *Gelidium* and *Pterocladia*, the same vigorous regenerative growth would appear to be possible after harvesting.

10. *Reproductive structures in Gelidium, Pterocladia and Suhria*

Detailed accounts of the reproductive structures of the three genera have been given by Dixon (1958b) and Fan (1961). Tetrasporangia in *Gelidium* and *Pterocladia* are formed from terminal cortical cells on the lateral filaments. The mother cell enlarges and divides, forming four spores arranged in a cruciate or tetrahedral pattern. The sporangia of *Suhria* produce two binucleate spores per sporangium (bispores), and similar structures are described for *Gelidium pristoides*.

Carpogonia are formed in either terminal or modified lateral ramuli. The organs arise from lateral cells close to the main axis, and the trichogynes project beyond the surface of the thallus. The carpogonium enlarges after fertilization and by subsequent amalgamation growth gives rise to a large fusion cell from which the gonimoblast filaments arise, and these in turn bear terminal carposporangia; the developing carposporophyte is nourished by means of nutritive cells formed after fertilization. A number of carpogonia in a fertile axis may be fertilized at the same time, and the " cystocarp " which then develops may be formed from a number of carpogonia.

Spermatangia arise in much the same way in all genera, but the male plants are identical with the female plants and those bearing tetrasporangia. The spermatangia are formed from modified elongated cortical cells, and the spermatia are abstricted from the apices.

11. *Gigartina*

Autecological studies on *Gigartina stellata* in the British Isles were carried out by Marshall *et al.* (1949) in a detailed study of the seasonal life of the plants. New frondage is formed early in spring, and becomes prominent in May because of its different colour. As growth continues the plants darken in colour, and the " cell rows " which constitute the cortex of the frond increase in number from the four to five cells in spring to ten in early autumn. Cell wall thickness is also greater during the autumn months. The erect fronds develop from a disc-like holdfast, and are perennial structures once formed. The layering of the tissues in the holdfast can be correlated with the annual growth of the plants, since the early spring growth is more pronounced than that observed

in the following summer. Vegetative growth of the erect fronds has been observed to continue into autumn. The papillate outgrowths which bear the sexual organs commence development in the spring. Regenerative growth has been observed from injured regions of the thalli, and also from the damaged fruiting regions of the fronds. Frequently this regenerative growth takes the form of a proliferation of new frondage (similar to that described for *Fucus*, p. 137).

Sexual organs only have been observed in the life-history of *Gigartina*, and the absence of tetrasporangia makes the sequence of events in the life-history somewhat problematical. Both male organs and carpogonia have been found in spring, summer and winter, but the main period of reproduction appears to be in the summer months. The cystocarps mature in July and August, and the main period of spore discharge occurs between September and December. Subsequent deterioration of the fronds follows the decay of the spent cystocarps. The lack of tetrasporophytes suggests a reduction division occurring in the carposporangium, but there is no convincing cytological evidence for this. It appears that the spores give rise to young *Gigartina* plants directly, with a disc-like primary stage in development (Marshall *et al.*, 1949; Powell, 1949). In experiments where spore settlements were obtained on glass slides set out on the shore, the juvenile plants of *Gigartina* were observed to grow under a film of diatoms and other algae. In tests with a variety of substrata the young plants were observed to favour quartzite grains. Fusion of neighbouring discs was observed and the development of erect branches from around the edges of the disc (see also sporeling development in *Gracilaria*, p. 197). The first dichotomies by the apices of the young plants were observed 12 months after first settlement of the spores. Serious damage on the young frondage was seen to result from the activities of browsing molluscs.

Experimental studies on the methods of harvesting and effects on the standing crop have also been reported (Marshall *et al.*, 1949). The experiments were designed to study the effects of bad harvesting, either by hand or by mechanical means, in which not only the frondage but also the holdfast regions as well were removed. In most experiments it was found that hand-picking the fronds enabled an adequate recovery of the crop, and after a sufficient time (12 months) the plants on the " picked " areas were much the same in appearance as the untouched controls, whereas experimental areas cut with shears failed to show the same degree of recovery. Cropping by shears removes most of the frondage, so that there is little protection for the young developing branches.

Noticeable changes in the numbers and condition of the erect

fronds are also seen in plants which are not subjected to regular cutting, the weakened fronds after fruiting being more readily detached in the winter months. Crop recovery is dependent on the holdfasts remaining undamaged after cutting.*

July, August and early September have been suggested as the best times for picking the fronds, since these are the months when the gel strength of the extracted agar is high after the growth of the fronds from the preceding spring. Harvesting at about this time would also enable new frondage to become fertile later in the year. Quite frequent annual cropping over the years seems to be possible. Attempts at increasing the area of the lower shore where *Gigartina* grows have been made by removal of the neighbouring algae, but have proved unsuccessful because of colonization by more rapidly growing algae.

12. *Chondrus*

The frond and holdfast structure of *Chondrus* are similar to those of *Gigartina*. Regeneration of new frondage from the cut surfaces of the erect branches and from the holdfast region is frequently observed. During the annual life of the plant considerable amounts of new branch formation were observed during spring and summer for plants growing in the British Isles (Marshall *et al.*, 1949). In the field, new growth can generally be distinguished by its different colour and the lack of epiphytes. Plants growing in intertidal pools usually bear an annual frondage, whilst in sublittoral habitats plants up to 3 years old may be found. The intertidal plants are those mainly harvested on European shores, whereas in North America the sublittoral plants are the principal sources.

Plants bearing tetrasporangia, as well as those bearing sex organs, are known to occur in the annual life of *Chondrus*. Observations on fruiting periodicity for plants in the British Isles have been reported by Marshall *et al.* (1949) and by Newton *et al.* (1957). Tetrasporangia are mainly produced through winter and spring, and cystocarps are found during the summer months. Discharge of tetraspores and carpospores is followed by degeneration of the spent fruiting thalli, so that the fruiting time is important for purposes of harvesting. In sublittoral habitats it appears that the cystocarpic plants occur in quantity during the winter months.

Experimental studies on the methods of harvesting and their effects on the standing crop of *Chondrus* have been reported (Marshall *et al.*, 1949; MacFarlane, 1952a). Careless harvesting has been found to have a more serious effect on *Chondrus* than on *Gigartina*, since the holdfast region of the plant is more easily detached in the former alga.

* Regulations governing *Chondrus* harvesting are now in existence in Nova Scotia.

Picking the fronds which leaves the holdfasts undamaged ensures that the density of the erect frondage will have recovered by the next year. MacFarlane tested this by a number of methods; in the first both fronds and attaching discs were removed, and in the second the fronds were cut by means of shears very close to the holdfasts. Methods of raking were also tested, either by detaching the frondage without injury to the holdfast, or by " over-raking ", with damage to and removal of the attaching discs. The method which did least damage to the attaching discs again ensured the most complete recovery of the plants, and dense frondage was available after 6 months, whereas any serious damage to the attaching discs delayed recovery for up to 2 years. The summer months are the best times for harvesting *Chondrus* in the British Isles, but the plant is a less valuable source than *Gigartina*. The large quantities of *Chondrus* available on the shores of Nova Scotia have already been described (p. 130).

13. *Furcellaria*

The importance of *Furcellaria fastigiata* f. *aegagrophila* to the Danish seaweed industry has already been mentioned (p. 128). The most complete biological study of this free-living form was that made by Austin (1960a) whilst continuing studies carried out on the seasonal life of the attached forms of the same species (Austin, 1955, 1960a,b). Since this plant is of potential economic value in other localities (Kireeva, 1958) the extent to which the standing crop can sustain the regular and intensive harvesting carried out in the Central Kattegat is clearly of some importance. Austin found that 99% of the 1–1½ cwt " haul " obtained after a 10-min " sweep " consisted of the free-living plant. The lengths of the individual fronds varied from 1–16 cm (mean 6 cm), compared with lengths of 15–25 cm as seen on attached forms of the species growing in the same area. The branches of the free-living form were appreciably thinner than those of the attached form, and also characterized by numerous dichotomies. Large numbers of adventitious branches were also observed, bearing basal " hapteron "-like outgrowths which served to attach the branches to those of neighbouring plants. Numerous small outgrowths or " propagulae ", readily detachable, were also observed on the upper branches, and were considered to be a means of vegetative propagation.

All of the free-living material examined was sterile, so that maintenance of the standing crop is brought about solely by vegetative means. This can in part be explained by the process of fragmentation as new branches are formed after degeneration of the older parts of the plant. In addition regenerative growth and proliferation are seen

with the free-living form, and these are properties shared with the attached forms of the plant, which also show regenerative growth after wounding or casting of spent sporogenous tissue. Regenerated branches which arise by active growth of the central axial filaments of the main axis are distinguishable in later years because of the basal ring formed by the remnants of older tissue which survive. The propagulae referred to above may become branched prior to their detachment from the parent plant; in the mass of free-living material these propagulae apparently form plants of a distinctly radial form. The free-living forms of *Furcellaria* were considered by Austin (1960a) to be able to survive because of a number of features of their biology. The plants must be able to grow without attachment to the substratum, and live without orientation to light or gravity. The thalli must be able to survive almost continual rolling on the seabed, and partial burying in the mud and sand. Finally, the plant must be able to carry out constant vegetative multiplication without loss of clonal vigour. These same features must also apply to other free-living seaweeds, e.g. *Phyllophora nervosa*.

It would appear that the free-living form of *Furcellaria* is able to contain by vegetative means the changes in standing crop following the intensive harvesting by the collectors. Up to now there is no evidence that the biomass has been critically lowered by the exploitation of the last 14–15 years (p. 128). It is possible that this removal of part of the population may have proved beneficial and encouraged growth of new and more vigorous young branches. There seems no evidence that the attached forms make any contribution to this free-living population. The possibility remains, however, that the spores produced by the attached intertidal forms drift down into sublittoral habitats and there germinate to form the unattached plants directly. Burrows (1958) has shown that the sublittoral algal population in Liverpool Bay can be appreciably added to in this way. Austin (1955, 1960b,c,d) has made a detailed study of the reproduction of the attached form in the British Isles. The species show a typical alternation between tetrasporophytes, male and female plants and carposporophytes. The diploid tetrasporophytes are far more numerous than the gametophytes, and the male and female plants occur in approximately equal numbers. Fruiting appears to be a protracted process; thus, the tetrasporangia are initiated in April and steadily increase in size throughout the summer and early autumn. The first division of the sporangia occurs during a limited period in November (9th to 15th day), and this includes a reduction division. The periodicity is apparently linked with a neap tide cycle, and divisions of the nuclei were restricted to a few hours per day (9 p.m. to midnight). Liberation of the tetraspores occurs about

mid-December, and the peak period of discharge is also a relatively short one (between 26th December to 8th January). Male plants are conspicuous during autumn, winter and spring, and spermatium production extends from December to April. Carpogonia are initiated in January and fertilization soon follows. Maturation of the carposporophyte is a protracted process, and carpospore liberation takes place nearly 12 months after fertilization. In both cases, spore emission periods are short, so that any recruitment of spore material to the free-living population would similarly be of short duration.

14. *Gracilaria*

Gracilaria verrucosa is the species mainly used as a source of extractives, particularly on the south-east coast of the United States (Tseng, 1947) and in Australia and New Zealand. A small agar industry has also been established in South Africa (Isaac and Molteno, 1953) and in Japan. *Gracilaria verrucosa* is a species of world-wide distribution, and plants reach an appreciable size in Australia and on the Egyptian coast. Tseng (1947) reports that plants up to 6 ft in length may be found in suitable habitats on the coast of North Carolina, and plants up to 12 ft long have been reported from near Vancouver, British Columbia. It appears to grow best in sheltered bays, particularly in river estuaries with sandy or muddy floors, and where there is shelter from wave action. action. Jones (1959a) found that plants in North Wales grew best in localities with strong water currents, but also with shelter from wave action. In many localities in the British Isles the plant is a common component of the sand-pool flora. The species has been recorded in Norway only since 1935, growing unattached on muddy substrata in land-locked bays where in the summer water temperatures can reach 16°–20°C. The plants were found to be able to tolerate water with high H_2S content (Stokke, 1956), but plants are rarely more than 10–15 cm in length.

Variations in size, colour and structure have been reported, and length of frond and width of branch have been used as a means of distinguishing between the different forms (May, 1948; Jones, 1962). Whilst frequently growing on rocky substrata and attached by a discoid holdfast, free-floating plants are frequently reported. Pillsbury (1950) described how *Gracilaria* plants could become cemented to calcareous worm tubes and the plants can reach lengths of many feet without a distinct holdfast system, and can also be many kilograms in weight. Ohmi (1958) reported from Japan that *Gracilaria* occurred in great profusion as a free-living plant in shallow turbid waters subject

to some disturbance because of their shallow nature, and of low salinity due to influx of fresh water, e.g. north-east side of Tokyo Bay. Minio (1949) described its occurrence in the lagoon of Venice. All evidence points to the species being able to tolerate a wide range of environmental conditions.

Very few plants are fertile in Norway and the standing crop is maintained by vegetative propagation, and regenerative growth was obtained from fragments 2 mm to 2 cm in length (Stokke, 1957). Such development, however, was obtained at temperatures between 14°–18°C, and not at 10°C. Humm (1944) described how free-living *Gracilaria* plants in the seas offshore in North Carolina showed rapid growth, and the standing crop formed the basis of an agar industry. Causey *et al.* (1946) showed that growth of *Gracilaria* in North Carolina took place in a temperature range of 10°–25°C. In South Africa the species is a component of both the cold-water flora on the west coast, and is also to be found in the warmer seas near Port Elizabeth (Isaac, 1956). In both these regions the plants grow best in sheltered waters, and the mean annual temperature range approaches 15°C. Detached fragments of *Gracilaria* two inches in length produced new apical and lateral branches. Jones (1959a) measured the change in weight of both tetrasporic and cystocarpic plants whilst immersed in the sea, and found that increase in fresh weight was slow during autumn and winter, and showed a more noticeable increase in May. This increase in weight was maintained through June and the rate of change started to slow down in July and August, and into the autumn. The nature of the growth process in the tetrasporic and cystocarpic plants appeared to differ somewhat, since the increased growth rate was later than with the plants bearing tetrasporangia. From September the cystocarpic plants were observed to decrease in weight, whilst at the same time the tetrasporic representatives continue to increase in fresh weight, and this appears to result from the more severe degeneration of the spent cystocarpic branches compared with those which have borne tetrasporangia. In the British Isles this increase in growth rate was found to coincide with sea temperature increases from 5°–10°C; growth continues at temperatures of 15°C, as observed by Causey *et al.* (1946) for plants in North Carolina. In this case increased growth was observed during spring and summer, then falling-off in the autumn. Free-living plants showed a tenfold increase in fresh weight in a period of 14 days during the main growth period.

Jones (1959a) found the the fruiting cycle showed some correlation with the different phases of growth. Tetrasporangium production commences in spring, reaches its peak in the summer, then

falls off through the autumn. The formation and release of spermatia follows a similar pattern. Plants bearing cystocarps increase in number during the spring, but peak production is reached during the autumn. Minio (1949) stated that in the lagoon of Venice the formation of cystocarps on *Gracilaria* followed a period of intensive vegetative growth, and that their formation was stimulated by the diseased condition of the fronds.

Some factors influencing the growth of *Gracilaria* plants were studied by Causey *et al.* (1946) and Jones (1959b). Growth was better in flowing water, and, whilst early growth was stimulated by high levels of illumination, the slower growth subsequently observed was due to bleaching effects through loss of the red pigment phycoerythrin. Under shaded conditions the red pigment was seen to return. *Gracilaria* sporelings were found to be susceptible to intensive illumination, a feature frequently observed with sporelings of red algae (Boney and Corner, 1962, 1963). Coalescence of young sporelings has been observed under experimental conditions (Jones, 1956), and this was observed to bring about an early stimulus of erect frond production when compared with isolated sporelings. With large "rafts" of coalesced sporelings erect fronds were obtained evenly spaced over the encrusting growths, and this phenomenon has been observed with sporelings from carpospores and tetraspores. Similar types of growth have been observed under field conditions, and these would have clear advantages where an unstable substratum such as sand may on occasions cover the young plant.

Studies on spore emission and attachment have been reported by Segawa *et al.* (1955a,b) and Sawada (1956). Spore emission appears to be influenced by osmotic changes in the surrounding medium, and is initiated by changes in the inner pressure of the spore material. The degree of drying undergone by the frond also appears to be important, since dried fronds release their spores more rapidly than those which remain immersed. A large-scale emission of spores from cystocarps on the same plant is sometimes observed, and this could result in dense settlements on restricted areas of the shore, and this would encourage the spore coalescence reported above.

Gracilaria plants are cultivated in Tokyo Bay by suspending branches of the plant in twists of rope in waters rich in nutrients. Growth of the attached plants is also encouraged by supplying suitable substrata, e.g. shells, small stones etc. (Scagel, 1961). In Tokyo Bay the plant is harvested mainly in September and October. The data from other localities suggest that the summer is the best time to collect the plants.

15. *Eucheuma*

A number of species of *Eucheuma* are used as sources of agar, and the Malayan term for this genus is now used for its extractive (p. 111). *Eucheuma* plants have flattened or terete much branched thalli, and reproductive organs are borne in small lateral branchlets. Species of *Eucheuma* have been collected for agar extraction in Western Australia (Chapman, 1950), although there appear to be difficulties in collecting large quantities of the seaweed; some utilization is also reported from Indonesia. This genus has for long been used on relatively small scale extractions in Asia, and some knowledge of its autecology would be welcome.

16. *Iridophycus:* (= *Iridaea*)

This genus is mainly found in intertidal habitats in the southern hemisphere and on the shores of the North Pacific Ocean. It is harvested as a source of extractive in Japan, and recent work on the biology of *Iridophycus cornucopiae* has been reported (Hasegawa and Fukuhara, 1952, 1953, 1955a,b,c; Fukuhara, 1958a). Plants bearing sexual organs and those with tetrasporangia are found throughout the year, but there are periods when one or the other predominates. Young fronds appear on the discoid holdfast in October and November, and whilst growth is slow in the following months, the rate increases rapidly in March and April, and maximum annual growth is observed in May and June. Some deterioration of the fronds is observed in August and September, but new frondage is formed at the same time, so that " summer " and " winter " fronds are observed. The best time for harvesting the plant is in May and June, since the main fruiting period is during the autumn and winter. Plants bearing cystocarps are said to be heavier than those with tetrasporangia. Released spores show a rapid attachment to the substratum after shedding, and from the primary disc a number of growing points are formed, each " point " producing two fronds. Fukuhara (1957, 1958b) was unable to recognize any diurnal fluctuation in the quantities of spores released, nor any effects due to tidal periodicity or water temperature. The optimal range for spore germination was found to lie between 7°–15°C, although some development was possible over the ranges of 3°–7°C and 15°–23°C. Sea temperatures closely approximate the optimal range found in experiment during the main periods of spore liberation.

17. *Ahnfeltia*

Ahnfeltia plicata is characterized by the possession of a tough wiry thallus with dichotomous branching. The erect branches grow from a discoid attaching system consisting of small cells, and the structural features of the slender branches include a medulla of elongate and thick-walled cells, with radiating filaments of smaller cells forming the cortex. Annual growth rings are formed by the division of outer cells of the cortex. The plant is relatively slow-growing, and is found in rock pools in the region of low water mark, and somewhat larger plants are found in sublittoral habitats. Spore production is by way of specialized structures, the nemathecia, which are yellowish-white in appearance. These arise by the increased cell division of certain areas of the cortex, and the threads of cells so formed terminate in monosporangia. The problematical nature of these structures has engaged much attention, together with the absence of any female organs in the species. *Ahnfeltia* has some limited usage as a source of extractives, and more detailed studies on its productivity and maintenance would be valuable, particularly as it appears to be rather slow-growing.

18. *Phyllophora*

Reference has already been made to the *Phyllophora* beds of the Black Sea (p. 125) and to the three species concerned, *Phyllophora nervosa*, *P. brodiaei* and *P. membranifolia*. The standing crop consists of free-living plants, and is apparently maintained by vegetative growth. The attached forms of the species are small, dichotomously branched and foliose in appearance, with discoid holdfasts. Plants are perennial, and new pale-coloured frondage is observed each year. The attached plants are characterized by proliferative marginal growth, and this indicates the source of new frondage in the free-living forms. Structurally the plants are characterized by a medulla of thick-walled cells and a cortex of radiating filaments. The life-histories of the genera are of a specialized, reduced nature.

19. *Gloiopeltis*

The cylindrical thalli of *Gloiopeltis* are found on Pacific shores, and seem well able to withstand desiccation effects in the intertidal zones. The plants are particularly valued in Japan as sources of glue (" funori ") and studies on the biology of two species have been reported, *Gloiopeltis furcata* and *G. tenax*.

According to Fukuhara (1956) *Gloiopeltis furcata* tends to dominate the shore zone it inhabits throughout the year. Young plants appear in late summer and early autumn, and the best time for harvesting is the

following May, when the plants reach maximum size and are the most numerous on the shore. Thereafter there is a decline in population, and plants bearing carposporangia and those with tetrasporangia are found. The larger plants are found in the lower part of the shore zone. Since the plants grow in habitats where there is some degree of exposure to air, large boulders are sometimes placed on shelves of rock which may not be sufficiently uncovered by the sea at times of low water in order to provide more suitable conditions for the growth of *Gloiopeltis* (Yendo, 1914). Culture studies have shown that spore attachment by *Gloiopeltis* occurs in considerable quantities on quartzite, and few other algal spores can so effectively attach themselves to this type of rock.

Prolonged ecological observations on *G. furcata* have shown that this high-zoned plant first forms a closed community on the shore, but may in time become overgrown by *Ulva pertusa* (Uzike, 1956). In experiments in which " belt transects " were cleared down the shore and all the seaweed growth removed, the zones of the dominating species (including *G. furcata*) tended to widen appreciably after re-colonization (Katada and Matsui, 1953).

Suto (1949) observed some periodicity in spore release with this species. Fertile fronds collected at low tide were observed to release their spores in the laboratory 30 min before the next flood tide. If the spores obtained in this way were then " sown " just before a flood tide by broadcasting them over the shore, it was estimated that about 30% of the sown spores would attach themselves to the rock. In the following autumn young plants would appear, and these would occur in numbers equal to about ten plants per cm^2 of substratum. Suto (1950a) stated that a daily release of spores was obtained, and that attachment was most likely immediately after release from the sporangia. Matsui (1956, 1957, 1959) reported on the discharge of spores from both *Gloiopeltis furcata* and *G. tenax*. With *G. tenax* exposure to air and drying tended to induce release of tetraspores, whereas with *G. furcata* drying delayed the time of spore shedding. With both species, however, an increased spore output was observed when the fronds were dried under " shade " conditions. In general, the quantities of spores released were observed to increase appreciably at the time of " spring " tides. Information of this nature is of value for the methods of sowing spores described above.

VI. Quantitative Changes in Seaweed Extractives and other Constituents due to Season and Environment

The principal extractives of brown and red algae were briefly discussed earlier (p. 111). Any quantitative estimate of the seasonal

fluctuations will clearly be of importance in order to establish the best time for harvesting. The principal storage products of marine algae have been the subject of a recent review (Meeuse, 1962). Studies on the metabolism of some marine algae by Bidwell (1958), Bidwell *et al.* (1958) and Bidwell and Ghosh (1962) have elucidated some of the metabolic pathways involved. Thus in studies with *Fucus vesiculosus* using $^{14}CO_2$ it was shown that mannitol was the major soluble photosynthetic product, and that alginic acid was more rapidly accumulated than either laminarin or fucoidin. Seasonal fluctuations in the alginic acid content of the middle lamellae of the cell walls show that this compound is directly involved in metabolism, and resembles the starch of higher green plants by its repeated breakdown and re-synthesis. Mannitol was considered to be more a temporary storage product than an active metabolite, and there was no evidence that it is a precursor of alginic acid. Quillet (1954, 1957) considered mannitol and laminarin to be closely integrated assimilatory products. The different metabolic pathways in the red algae have been verified by studies on enzyme activity (Bean and Hassid, 1955; Jacobi, 1962). A critical study of methods of analysing brown algae has been made by Baardseth and Haug (1953).

A. *Brown algae: the Laminariales*

The metabolic products under consideration here are the principal extractives, e.g. alginic acid, mannitol and laminarin. In the following pages the times of maximal and minimal production will be described with regard to the best time of harvesting. The actual quantities of extractive involved (as % of dry weight of plant material) are given in detail in the references from which the data have been drawn, and are not directly referred to in the succeeding pages. In general, however, the amounts involved are of the following order. Alginic acid may range from 8% of the dry weight to values of 40%, the quantities varying with different seaweeds. With mannitol the range may be from 6% to just over 20%, and with laminarin from less than 1% to 24%. Some of the data relevant to this are summarized in Table X.

Regular analyses of three species of *Laminaria* from the British Isles were carried out by Black (1948a, 1950, 1954a). Separate analyses were made of stipes and fronds. With all the species the widest seasonal fluctuations were shown by the lamina, the seat of assimilation. Thus with *Laminaria hyperborea* the alginic acid content of the stipe showed little seasonal variation, whereas in the frond it was at a maximum value in the spring and at a minimum in the autumn. Laminarin was mainly present in the frond, and was at a maximum in the autumn, but

it was not present until the mannitol reached a certain critical value. The small quantity in the stipe showed little seasonal variation. The mannitol content of the frond increased throughout the summer, and that of the stipe showed a smaller value in the spring when new frondage was being formed. The protein contents of the frond were highest at the beginning of the year. The seasonal fluctuations of the reserve products of *L. digitata* showed maximal values earlier in the year than those of *L. hyperborea*. The alginic acid content of the frond was at its lowest value in midsummer, whilst that of the stipe fluctuated very

TABLE X. RANGE OF VARIATION (AS % COMPOSITION ON A DRY WEIGHT BASIS) OF THE MAIN EXTRACTIVES OF BROWN ALGAE. SHOWN AS MINIMUM AND MAXIMUM QUANTITIES OBTAINED OVER YEAR.
(A = alginic acid; B = mannitol; C = laminarin)

Species	*Minimum*			*Maximum*			*Authority*
	A	*B*	*C*	*A*	*B*	*C*	
Laminaria saccharina .	13	4·5	0	22·5	22	23	Black, 1948a
L. digitata . . .	19·5	6	0	27	26	21	Black, 1948a
L. hyperborea . .	14·5	5	0	24·5	18	18	Black, 1948a
Alaria esculenta . .	21	4	0	42	12·5	34	Haug and Jensen, 1954
Ascophyllum nodosum .	24·5	6·5	2·5	28·5	12·5	7	Black, 1948b
Fucus serratus . .	17	6	2·5	22	16·5	10·5	Black, 1949
F. vesiculosus . .	13·5	9	2	17	16·2	5	Black, 1949
F. spiralis . . .	14·3	6	1·5	16·5	11·5	6·75	Black, 1949

(All data for whole plants; differences between plants from open sea and sheltered localities can also be seen in the references quoted, and the values for fronds and stipes separately.)

little. The laminarin content of the frond was negligible between January and April; the peak quantities were observed in summer and autumn. Mannitol in the frond was at its highest value in June, with a second peak in early autumn, and fell to a minimum in early winter; the stipe content followed a similar variation but between much narrower limits. The protein content of the frond was higher in the early months of the year, and low in summer and autumn. The stipe values showed little variation. With *Laminaria saccharina* frond variation was the most significant, and the alginic acid was highest at the beginning of the year, and at a minimum in the summer. There was little seasonal

variation in the stipes. Laminarin was absent in the late winter and early spring, as with the other species, and reached its peak value in July and August. The mannitol content of the fronds showed summer and autumn peaks similar to that of *L. digitata*, and the protein contents were highest in the spring and low in early autumn. As noted earlier, growth of *Laminaria* plants was most rapid in the first 6 months of the year, and the older tissues on the distal end of the frondage was continuously worn away. In general, when the rapid growth phase commenced the protein content was high and thereafter fell steadily. By contrast, laminarin was present in negligible quantities and the mannitol values were low at this time. Both compounds reached peak values at similar times. Whilst the rate of growth decreased in the summer the mannitol and laminarin accumulated because of continued assimilation, and with the increased protein content of the autumn months there was an accompanying fall in the laminarin and mannitol. The times when the alginic acid contents were high roughly correspond with the peak values for protein. Seasonal fluctuations were also observed in the mineral salt content and total dry weights of the plants. Differences due to environmental factors have also been observed in addition to the seasonal variations.

The seasonal variation in the biochemical composition of species of *Laminaria* and *Alaria esculenta* in Norway has been described by Haug and Jensen (1954). In general the results were similar to those obtained by Black (p. 201). The fronds of *Laminaria* plants showed alginic acid maxima in spring and minima in the autumn, whilst with *Alaria* the highest yields were observed in March and lowest in autumn. The stipe contents fluctuated very little. Mannitol contents were at a maximum from June to November, but were very low from February to May, and the laminarin contents showed extreme variations, being absent from February to June, then rising to a maximum in autumn before falling again. The alginic acid content of *Alaria esculenta* showed a seasonal fluctuation similar to that of *Laminaria*, and in both genera the protein contents followed a similar course to that of alginic acid.

Laminaria plants have been analysed in Japan (Suzuki, 1955). Mannitol was at a minimal value in spring, and then increased throughout the summer. The protein and fat contents were at a maximum in spring and a minimum in the autumn. The seasonal variation of alginic acid appeared not to be so readily distinguishable. Seasonal analyses of *Alaria crassifolia* and *Undaria pinnatifida* f. *distans* have also been reported (Togasawa, 1954; Togasawa and Mino, 1954; Takagi and Murata, 1955). Analysis of carbohydrates showed that the reducing sugar content of the sporophylls was appreciably higher than that of

other parts of the plants, and similar results were obtained for the mannitol, protein and fat contents. Evidence was also obtained of changes in the metabolic rate with growth of the plant, and of different concentration gradients with age.

The effects of depth of immersion on the chemical composition of the *Laminaria* species common to Scotland have been reported by Black (1950b). In general the variation in the fronds was far more noticeable than that of the stipes. For *Laminaria hyperborea* the alginic acid content showed little significant variation with depth in both stipe and fronds, and whilst the stipes of *L. digitata* contained similar quantities of alginic acid, the fronds had a tendency to show a somewhat lower content with increasing depth during July, and some measurable variation was also observed with *L. saccharina*. The mannitol reserves were found to increase to depths of 6–10 m, and then to decrease. By contrast, the laminarin content fell progressively with depth. Where any noticeable fluctuation occured in alginic acid the variation is usually the inverse of mannitol, in keeping with Bidwell's (1958) observations. Differences of this nature are in part due to the degree of sublittoral illumination, although other factors, probably associated with available nutrients, are also involved. The most marked differences were observed during the summer.

Black (1954a) studied concentration gradients in the fronds of *Laminaria saccharina* at different seasons. During the rapid growth period (spring) little variation was observed in the composition of the tissues along the frond. During the slow-growth period (August), and in the autumn, considerable variation in chemical composition was observed because of the variable age of the tissues. At the base of the frond above the meristematic region, laminarin was absent, and proteins and alginic acid were of very low value; mannitol was present, and the mineral content high. During the period of slow growth the upper two-thirds of the lamina contains a reserve of laminarin and proteins, since this is the period when continued photosynthesis and a reduction in the rate of cell division leads to a steady accumulation of reserves. The iodine and fucosterol contents were found to be at a maximum in the transition region between stipe and frond. A study of chemical and growth gradients was made also on *Laminaria hyperborea* (Black *et al.*, 1960). Larsen and Haug (1961) investigated the biochemical gradients in the stipe of *Laminaria hyperborea*, using the peripheral tissue, the outer and inner cortex and the medulla. Whilst the iodine and protein contents of the outermost layers were high, the alginic acid and ash contents were low; the iodine and protein quantitatively increased from the transition region to the holdfast complex, whilst in the outer

cortex the gradient was in the opposite direction. The alginic acid content was higher in the inner regions of the stipe. Young and Langville (1958) studied the inorganic elements in *Laminaria digitata* from the Atlantic Provinces of Canada, and found that the trace elements were present in greater quantity in the stipe than in the frond. A further observation was that the plants showed a property of accumulating arsenic from the surrounding medium to appreciably high quantities.

Reports are available on the seasonal analyses of *Macrocystis*. In earlier years the plant was extensively used as a source of potash, and detailed analyses were carried out with this in mind (summaries in Chapman, 1950, and Cribb, 1954). Present day usage is mainly concerned with the alginic acid content. Sannie (1950) reported that *M. pyrifera* from the Kerguelen Islands contained 17·65% alginic acid and 4·15% mannitol in the dried fronds, 18·79% and 5·78% in the stipes, and 20·94% and 6·07% of the two compounds respectively in the bladders. Cribb (1954) analysed *M. pyrifera* plants from Tasmania and found an alginic acid variation of between 16·2% in the winter and 29·4% in late autumn; for mannitol the variations ranged from 6 to 11·55%. Wort (1955) described the results of a seasonal analysis of the chemical composition of two giant kelps, *Macrocystis integrifolia* and *Nereocystis leutkeana*, from the seas off British Columbia. The analyses included dry weights, ash, total nitrogen, total phosphorus, trace elements, alginic acid, fucoidin, mannitol, laminarin and lipoid substances. In general the fronds showed higher contents than the stipes, and in both structures there was a parallel seasonal variation. The dry weight and ash contents were maximal in late autumn and winter, and at a minimum in spring. The mannitol, laminarin and fucoidin contents were highest in summer. The quantity of alginic acid showed little variation throughout the year. Cribb (1954) indicated the probable source of errors in the analyses of *Macrocystis pyrifera* reported above, and it is possible that, as with the related species in British Columbia, there are no wide variations in alginic acid content over the year.

B. *Brown algae: the Fucales*

Black (1948b, 1949) studied the seasonal variation in chemical constituents of the fucoid algae commonly found on Scottish shores. Thus, with *Ascophyllum nodosum* it was found that the principal extractives (mannitol, laminarin, alginic acid) showed slight seasonal variation; in consequence from the chemical aspect the plant could be harvested at any time of the year. The slight increases in mannitol

and laminarin contents show three annual peaks, in June, September and December. The alginic acid content of the thalli was generally higher than that of the *Laminaria* species examined (p. 202), and *Ascophyllum* plants in open sea habitats contained more alginic acid than those growing in sheltered sea lochs. By contrast, the mannitol and laminarin contents of the sea loch plants were greater. Barry and Flood (1958) reported that the alginic acid in 3–6 year old plants of *Ascophyllum* growing in Dublin Bay was at a minimum in April but rapidly increased to a maximum value in June. Larsen and Haug (1958a) showed that habitat conditions, notably variations in salinity, had a marked effect on the chemical composition of *Ascophyllum nodosum*.

Results for the other shore fucoids (*Pelvetia canaliculata*, *Fucus spiralis*, *F. vesiculosus*, *F. serratus*), have shown some gradation in the seasonal variations in chemical composition which can probably be linked with the degree of immersion in their shore habitats. Minimum dry weights, corresponding with minimum ash, mannitol and laminarin were observed in January and February. Thence through spring and into early summer the ash, mannitol and the dry weight increased. During July and August, *Pelvetia canaliculata* and *Fucus spiralis* showed a fall in dry weight, laminarin and mannitol, but with *F. serratus* and *F. vesiculosus* this fall was delayed until autumn. In fact, the mannitol and laminarin reserves showed two annual peaks in all representatives, e.g. early summer and late autumn. The plants which are immersed for longer periods (*F. serratus* and *F. vesiculosus*) have higher mannitol contents than *F. spiralis* and *Pelvetia canaliculata*. Alginic acid appears to reach a maximum value in January and February, but decreases during the summer months. The seasonal variations seem to be far more marked in plants growing in the zones lower down the shore, e.g. *Fucus vesiculosus* and *F. serratus*. It is possible that there is a more regular pattern of metabolism and turnover of reserves in plants found in the higher shore zones. The growth rates of the plants found in the upper shore zones are slower than those lower down, however, and recuperation is a slower process.

Moss (1948) studied the chemical composition of plants of *Fucus vesiculosus* collected from different habitats, ranging from that sheltered from wave action to one receiving the full force of the open sea. In general, chemical composition could be related to the variation in thallus form and structure associated with a habitat, and some gradation was observed along the length of the thalli. Generally the younger (distal) tissues contained the larger quantities. However, when plants are harvested the bulk of the frondage is removed, so that total weights are of greater significance, and these indicated that the alginic acid and

mannitol contents were about 2% higher in plants growing in habitats exposed to severe wave action. A comparative study was also made of the chemical composition of the receptacles and sterile apices of plants from the three contrasting habitats. Whilst the receptacle composition varied little with habitat, the sterile tips showed a similar variation to that described above for the larger fronds. When a seasonal analysis was made of receptacles and sterile branches marked differences were observed. Thus the mannitol content of the sterile tissues was low in December, but steadily increased through spring and summer; by contrast the mannitol in the receptacles was highest in December and thence fell progressively; but the minimum content of the sterile fronds was higher than the winter maximum of the receptacles. Similarly the alginic acid content of the sterile tissue was appreciably higher than that of the receptacles in winter, and in both cases the quantities fell progressively, reaching a very low value in the case of the receptacles. Here again, the maximum value observed in the receptacles was lower than the minimum value of the sterile tips. These lower values in receptacle tissue were also seen in dry weights, total ash and crude protein. The observations recorded above are of some significance, since the fruiting tissue makes up a considerable proportion of fertile plants (estimated at half the fresh weight), and the fruiting plants will clearly be of lower value on harvesting.

Whilst *Himanthalia elongata* is of little economic value, the unusual form of the plant with the cup-shaped frond and the strap-like receptacles enables a more critical analysis to be made of sterile and fertile tissues (Moss, 1952b). Thus the mannitol content of the sterile tissue falls with growth of the thongs, and the laminarin content was low in both tissues. Whilst the developing fronds show steady increases in mannitol, crude protein and alginic acid, the young thongs have higher protein and alginic acid contents than the fronds which produce them. These results further demonstrate the differences in extractive content of fruiting and sterile tissues.

Macpherson and Young (1952) reported the seasonal variation in chemical composition of fucoid algae from the Maritime Provinces of Canada (*Fucus vesiculosus*, *F. evanescens*, *Ascophyllum nodosum*). Of the principal extractives, mannitol was highest in summer and autumn, whilst the laminarin changed little over the course of the year. Alginic acid was at a maximum in the winter, falling in spring and summer; the total ash content was the converse of this.

Black and Dewar (1949) made a detailed investigation of the more significant physical and chemical properties of the sea, and attempted to correlate these observations with the annual changes in the chemical

constitution of *Laminaria saccharina* and *L. hyperborea*. These biochemical changes have been summarized in earlier pages, and include the change from the low mannitol and laminarin contents of late winter to the higher values in late spring and in the summer and then, in the case of mannitol, remaining relatively constant, whilst laminarin tends to increase. Alginic acid is highest early in spring, but decreases as summer progresses, and the protein reserves show a similar course. In autumn the protein content increases again, and subsequently there is a rise in the mannitol and laminarin reserves. The well known cycle of changes in the nitrate and phosphate contents of the sea show two annual peaks, e.g. in spring and autumn, and these cyclic changes have been correlated by Black and Dewar (1949) with the major changes in chemical content of the algae. They also suggested that the higher summer temperatures may be of significance because of increased rates of respiration; the depletion of nutrients and the accompanying restriction of photosynthesis was considered to be of greater importance. However, Bidwell *et al.* (1958) observed that addition of inorganic salts appeared to have little effect on the photosynthesis of *Fucus vesiculosus*, and Macpherson and Young (1952) observed that the fluctuation in mannitol content paralleled that of the sea temperature. Whilst depletion of nitrate and phosphate may indeed be limiting factors, it is also possible that other elements (of a micronutrient nature) may fall below a critical value and hence be significant.

Vitamin content of brown algae

Reference has already been made to use of brown seaweeds as sources of meals for animal feeding (see also p. 215). This is in part due to their vitamin content, and the results of studies on the seasonal variation of certain of these are available. Thus, with the vitamin B group, seasonal fluctuations in the niacin content have been described from Norway (Larsen, 1958). For *Laminaria hyperborea* the minimum quantity is observed in the winter, thence rising to a maximum in April and May, and similar fluctuations are observed in the stipe and frond. Results for *Laminaria digitata* and *L. hyperborea* were much the same. In *Ascophyllum nodosum* the maximum content is found in the spring, with a smaller peak in the winter, and with *Fucus serratus* the maximum content is observed in late spring. In both these species the niacin content appears to vary with salinity (Larsen and Haug, 1958a), and younger tissues have a higher content. No significant loss was observed on drying the weed for preparation of the meal. For *Laminaria* species the biotin content was also at a maximum in spring, with a second peak in autumn, whilst with *Fucus serratus* the two

maxima were earlier in the year and that of *Ascophyllum nodosum* showed little annual variation. In regions of low salinity, however, the biotin content of the thalli was higher, and younger tissues had a higher content than older fronds. The niacin and biotin contents of thalli of *Alaria esculenta* followed much the same course over the year, with maxima recorded in December, and the lowest values in June when frond size is at a maximum. The suggestion has been made that harvesting of *Laminaria angustata* should be restricted to third year plants, so permitting growth, spore production and gametophyte formation in the first two years (p. 160). It would hardly be practicable to pick out the older plants from dense populations, so that harvesting will normally include plants of all ages. The time of cutting could then depend on the type of extractive required, and the extent to which this fitted in with other aspects of the seasonal life, e.g. fruiting. For the *Laminaria* plants analysed from European shores, the alginic acid contents would appear to be highest in the earlier months of the year, and mannitol and laminarin values are at a higher level later on. Stipe variations over the seasons are of a much lower order than those of the fronds, and similar observations were obtained when plants from different depths were considered. Analysis of *Laminaria* plants from Japan did not show the large-scale fluctuations in alginic acid content. Nor did the *Macrocystis* plants analysed show any wide variations in alginic acid content over the seasons. With the fucoid algae a similar overall cycle of relative abundance of the main extractives was observed, the one exception being *Ascophyllum* in that the alginic acid remained at much the same level throughout the year. The effect of depletion of reserves in formation of the fruiting receptacles of the fucoid algae is a feature of their seasonal biology of marked significance for harvesting.

C. *Red algae*

Studies on the seasonal change in the organic extractives of red algae have shown that differences in gel strength are to be found, as well as quantitative variations. The data, however, are less readily available for the members of this class than for the brown algae. Earlier observations have shown that in *Chondrus* there is a seasonal variation in carbohydrate content, ranging from an early spring minimum to high values through summer and autumn (Butler, 1936). Young and Langville (1958) found that the total ash content of *Chondrus* was high in winter, falling to minimum values in summer, a result of the observed spring growth. For *Gigartina stellata* changes in the gel strength of the extracts with the seasons have been observed (Marshall

et al., 1949). During April, when new frondage was forming, both yield and the gelling qualities of the extract were low for plants from an area harvested the previous year, but the physical properties of extracts from untouched plants collected at the same time of year were somewhat higher. Material collected a few weeks later gave extracts with a higher gel strength. Subsequently the gel strength increased, and reached a maximum value in September, falling off slowly through the winter and then more rapidly with the approach of spring. A similar seasonal analysis for *Chondrus* showed that both yield and gel strength tended to improve from spring to autumn.

For *Gelidium cartilagineum*, the agarophyte used in the United States, Cooper and Johnstone (1944) have shown that the major yields of agar are obtained from plants harvested in the summer months, the time of the year when the peak period of assimilatory activity is recorded. For *Gelidium amansii*, the agarophyte collected in Japan, Asano *et al.* (1951) have reported a steady rise in agar content through the summer, reaching a maximum in autumn and then falling again in the winter months. The crude protein contents were the inverse of this. Both from the point of view of chemical contents, and in order to allow the necessary regeneration of new frondage, harvesting of *Gigartina* in the British Isles would best be carried out in July, August and early September. In Japan, the autumn would appear to be the most favourable time for collecting *Gelidium amansii*. With the red algae, as with other crop plants, the time of harvesting must take into account the best season for maximum yield of agar, to enable regeneration of new frondage, and not to interfere with spore output. These times would appear to vary with *Gelidium* species from different parts of the world.

VII. Techniques used in Harvesting Seaweed

Many of these have been indicated in earlier pages. Mechanical cutters will be effective only when dense crops of weed are available, e.g. *Macrocystis* and *Laminaria* sp. Large-scale harvesting of *Macrocystis* on the coast of California has been described by Tseng (1947), and methods for harvesting *Laminaria* have been investigated by the Institute of Seaweed Research in Scotland (Booth, 1964a). Dredging by means of outboard trawls has been utilized with free-living seaweeds, e.g. *Furcellaria fastigiata* f. *aegagrophila* in Danish waters (Austin, 1960a). Intertidal fucoid algae are reaped by means of hand cutting methods such as those described above where there are fairly dense "stands" of the seaweed. The need for controlled cutting of the fucoid algae has already been described, whilst the continued heavy cropping of the *Furcellaria fastigiata* f. *aegagrophila* seems to have left

the standing crop little changed. There are indications that controlled harvesting of *Macrocystis* can be advantageous during the active growth period.

Large quantities of certain sublittoral seaweeds, e.g. *Chondrus* and *Gracilaria*, are successfully harvested from shallow-draught boats using long-handled rakes with tines set at a sharp angle. Long poles with hooks are used in Japan to collect the kombu blades. These tend to detach the holdfast as well, so that regenerative growth is not readily observed. These methods pick up large quantities of seaweed in the North American seas, and in the instance of *Chondrus* remove sufficient quantities of the frondage without injury to the attaching systems (p. 193). Long-handled cutting knives have been used for harvesting *Macrocystis* fronds in Tasmania (Cribb, 1954), and long-handled rakes with *Undaria* plants in Japan.

Some harvests depend on cast weeds, e.g. *Chondrus* on Prince Edward Island (MacFarlane, 1964a), and *Pterocladia* on the North Island in New Zealand (Newton, 1963), in which cases a fairly rapid collection is necessary before the seaweed is damaged by exposure to air.

Many of the seaweeds are still harvested by hand, and this would still appear to be the most economic mode of collecting. The collecting may be carried out under the sea by means of divers, whilst hand-picking of fronds of plants growing on the shore has been found to be advantageous in allowing regrowth of new frondage. *Gelidium* sp. and *Pterocladia* sp. are harvested in this way. *Porphyra* plants are hand-picked from the " hibi " in Japan, and this has the advantage of ensuring that mainly the fully-grown thalli are collected. Pickings of the cultivated plants can be made three or four times between November and March. In the British Isles the *Porphyra* plants used as sources of laver are collected directly from their shore habitats, and in some cases the collectors are known to " farm " the regions of shore from which they harvest the *Porphyra* (Hampson, 1957). The quantity of any material harvested will depend on the initial spore attachment. It is important to know the relationship between the quantity of spores released and the hydrographical and meteorological conditions which occur at the time. Suto (1952) suggested that if quantitative estimates can be made of the spore output of a particular plant and when the maximum release is observed in the sea, then announcements made over the radio from local research stations could indicate to fishermen the best times to obtain dense spore settlements on suitable surfaces.

The large-scale exploitation of seaweeds requires adequate processes of drying, since there will be deterioration of the harvested material if this is not expeditiously carried out. Studies specifically concerned

with this have been carried out (Gardner and Mitchell, 1956), and there are many reports on the techniques of seaweed drying carried out on both a large and small scale, e.g. Tseng (1947) on *Gracilaria*, Cribb (1954) on *Macrocystis*, Marshall *et al.* (1949) on *Gigartina* and *Chondrus*, and Newton (1963) on *Chondrus* drying in Nova Scotia. With agarophytes the product can be appreciably affected by the initial drying processes.

Direct observations on sublittoral seaweed populations by divers using self-contained breathing apparatus have been reported in earlier pages, and the part such techniques can play in cultivation and harvesting of seaweed crops offers considerable scope. Studies on sublittoral populations have been successfully pursued by the use of remote-controlled still cameras (Vevers, 1951) and with underwater television (Barnes, 1963). The effects of weeding unwanted algae from beds of harvestable weeds appear not to have been described in detail, but preliminary work in this direction has been reported. Thus " farming " the seabed is a reasonable probability.

VIII. Weeding

A number of reports are available on the effects of " weeding " unwanted algae from the areas in which harvestable seaweeds are growing. In some cases these methods have not been too successful. Thus where regions of the shore bearing stands of *Chondrus* and *Gigartina* have been cleared of other algal growths from the neighbouring rock in order to encourage the spread of the two species, the fucoid algae and other species tend to come back in quantity. Only in the instance of *Chondrus* was there any noticeable recolonization (Marshall *et al.* 1949). When the shore in the vicinity of *Ascophyllum* is cleared other fucoid algae subsequently predominate (p. 223). The *Chondrus* beds in Nova Scotia may suffer from an overgrowth of the filamentous green alga *Chaetomorpha*, and this can cause devaluation of the crop; too severe a harvesting of the *Chondrus* beds can result in their replacement by *Laminaria* plants. Similarly, where large areas of *Chondrus* are destroyed by unfavourable weather conditions the plants are often replaced by *Chordaria flagelliformis*, and several years may elapse before the *Chondrus* stocks are again built up (MacFarlane, 1952). Chapman (1950) has described how beds of *Laminaria japonica* in Japan may be invaded by the marine angiosperm *Phyllospadix*, and the *Laminaria* " grounds " subsequently require " weeding ".

In Japan coralline algae such as *Amphiroa ephedraea* can be harmful because of their growth on agar-producing algae. Ogata (1952, 1953) studied the distribution of *Amphiroa* spores in the sea in relation to

regions of the shore where the agar-weeds were growing. He found that spores occurred in greater numbers near the top of a submerged reef bearing large numbers of *Amphiroa* plants, and that these spores tended to drift in the surface layers over considerable distances from the point where they were released. The harmful effects of *Amphiroa* and related genera is due to their calcareous nature, and calcification of the cell walls is observed at the earliest stages of plant growth (Furuya, 1960).

IX. Seaweeds as Food and Manures

A. *Seaweeds as human food*

Although marine algae have been utilized as a direct source of food by coast dwellers in different parts of the world for many years, in general the seaweeds are a poor source of nutrition. Whilst the carbohydrate contents of the thalli are fairly large, the quantities of protein and fat are relatively small; and although carbohydrates are good sources of energy, the algal polysaccharides are poorly digested by man. The food value of marine algae lies mainly in their mineral salt and vitamin content. The variety of ways in which seaweeds are directly utilized have been described by Chapman (1950), Newton (1951, 1963) and Scagel (1961). The value of the trace element content of *Macrocystis* in treatment of anaemia, debility, digestive upsets and geriatric conditions has been reported by Siefert and Wood (1956).

In recent years there have been reports of tests on the edibility of marine algae used as human food. Thus the flavour of the green seaweeds *Ulva pertusa* and *Enteromorpha prolifera* has been studied by Obata *et al.* (1951), and was considered to be due to the production of dimethyl sulphide. The flavour was scarcely detectable in fresh thalli, but gradually developed when left in seawater. On sterilized portions of the thallus the flavour developed only after inoculation with bacteria. The free amino-acid content of *Porphyra tenera* was analysed by Tsuchiya and Sasaki (1957), who identified a number of " sweet " and " flavour-producing " amino acids. Miura (1961), describing a new species of *Porphyra* (*P. tenuipedalis*), found its taste inferior to that of other Japanese lavers. Hampson (1957) reported that in South Wales the lavers collected during the summer months showed a definite loss of flavour, and a souring of the thalli took place in hot weather. This is put forward as a reason for people not utilizing the lavers when there was no " r " in the month. Information is also available on the extractive content and quality of kombu in Japan (Oishi *et al.*, 1961a). The ratio of amino-nitrogen to total nitrogen was always higher in better quality kombu. Further investigations were carried

out on the amino-acid composition of kombu (Oishi *et al.*, 1961b). Studies on the " quality " of edible *Laminaria* spp. showed that the lamina base was of greater value than the apical or marginal portion (Okamura *et al.*, 1962).

Observations have also been made on the effects of processing and storage on edible seaweeds. Katayama and Tomiyama (1950) reported on the ascorbic content of *Porphyra tenera* due to ecological conditions and processing treatment. Whilst seasonal and environmental changes were not observed, the vitamin C content fell after prolonged storage at high temperatures; storage in a dry atmosphere was satisfactory, but marked changes followed exposure to increased humidity. Tsuchiya *et al.* (1961) studied the effects of low temperature storage on *Porphyra tenera*. They found that colour, flavour and edibility remained unchanged when material was vacuum sealed in CO_2 at —20°C over storage periods of 2–12 months. Oishi *et al.* (1963) observed that satisfactory storage of kombu required initial drying at 80°C for 2 h. In South Wales the tougher " winter " thalli of *Porphyra* require more boiling than the early spring growth, and the saleable value of the pulped weed (and its edible value) are improved by the addition of black colouring matter (Hampson, 1957). Seasonal changes have also been reported in the vitamin B_{12} content of seaweeds (Hashimoto and Sato, 1954), and in the amino-acid content of *Porphyra* (Murata, 1963). Tests on the effects of adding dried seaweed meal (*Fucus vesiculosus*, *Cystoseira barbata* and *Laminaria japonica*) to wheat and rye bread have been carried out (Alfimov, 1961). Added at various stages in baking and treated so as to remove odour and taste, the seaweed meals significantly increased the calcium and iron salt contents of the bread. It is well known that people long used to a seaweed diet are more capable of digesting the algal polysaccharides. It is interesting to note, therefore, that tests on digestibility of marine algae have been carried out in Japan (Kimura, 1952). In the experiments digestibility was measured in terms of the absorption rate for polished rice when fed mixed with three test plants, *Laminaria japonica*, *Undaria pinnatifida* and *Porphyra tenera*. The results indicated that the carbohydrate and fat values were higher in *Laminaria* and *Undaria*, and the protein value higher in *Porphyra*. Numerous analyses of the amino-acids in marine algae are available (Lewis and Gonzalves, 1960; Lewis, 1962; and Fowden, 1962). The suggestion has been made that predigestion of algal polysaccharides by enzyme additives might increase their nutritional value in human diet (Schifner *et al.*, 1962). Relevant to this work are the studies on degradation of algal polysaccharides by browsing molluscs and sea urchins (p. 114). Another development is yeast production in boiling

water extracts of certain seaweeds (*Ecklonia cava*), although not all marine algae are useful (Tomiyasu and Zenitani, 1952).

B. *Seaweed as animal fodder*

The use of intertidal seaweeds and saltmarsh plants as fodder for cattle, pigs and sheep has for long been the practice in small islands and coastal districts (Chapman, 1950; Newton, 1951, 1963). Direct grazing on the shore is carried out, or the seaweeds are used chopped and boiled in winter-feed supplements. The following species are known to be eaten by domestic animals: *Pelvetia canaliculata*, *Fucus vesiculosus*, *Alaria esculenta*, *Laminaria* sp., *Chondrus crispus*, *Rhodymenia palmata*.

Ascophyllum nodosum f. *mackai*, a free-living form, is eaten in salt-marsh habitats. There is some evidence that seaweeds cause flesh-tainting but this seems to disappear after a period of grass-feeding. Numerous analyses of seaweeds have been carried out (Newton, 1963). The use of seaweed meals has been intensively studied in recent years in order to establish their actual nutritive value. Most evidence points mainly to their value as sources of vitamins and trace elements, and in this respect their value is higher than that of dried grasses (McInnes, 1956; Black and Woodward, 1957; Booth, 1964b). Protein values fluctuate too much in the course of the year for this to be an additional factor of value. The value of seaweed meals also lies in the fact that any season of harvesting makes little difference as far as the important constituents are concerned.

Experiments in which dried seaweeds were tested as supplementary feeding for young mink have been reported (Kovalevskaya *et al.*, 1961). The animals digested the algae poorly, and in general the diet affected growth, fertility and fur production. Too high a percentage of algae caused excessive loss of fur. In this instance it was suggested that the algae were no substitute for high calorie value animal foods.

A condition similar to " swayback " has been described for lambs borne of sheep fed mainly on *Rhodymenia* and *Alaria*, particularly during the latter half of the gestation period. This condition, due to demyelination, was prevented by using more hay and adding copper supplements in Iceland (Hallsson, 1964). In the Orkneys a similar condition is observed at lambing time if sheep are not taken off seaweed feeding (*Rhodymenia*) 6 weeks prior to lambing. In North Ronaldshay no record of swayback is given although sheep are fed for 9–10 months, and this is possibly because *Laminaria* sp. are mainly used (Discussion in Hallsson, 1964).

Seaweed meal made from *Ascophyllum nodosum*, *Fucus vesiculosus* and *F. serratus* was subjected to an extensive range of tests with sheep

and hens (Jensen, 1958). With ewes there was no significant effect on fertility, although those ewes fed with seaweed meal produced lambs which showed a significantly higher rate of growth. Increased wool production was a further result of seaweed meal supplement. The laxative effects of too high a supplementation with seaweed meals have frequently been described. Experiments with very young chicks showed that seaweed meal had similar effects to commercial grass meal when added to rations deficient in vitamins, and evidently all the vitamin A and D requirements can be supplied from this source. Jensen (1964) observed that the ascorbic acid content of *Ascophyllum nodosum*, *Fucus serratus* and *F. vesiculosus* showed a seasonal variation, being highest in spring, and declining through summer and autumn to the lowest value in winter. The vitamin contents of algae have also been described by Freudenthal (1956) and Hundin and Ericson (1956). The importance of carotenes as precursors of vitamin A is well known, and the carotene and carotenoid contents of marine algae have also been studied (Haug and Larsen, 1956; Liaaen and Sørensen, 1956).

The use of seaweed in poultry food has been extensively studied. Thus Høie and Sandvik (1956) and Black (1954) reported that *Laminaria hyperborea* and *Ascophyllum nodosum* meals caused heavy water consumption and loss in weight if used in too high a quantity, and a rapid decrease in egg production has also been recorded. Høie and Sandvik (1956) and Sunde (1956) have reported that seaweed meals lead to an enhanced iodine content in the egg yolk. Yolk colour is also improved.

The value of seaweed meals as sources of trace elements and vitamins seems to be well established. There is also much evidence that the proportions of seaweed meal mixed with other feeding stuffs must be carefully watched, since harmful side effects have been recorded. *Ascophyllum nodosum* is extensively used as a source of seaweed meal, and for reasons given earlier (p. 144) heavy cropping of this plant can lead to a marked fall in the standing crop.

C. *Manurial value of seaweeds*

Newton (1963) has summarized data on the utilization of the large marine algae as manure. Direct utilization and methods of composting are practised in many coastal areas. Dried seaweed meals are marketed by a number of firms, using in particular *Ascophyllum nodosum* and *Macrocystis pyrifera*. Chapman (1950) quoted results of work in which the rates of decomposition of *Macrocystis*, *Ecklonia* and *Durvillea* were tested after being dug into the soil. All three were completely decomposed within 3 months, and the bulk of the Na^+, K^+ and Cl^- released

in 14 days. By contrast *Carpophyllum* remained recognizable after 1 year. *Macrocystis* application increased the yield of dwarf beans. Little (1953) reported on decomposition rates and manurial values of some common brown seaweeds. Many of the earlier studies on the manurial value of seaweeds were based on mineral analysis, but there is evidence that seaweed application is not of value as a sole dressing. Francki (1958) stated that manurial values should not be based solely on mineral analyses. Thus application of dried *Pachymenia* and *Durvillea* meal to soils of pH 6 or below resulted in the release of manganese in toxic quantities. Francki (1960a,b), in further investigation of the manurial values of desiccated, sun-dried meals of *Pachymenia himantophora* and *Durvillea antarctica*, used tomato plants, beetroot and radish as a means of bioassay. The general effects of applying *Pachymenia* were found to be those associated with manganese toxicity and phosphorus deficiency, particularly in soils of low pH where a noticeable depression of growth was observed. Results with *Durvillea* indicated metabolic changes normally associated with an inadequacy of available nitrogen, suggesting an immobilization of the element. Whilst *Durvillea* had no effect on soil consistency, *Pachymenia* induced marked waterlogging of soil and a general impermeability. From these results the suggestion was made that changes in ionic milieu, physical structure, soil microorganisms and production of growth-affecting organic compounds might follow dressing with these marine algae. Milton (1964) has stated that direct application of seaweed meals does indeed cause an inhibitory action on plant growth, but that this is overcome after a latent period of 15 weeks. In this initial period the nitrate and ammonium contents of the soil diminish, suggesting selective growth of microorganisms, but this is followed by a period of increased nitrogen content with a consequent stimulatory effect on plant growth. Partial composting with loam and urea cuts this induction period down to 14 days. Pressurized alkaline hydrolysis and liquefaction had the same result as composting, and retained the trace element content. Such an extract can be used as a direct soil dressing or applied as foliar sprays. In general there is a marked improvement in root growth and in crumb structure of the soil. The beneficial effects of seaweed applications for their trace element content has been commented on by Booth (1964b). Myklestad (1964) stated that *Laminaria hyperborea* was more valuable as a soil dressing in Norway on either newly cultivated ground or on soil in a poor condition; its value on well manured soil was far less marked. MacFarlane (1964b) observed that fields in the Channel Islands dressed with seaweeds showed marked water retaining properties during summer droughts.

Mowat (1963, 1964) commented on the auxin and gibberellin content of plants utilized as dried seaweed meals.

Whilst in general the value of using the large brown seaweeds as manures have long been realized in coastal areas, more recent work has indicated the ways in which the efficiency of the soil dressing can be markedly improved, and the methods of liquefaction offer a potential source of exploitation which could be of very marked value in horticultural practices. The fact that not all seaweeds are of equivalent value has been well underlined.

X. Factors Influencing the Seaweed Crop

A. *Diseases of economically important seaweeds*

1. *Fungal diseases*

There have been some reports in recent years on the effects of fungi on *Porphyra*. Thus Arasaki (1947) described a *Pythium* disease of *Porphyra tenera* which destroyed significant quantities of the population and was a potential threat to its large-scale culture. Optimum conditions for growth of the fungus were a sea temperature of 20°–24°C, and salinities in the range of 4 to 8°/$_{oo}$. Fungal infection in the early fall could be followed by a second " wave " of the disease. The disease was not readily observed on immature thalli; Johnson and Sparrow (1961) questioned whether the fungal growth was more in the nature of an invasion of senescent *Porphyra* plants. Suto (1952) observed that fungal infection of *Porphyra* tended to be more prominent during mild winters when the sea temperature was higher than average. A more recent report of *Pythium* infection has been made by Tsuruga and Nitta (1960). Chytrid infection of the *Porphyra* thallus has also been reported (Arasaki, 1960; Arasaki *et al.*, 1960). This infection has been frequently observed in the culture fields in the seas of central and northern Japan during the main growing season, November to March. An intracellular species of *Olpidiopsis* has been identified as the causative agent, infecting the host plant at all stages in its life-history except the *Conchocelis*-phase. Whilst damage to mature fronds is slight it could have more severe effects on sporelings and young thalli. Arasaki *et al.* (1960) have reported that gall-like growths can result from chytrid infection. Suto (1952) reports that fungal disease of *Porphyra* on " hibi " may be controlled by exposure of the plants to the air for daily periods of 3 hours. Laver diseases are known to be more prevalent when the plants are grown in deeper water (Kurogi, 1963b).

Other diseased conditions of uncertain cause have been reported.

Thus the " perforating " disease reported by Suto and Umebayashi (1954) could reduce the market value of the laver if of widespread occurrence. A diseased condition of the *Conchocelis*-phase has been described in which orange-yellow spots appeared in the filamentous growth within the shell. Infected cells had a high bacterial content, but whether this was the cause, or was secondary to the disease, is not known (Nozawa and Nozawa, 1957). A " yellow-patch " disease of *Conchocelis* has been reported (Horida *et al.*, 1960). There are no reports on the severity of infection, or of possible spread of such conditions in the *Conchocelis* culture tanks.

The occurrence of saprophytic fungi on fucoid algae has been reported for many years (see review by Wilson, 1960; also Johnson and Sparrow, 1961). Fungal hyphae have been observed in anatomical investigations of seaweed meals (Baardseth, 1964). The pyrenomycete *Leptosphaeria chondri* (Rostr.) Rosenvinge has been found on decaying cystocarpic and tetrasporic pustules of *Chondrus crispus* (Marshall *et al.*, 1949). Certain of the fungi associated with decaying seaweeds have been found to show some laminarase activity (Chesters *et al.*, 1956), and some degree of hydrolysis of sodium alginate was also recorded. The same workers found that actinomycetes could hydrolyse insoluble laminarin, and also showed some alginase activity.

2. *Bacterial diseases*

" Black-spot " of *Macrocystis pyrifera* has been attributed to bacteria, and can wipe out a kelp bed with the result that several years will elapse before the standing crop returns to its pre-infection status (see Chapman, 1950). A tenfold decrease in the available *Macrocystis* has been reported after severe infection of the kelp beds. The disease appears to be associated with high sea temperatures (18°–20°C), and the floating fronds are more susceptible than those in deeper water. Harvesting in May and June keeps down the incidence of the disease. Bunt (1955), describing the decomposition of thalli of *Durvillea antarctica*, reported that penicillea, bacteria, protozoa and yeasts were responsible, and that the yeasts attacked the stored carbohydrates within the tissues. Ando and Inoue (1961) reported on a diseased condition of *Laminaria japonica* which appeared initially as small perforations in the lamina and resulted in degeneration of the bulk of the frond. A vibrio-type of bacterium producing an active alginase, and with a pathogenic action on *Laminaria*, was isolated from the infected fronds. The disease caused extensive damage to the kombu beds near Wakkanai, Hokkaido, during 1959.

B. *Effects of various contaminants on seaweeds*

1. *Sewage and oil pollution*

It is well known that coastal waters are richer in plant nutrients than the open sea due to land drainage and disposal of sewage from large population centres. The rank growth of *Ulva* plants, with thicker fronds and high nitrogen content, in the vicinity of sewage outfalls in the Belfast Lough was described many years ago by Cotton (1910), but there are indications from work in recent years that sewage and industrial waste can prove harmful to marine algae. Thus Katayama and Fujiyama (1957) reported that *Porphyra* plants in laver fields in the sea off Fukuyama developed a tumour-like growth (or crown gall) in heavily polluted waters during December and January. In clearer waters onset of the disease was delayed by one or two months. Analysis showed that severity of tumour growth could be correlated with increase in the DNA content of the thallus. Whilst no specific cause was mentioned, the effect was attributed to toxic effluents. A similar report from Tokyo Bay described a cancer disease of the *Porphyra* fronds with abnormally swollen cells and gall-like growths. This, together with a chytrid infection, appreciably lowered the " nori " yield in the subsequent harvest (Arasaki *et al.*, 1960). Polluted harbour water appears to have a harmful effect on *Chondrus crispus* (Grenager, 1957), although *Porphyra perforata* (Kylin) appears to favour polluted waters during winter and spring (Denmark, Holland, German Baltic, Nice; Den Hartog, 1964). *Gigartina* forms richer growths where sewers enter the sea on Great Cumbrae, Scotland (Marshall *et al.*, 1949). Fuel oil contamination has a distinctly harmful effect, and harvestable crops of *Gigartina* and *Chondrus* were reported to be ruined on shores in the sheltered waters of Plymouth Sound, and in certain localities on the west coast of Scotland both oil and tar pollution of *Gigartina* beds was reported (Marshall *et al.*, 1949).

Marked reduction in the *Macrocystis* population near sewage outfalls has been reported from California (North, 1964). This was attributed either to toxic effluents, or to the greater turbidity of the water cutting down submarine illumination to the extent that growth of the giant kelp could not keep pace with the browsing action of associated animals. By enclosing young *Macrocystis* plants in wire cages (1-cm mesh), and removing the " browsing " pressure the plants were able to grow successfully in the vicinity of the sewage outfall. Clearly not all sewage effluents are harmful, but with edible products there will be obvious concern for both the hygienic condition and appearance of the product.

2. *Toxic chemicals*

Whilst marine algae are known to accumulate trace elements to a considerable degree there is, nevertheless, some evidence that harmful elements may be assimilated. For example, the arsenic content of dried *Porphyra* was found to be at a higher level in culture fields where there was contamination from industrial effluents (Kawashiro *et al.*, 1961). Certain aspects of the metabolism of *Porphyra tenera* were adversely influenced by low concentrations of industrial waste, e.g. " black liquor " from pulp mills, waste from distilleries, resinous wastes (Tsuruga, 1963b); these effects were more noticeable in shallow inshore waters. The possibility that certain herbicides may prove harmful on draining into the sea must also be borne in mind (Boney, 1963), although experiments with low concentrations of pentachlorophenate, a herbicide used in paddy fields, when measured in terms of appearance showed the enzyme activity and phosphorus metabolism of the *Conchocelis*-phase of *Porphyra* to be less sensitive than those of certain inshore fish (Tomiyama *et al.*, 1962).

3. *Radioactive elements in sea water*

The fact that there may be an increase in the quantity of radioactive material in the sea due to disposal of waste, and as a result of fall-out from atomic explosions, is clearly a problem affecting seaweed industries concerned with edible products. Studies on the uptake of radioactive elements by seaweeds can be considered from two aspects: those concerned with the uptake of material in natural habitats, and studies which are linked with the metabolic utilization of labelled compounds. The latter have also rendered information of value for work on seaweeds of economic importance. Palumbo (1959) reported the gross beta radioactivity of the marine algae at Eniwetok Atoll (during 1954–56), and showed that the highest levels of radioactivity were recorded in plants collected from shores of islands closest to the site of the test detonation and in the downwind path of the fallout. Both the algae and the bottom sand retained more radioactivity than the surface waters, and the algae tended to absorb isotopes with the longer half-lives. Akamsin *et al.* (1960) observed that *Cystoseira barbata* accumulated appreciable quantities of natural radioactivity from the surrounding medium, and suggested its use as an indicator of the quantities of artificial radioactivity in sea water. Subsequently Polikarpov (1961) measured the uptake of strontium-90, caesium-137 and cerium-144 by *Cystoseira barbata* and the rate of loss of these isotopes when the thalli disintegrated in the sea. Work on various aspects of this problem has also been carried out in Japan. Thus Tsuruga and

Takeuchi (1960b) reported on the radioactivity of *Porphyra tenera* from Hiroshima Bay, and Tsuruga (1962a,b) subsequently studied the absorption of ruthenium-106 by marine algae. Measurements of activity due to this isotope were made on *Porphyra tenera* and a number of other algae in 1958 and 1959. Contamination was considered to be due to fallout from test explosions, since the influence of the 1945 A-bomb on the city should by now have gone, and there was no discharge of radioactive effluent from the neighbourhood. Other seaweeds with a fairly high ruthenium-106 content were *Gloiopeltis furcata, Monostroma nitidum* and *Polysiphonia fragilis*. These are plants of the intertidal belt, and it was suggested that the fallout contamination could be present in rainwater. Under field conditions species of the lower-zoned *Sargassum* showed lower values for ruthenium-106, although when measured in the laboratory it accumulated very high concentrations of the nuclide. In general the seaweeds rapidly absorbed the nuclide. In a review of radioactive wastes and marine algae (planktonic and benthonic) Eppley (1962) showed that there was both active and passive uptake of ionic and particulate forms of radionuclides.

Other relevant studies involving radionuclides and marine algae include the pioneer experiments by Spooner (1949) using strontium-90 and yttrium-90 with brown, green and red algae. This work showed that whilst *Fucus serratus* extracted strontium-90 from seawater by a process of ion exchange, *Ulva lactuca* and the red algae *Gigartina stellata* and *Rhodymenia palmata* extracted small and negligible amounts of the element. By contrast, yttrium-90 was heavily taken up by the red and green algae, but to a much lesser extent by *Fucus serratus*. The uptake of cobalt-60 by *Rhodymenia palmata* was studied by Scott and Ericson (1954). The element formed stable complexes within the cells as either a direct or indirect result of photosynthetic activity, and these compounds were in a state of continuous metabolic turnover. More cobalt-60 was taken up than could be linked with vitamin B_{12} manufacture by the plant. Bowen (1956) studied the strontium-90 contents of sea water and marine algae, and considered that even though the algae were natural ion-exchange systems, the hazard from strontium-90 in sea water was (at that time) negligible when compared with the amounts of naturally radioactive material present.

Other experimental studies on the uptake of radionuclides by seaweeds include the work of Kelly and Baily (1951) on the uptake of radioactive iodine by *Ascophyllum nodosum* and of Shaw (1959) on the uptake of iodine-131 by *Laminaria digitata;* this latter paper also summarizes earlier work on the uptake of radio-iodine by marine algae. The potentialities of using the uptake of zinc-65 as a means of measuring

primary production by benthonic marine algae has been investigated by Bachmann and Odum (1960). Jones (1960) studied the accumulation of radio-ruthenium by *Porphyra laciniata*. The mechanism of uptake of zinc-65 by *Ulva lactuca* has been described by Gutknecht (1961). Zinc accumulation was an active process related to the rate of photosynthesis and is governed by pH and the zinc concentration of the medium. Freshly killed *Ulva* thalli initially absorbed more of the element than living plants. Mauchline and Templeton (1964) have recently reviewed several aspects of the distribution of artificial and natural radioisotopes in the sea and their uptake by plants and animals.

C. *Browsing by animals*

The competitive interaction between browsing herbivores and seaweeds has been studied for many years. Some of the more striking results in this direction have come from large-scale experiments carried out on the shores of the Isle of Man (Jones, 1948; Lodge, 1948; Burrows and Lodge, 1950; Southward, 1953, 1956). In one of these experiments an area of shore 10 m wide and 150 m in length was cleared of all vegetation and limpets; the area extended approximately from M.H.W.N.T. to M.L.W.S.T. Recolonization then followed successive stages, starting with a diatom film, then a dense cover of green algae (*Ulothrix*, *Ulva*, *Enteromorpha*). Germlings of fucoid algae developed under the green algae and resulted in almost pure stands of *Fucus* species after about 2–3 years, consisting of *Fucus spiralis*, *F. vesiculosus*, *F. serratus*, and of hybrids between them. Somewhat similar results have been obtained in clearance experiments carried out at other localities in the Isle of Man and on the Devon coast (Boney, unpublished observations). In this last case, removal of limpets was made from an area of shore in the region of M.T.L. 4 m wide and 10 m in length, and devoid of fucoid algae. As a result of the recolonization stages almost pure stands of *Fucus spiralis* and *Fucus vesiculosus* were obtained. Continued observations at each locality showed a re-invasion of the cleared area by limpets which appeared in increased numbers, showing rapid growth under favourable conditions. The gradual decrease in the fucoid population which then followed was due to the detachment of the old plants and the absence of any recruitment because of the limpet population browsing on the sporelings. In addition to their effect on the young plants, the limpets have been observed to feed on the stipes and holdfasts of older plants, and probably weaken them sufficiently to cause their subsequent detachment. A similar weakening effect is thought to be due to the browsing action of *Patina pellucida* on the stipes and holdfasts of *Laminaria* species (Kain,

1963). The results of the clearance experiments described above show that there is a dynamic relationship between the population of algae, limpets and barnacles in a wave-beaten shore. In earlier pages reference was made to the slow rate of recuperation by *Ascophyllum nodosum* plants after too severe harvesting. Observations have shown that this is in part due to the depredations of *Littorina* species, which browse heavily on the *Ascophyllum* sporelings (Knight and Parke, 1950). In sublittoral habitats the browsing activity of species of *Echinus* can severely depopulate the algal cover (Forster, 1959). The action of browsing herbivores on giant kelps (*Macrocystis*) was referred to in earlier pages. This included kelp-eating fishes, the gammarids *Amphithoë humeralis* and *Idothea resecata*, and sea urchins. Reference has also been made to the digestive tracts of some marine invertebrates possessing enzymes capable of splitting the polysaccharides of marine algae, and in some instances containing bacteria which can bring about this chemical breakdown. There are also many references to the animal populations associated with the holdfasts of kelps (*Macrocystis*, *Nereocystis*, *Laminaria* sp.), and that many animals browse on the surface of algae in order to feed on the diatom or bacterial film (summarized in Scagel, 1959). In this last case the damage to the algae can cause a weakening of the thalli. Saito and Nakamura (1961) have described the severe depredation of the thalli of *Undaria pinnatifida* by the sea hare *Aplysia juliana* Quoy and Gaimard, and made estimates of the quantities of plant tissue devoured by the mollusc. Both *Chondrus* and *Gigartina* can suffer damage from browsing animals and show a proliferation of new branches as a result (Marshall *et al.*, 1949). The dynamic interrelationship between browsing activity and the algal population on wave-beaten shores described earlier has its counterpart in the sublittoral balance between *Macrocystis* and its predators (p. 151); the more serious effects of the browsing animals on *Macrocystis* plants transplanted from another area are also relevant to proposals that large-scale transplantations of brown algae might be attempted, e.g. transplantation of *Macrocystis* to sublittoral habitats in Scotland.

XI. Conclusions

Anyone preparing a review of the seaweeds of economic importance must be impressed by the wealth of data accumulated over the years. In fact the statements which imply that there is little chance of development of a major industry seem belied by the masses of data which so frequently appear. The scope of seaweed utilization, both direct and for extractives, is at present extensive, and there is plenty of evidence

that there will be further large-scale exploitation. We may therefore enquire into the extent to which the available knowledge has made any positive contribution to a more effective usage of the available stocks of marine algae.

Whilst in many instances studies on the autecology of brown and red algae have indicated the best times when frondage can be removed without too serious an effect on subsequent plant growth, there are still quite common examples in which the cycle of events is still not known with any certainty, e.g. the problem of recuperative growth in *Ascophyllum*. For a number of species there is now a much clearer picture of the link between annual phases of growth and reproduction and the condition of the extractives. But there are still several representatives of potential value as sources of extractive for which more detailed studies of their autecology would be necessary before any large-scale usage is envisaged, e.g. *Gelidium* species at present used on a small-scale, *Eucheuma* sp., *Hypnea musciformis*.

Studies on the periodicity of sporing and on the quantities of spores liberated, and on the settlement, attachment and early growth stages have been carried out under both field and laboratory conditions, and have shown that for some species an extension of the areas under cultivation is possible. For example, the microscopic gametophytes of *Laminaria* can be cultured under laboratory conditions and the young sporophytes reared and later transplanted in the sea. "Nursery" growth of the young stages offers some scope for increasing the standing crops of some representatives. Culturing the *Conchocelis*-phase of *Porphyra* in oyster shells suspended in culture tanks on land as practised in Japan (p. 182) appears to have markedly increased the productivity. Sowing the spores on artificial surfaces under suitable conditions on land and then planting the germlings out in the sea appears to be a more successful method than collecting the spores from fruiting plants and using them directly. For a few species there is also some evidence that a combined knowledge of fruiting periodicity and of times of tides and directions of water currents enables some prediction to be made of the areas where maximal spore settlements can be obtained.

Crop plants on land are studied for information on the value of their products, and for knowledge of their growth and reproductive phases, and the ways in which all these are influenced by environmental factors. The effects of parasites (fungal and animal) and of nutrient deficiencies are also examined. Many of the topics of investigation carried out with the seaweeds of economic importance over recent years have been of a similar nature to those outlined above. There is a good deal of information now on the seasonal fluctuations in the extrac-

tive content of marine algae, and of changes associated with the growth or reproduction of the plants concerned. Destructive diseases, fungal and bacterial, can seriously deplete the seaweed crops. Sudden changes in local conditions, such as water turbidity, can, if prolonged, upset the balance which exists between growth of sublittoral algae and the browsing " pressure " of marine invertebrates. Sewage and toxic effluents are features to be closely watched in the vicinity of " stands " of harvestable plants. Information on seaweed growth and supply of nutrients is less clear-cut for large plant populations, although a good deal of data are available on both mineral and organic nutrients and their effects on the growth of young stages of algae. The increase in plant productivity as a result of the fertilization of a Scottish sea loch has been reported (see summary in Newton, 1963). The cultivation of edible *Laminaria* plants in the vicinity of porous containers of mineral salts, as reported from China (p. 159), has shown that marked increases in organic production can be obtained. To carry out similar methods on a large scale with underwater beds of seaweed would require a detailed knowledge of the currents in the immediate vicinity of the areas under cultivation.

One aspect for which there appears to be little quantitative data concerns processes of " weeding " unwanted algae from underwater beds of seaweed. Use of the self-contained breathing apparatus should enable some progress to be made in this direction, and observations will need to be extended over some time to obtain the more significant results.

Attempts have been made in recent years to investigate the effects of cross-breeding between different species of marine algae in order to obtain more valuable products. The methods pose appreciable difficulties of technique, e.g. the work carried out with the edible *Porphyra* species. However, the methods evolved in studies on the " nursery " growth of the very young stages make the practical applications of such cross-breeding experiments at least a possibility.

Consideration must also be given to the important factor of conservation. Whilst with the shore fucoids, particularly *Ascophyllum*, some degree of controlled harvesting is clearly necessary, the data are far less clear-cut for other species. If the experience of the last two decades is any guide, there will be no slackening of demand for raw materials. For example, a fivefold increase in the value of the annual output of seaweed colloids was reported from the United States for the years 1945–1955 (Newton, 1963). Large-scale harvesting of some species, e.g. *Furcellaria fastigiata* f. *aegagrophila*, has been carried out on an increasing scale over the years without apparently seriously depleting

the standing crop. There is always the possibility, however, that some environmental change or fungal or bacterial disease may seriously deplete the underwater beds of seaweed. Continued intensive harvesting over the years may affect the ability of the plant populations to withstand any future depredations. The need for conservation should be an essential part of any planning of large-scale exploitation of marine algae. There are cases where this need has been discovered at too late a stage for effective measures to be taken. Whilst there are many aspects of the biology of the economically important seaweeds which remain to be fully explored, a welcome trend in recent years has been shown in those studies which have linked taxonomic observations with the seasonal life of the plants in different habitats, with a close watch on the cycles of growth and regeneration, and on the morphological changes which may be involved. Work of this nature, together with studies on plant biochemistry, can lead to a more effective exploitation of the available resources.

XII. References

• (All references marked with an asterisk are in Japanese with English summaries.)

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Addendum

Some further references particularly relevant to the subject matter under review have become available since the script was forwarded to the publishers.

Further information on seaweed extractives is available in reviews by Hoppe (1962), Hoppe and Schmid (1962), Maass (1962), Schmid and Hoppe (1962) and Schulzen (1962). Kanazawa (1963) has summarized data on the occurrence of vitamins in algae. More data on seaweed

resources are available in the work of Tseng and Chang (1959) for the economic marine algae of the Yellow Sea and the East China Sea, and Varma and Rao (1964) have reported on the algal resources of Pambam. The standing crops of harvestable *Ascophyllum nodosum* on the shores of Southern Iceland have been described by Munda (1964), together with some account of their chemical composition. Diaz-Piferer and de Perez (1964) have given an account of the agar producing and agaroid algae of Puerto Rico. The extractive content of forty-four species is described, together with more detailed accounts of the biology of nineteen plants, including *Hypnea musciformis* and thirteen species of *Gracilaria*. Accorinti (1963) has given additional information on the seaweeds of economic importance in Argentina.

Further information is also available on aspects of the biology of certain of the marine algae described in the review. Thus Kain (1965) has reported on the results of a study of the effects of light and temperature on the early growth of the sporophyte of *Laminaria hyperborea*. Mitrakos (1964) has given an account of the effects of various environmental factors (pH of the medium and light) on the growth of the young carposporophytes of *Gracilaria verrucosa*. Further studies on the productivity of the Japanese agarophyte *Gelidium amansii* have recently been reported. Thus Yamada (1964) has described the uptake of phosphorus and nitrogen and Yamada *et al.* (1964) have reported on the rates of absorption of P^{32} compounds. The use of mineral salts as fertilizers for underwater beds of the agarophyte has been studied (Yamada and Iwahashi, 1964). A marked improvement was observed in both the colour and condition of " yellowed " *Gelidium* fronds after bags of fertilizer had been placed in the near vicinity of underwater beds of the agarophyte.

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MARINE TOXINS AND VENOMOUS AND POISONOUS MARINE ANIMALS

FINDLAY E. RUSSELL

Laboratory of Neurological Research, Loma Linda University, Los Angeles County Hospital, Los Angeles, California, U.S.A. and *Department of Zoology, University of Cambridge, England*

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I. Introduction

This review treats of the toxins of some of the more venomous and poisonous marine animals of the world. For the most part, it is concerned with the chemical, zootoxicological and immunological properties of the toxins, the animal's venom apparatus, and the mechanism of envenomation. Some attention has been given to the general biology of the animals, and to the problem of the poisoning in man by the various forms. A second purpose of the review is to present an account of several of the more interesting problems in the emerging field of marine toxinology.

Approximately 1000 species of marine organisms are known to be venomous or poisonous. For the most part, these species are widely distributed throughout the marine fauna from the unicellular protistan, *Gonyaulax*, to certain of the chordates. They are found in almost all of the seas and oceans of the world, and while their numbers may sometimes be quite large, they do not produce major ecological effects by virtue of their toxicity alone, nor are they other than a local danger to man's health and economy. It is generally believed that most of the venomous marine animals have been identified; although it must be conceded that a number of forms have not yet been adequately described, and certainly our knowledge of the potentially dangerous deep-sea organisms is meager indeed.

A. *Definitions*

It might be wise to consider a few words and terms that are peculiar to toxinology. The term *venomous animals* is usually applied to those creatures which are capable of producing a poison in a highly developed secretory organ or group of cells, and which can deliver this toxin during a biting or stinging act. *Poisonous animals* are generally regarded to be those whose tissues, either in part or in their entirety, are toxic. In reality, all venomous animals are poisonous but not all poisonous animals are venomous. Animals in which a definite venom apparatus is present are sometimes called *phanerotoxic* (Gk. φᾰνερός, evident + τοξικόν, poison), while animals whose body tissues are toxic are called *cryptotoxic* (Gk. κρυπτός, hidden). The rattlesnake, stingray and black widow spider are venomous or phanerotoxic animals, while the blister beetles, certain puffer fishes and toads are said to be poisonous or cryptotoxic.

Although the terms *venomous* and *poisonous* are often used synonymously, most investigators have tried to confine the use of the term venomous animals to those creatures having a gland or group of highly

specialized secretory cells, a venom duct (although this is not a constant finding), and a structure for delivering the venom. While there has been a tendency to employ the term *venom apparatus* to denote only the sting, spine, jaw, tooth or fang used by the animal to inject or deliver its venom, most biologists now use the term in its broader context, that is, to denote the gland and duct in addition to the sting or fang. Poisonous animals, as distinguished from venomous animals, have no such apparatus; poisoning by these forms usually takes place through ingestion.

Fish poisoning is synonymous with *ichthyotoxism*. It does not include that type of poisoning which may occur following ingestion of fish contaminated by bacterial pathogens. Halstead (1964) has divided ichthyotoxic fishes into three subdivisions: (a) *Ichthyosarcotoxic fishes*—those fishes which contain a toxin within their musculature, viscera or skin, which when ingested produce deleterious effects. This type of poisoning is generally identified with the kind of fish involved: elasmobranch, chimaeroid, ciguatera, tetraodon, scombroid, etc.; it also includes hallucinatory fish poisoning. (b) *Ichthyootoxic fishes*—those fishes which produce a toxin that is generally confined to the gonads. In these fishes there is a relation between gonadal activity and the production of toxin. Most members of this subdivision are fresh-water species. This group would include those fishes whose roe is poisonous. (c) *Ichthyohemotoxic fishes*—those fishes which have a toxin in their blood. Some fresh-water eels and several marine fishes make up this group.

B. *History and folklore*

Few areas in biology have had their beginnings as steeped in superstition and myth as have toxinology and the poisonous animals. The investigation of such complex substances as toxins, often capable of destroying life by complicated and sometimes undeterminable means, has by its very nature invited exaggeration, and sometimes pure fantasy. In early times the consequences of the bites or stings of venomous animals were often attributed to forces beyond nature, sometimes to vengeful deities thought to be embodied in the animals. To these early peoples the effects of venoms were so surprising, varied and violent that venomous animals and the injuries they inflicted were always shrouded with much myth and superstition. Even today considerable folklore about venoms still exists, and this is particularly conspicuous with respect to the methods of treatment for the injuries inflicted by venomous animals (Russell, 1961). The task of separating fact from

fiction is often a formidable one, and one not always lightened by the passage of time.

Egyptian medical records, possibly dating from 1600 B.C., contain some advice on the treatment of venom poisoning, and some descriptions of venomous animals. Perhaps one of the earliest references to toxic marine organisms is found in Exodus 7 : 20–21 : " . . . and all

FIG. 1. The stingray, *Dasyatis pastinaca* (L.), and the greater weever, *Trachinus draco* L. From Grevin (1571).

the waters that were in the river turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river . . . " (Moses, *c.* 1491 B.C.). This description is thought to refer to a " bloom " of toxic dinoflagellates.

But the most exhaustive and credulous early writer of natural and

medical history fact and fiction was Pliny, whose voluminous work *Historia Naturalis* contains numerous fascinating accounts of venomous and poisonous animals. Describing the stingray, Pliny writes:

> " So venimous it is, that if it be struchen into the root of a tree, it killeth it: it is able to pierce a good cuirace or jacke of buffe, or such like, as if it were an arrow shot or a dart launched: but besides the force and power that it hath that way answerable to iron and steele, the wound that it maketh, it is therewith poisoned."

So frequently quoted were Pliny's works that Hulme (1895) commented:

> " Several writers of antiquity influenced the mediaeval authors, but it is scarcely necessary to detail their labours at any length, since if they lived before Pliny he borrowed from them, and if they lived afterward they borrowed from him, so that we practically in Pliny get the pith and cream of all."

As Klauber (1956) has so aptly concluded: " Pliny's *Historia Naturalis* was the funnel through which we can watch the ancient folklore pouring down into the mediaeval and modern worlds."

Between the days of Pliny and the present a number of works on venomous and poisonous animals, including the marine forms, and their toxins have been published. These include the fine contributions by Grevin (1571), Autenrieth (1833), Bottard (1889), Phisalix (1922), Pawlowsky (1927), Evans (1943), Phillips and Brady (1953), Buckley and Porges (1956), Kaiser and Michl (1958), Nigrelli (1960), Keegan and Macfarlane (1963), Halstead and Russell (1964). By far the most comprehensive work on toxic marine animals is that being prepared by B. W. Halstead (1965–66). The author recently had the pleasure of browsing through Dr. Halstead's manuscript. The two volumes are the most complete and fascinating work yet prepared on this subject. They will greatly enhance our knowledge of the venomous and poisonous marine organisms.

C. *General chemistry and zootoxicology of marine poisons*

Marine toxins vary considerably in their chemical and zootoxicological properties. Some are proteins of low molecular weight, while others are proteins of obviously high molecular weight. Some of the toxic fractions appear to be amines or quaternary ammonium compounds or polypeptides or mucopolysaccharides, while the structure of still others is unknown. Some marine toxins contain enzymes but these substances are not nearly as common as they are in the reptilian venoms, nor are they to be found in such large quantities within a

single toxin. Another characteristic of many marine venoms is their relative instability. Some are very labile, even at temperatures down to 0°C. While the marine toxins as a whole are far more varied in their chemical composition than the venoms of terrestrial snakes or arthropods, there is some degree of consistency (or lack of consistency) within a particular phylum, which is not unlike that seen in the terrestrial venomous animals. The more simple marine forms have poisons composed of one or several components having deleterious biological effects; the higher forms have poisons containing more components, and in general these fractions appear to be more complex in structure and function.

The zootoxicological properties of marine toxins vary as remarkably as do their chemical properties. Some marine venoms provoke rather simple effects, such as transient vasoconstriction or vasodilatation, while others provoke more complex responses, such as parasympathetic dysfunction or multiple concomitant changes in the blood-vascular dynamics. The effects of the separate and combined activities of the fractions of these poisons, and of the metabolites formed by their interactions, is further complicated by the response of the envenomated organism. The organism may produce and release several autopharmacologic substances which may not only complicate the poisoning but which may in themselves produce more serious consequences than the venom.

The zootoxicological study of marine poisons is further complicated by the fact that qualitative as well as quantitative differences in the chemical composition of these toxins may exist, not only from species to species within the same genus, but from individual to individual within the same species. A venom may even vary within the individual animal at different times of the year or under different environmental conditions. Thus, discrepancies in the proposed mode of action of a toxin are likely to occur until our knowledge of the individual fractions of these complex substances is more complete. In addition, obvious difficulties in determining the chemical and zootoxicological properties have arisen because of the differences in the methods of extracting venom, the methods of storage (fresh, lyophilized or crystallized), and the problems inherent in the methods of bioassay.

Most of our information on the zootoxicological properties of marine toxins is based on studies with mammals, which, of course, somewhat limits its application as far as understanding the design of the toxin in the animals' armament. The venom of the black widow spider, for instance, did not evolve and adapt to the problems existing between that spider and mammals. Thus, it is not surprising to find

that its venom is twenty times less lethal to some arthropods than it is to the mouse, while on the other hand is is also ten times more lethal to certain other arthropods which have not adapted in the same manner. Some sharks appear to be relatively immune to stingray venom while others from completely different habitats are very sensitive to this toxin. Some non-venomous reptiles are not only immune to the venom of certain of the snakes of their area but actually feed upon these snakes. These various studies indicate that care must be exercised in applying data derived from studies in one group of animals to conclusions about the biological effects of a toxin in another group of animals, or to data on the design, use and adaptation of a toxin.

No comprehensive classification for toxins now exists. Our knowledge of the chemical and zootoxicological properties of these complex substances is not broad enough or consistent enough, at the present time, to permit the adoption of a single working classification. It would seem, in the absence of a useful and reliable method for classifying toxins that it might be wisest to develop a system based on the taxonomy of the animal. While such a classification would be somewhat bulky, and would need to be altered periodically with changes in taxonomy, it might serve our purpose during the interim in which we attempt to organize our data on the chemical and biological properties of these complex substances in a more thorough manner.

On the other hand, perhaps some consideration for classification might be proposed on the basis of the use to which the animal puts its toxin. In general, most venom delivered from the oral pole is used by the animal during an offensive act, as in the gaining of food. This is particularly evident in the snakes and only slightly less so in the spiders. The venoms of these animals tend to have a higher enzymatic content than those delivered from the aboral pole, as those of the scorpions and bees. However, most of these animals use their toxins in their offensive armament; whereas the toxins of most venomous fishes and the poisons of certain amphibians, which are usually derived from dermal tissues, are used in the defensive armament. These latter toxins contain little, few or no enzymatic constituents. However, a classification based on these premises at the present time would be dangerous, since our knowledge on the use and adaptation of venoms is not yet complete enough to permit accurate classification.

Until the fractions of marine toxins responsible for the deleterious effects have been isolated and studied individually, and in combination, we need to exercise extreme care in systematizing data which are based partly on biological assay methods, partly on biochemical studies, partly on clinical observations and partly on intuitive hunches.

II. Protista

Most of the toxic protista are of the order Dinoflagellata. These organisms are widely distributed throughout neritic waters and in the high seas from the polar oceans to the tropics. " Blooms " of toxic dinoflagellates sometimes occur during weather disturbances, or under certain other conditions, and result in the phenomenon frequently referred to as " red water ", " red tide " or " brown water ". However, the bloom may appear yellowish, greenish, bluish, or even milky in color, depending on the protistan involved and a number of ecological factors. When excessive numbers of these unicellular organisms collect there may be a mass mortality of fishes and other marine organisms in the area. Such occurrences are frequently reported (Kofoid, 1911; Hornell, 1917; Nightingale, 1936; Galtsoff, 1948; Connell and Cross, 1950; Fish and Cobb, 1954; Smith, 1954; Brongersma-Sanders, 1957; Grindley and Taylor, 1962). Halstead (personal communication, 1964) lists a number of conditions which favor plankton blooms. These include changes in weather conditions that bring about upwellings or other alterations in water masses, changes in nutrient salt concentrations, changes in water temperature and in sunlight; and changes in those factors which affect water turbulence, transparency, surface illumination, passive sinking of the phytoplankton themselves to depths beyond the photic zone, and the grazing action of the zooplankton population.

The possible causes for the mass mortality of marine life during and following plankton blooms has been the subject of considerable discussion. Among the factors that have been implicated are: oxygen depletion in the water, due either to the number of plankton present or to the release of decay products by these organisms and the dying fish; asphyxiation through a blanketing of the fish with a mass of plankton; or the production of a toxin by the protistan. It is known that the early larvae of certain molluscs and crustaceans which respire through their body surfaces are not affected by blooms, while those forms with specialized respiratory gills die when these organs become covered with the disintegrating bodies of the plankton (Motoda, 1944). It would appear that the evidence to date favors the conclusion that in most instances the mass mortality of fishes and certain other marine forms during these maxima can be ascribed to physical rather than chemical phenomena, although there is evidence to the contrary.

A. *Paralytic shellfish poisoning*

Paralytic shellfish poisoning is caused by certain molluscs and one or two echinoderms and arthropods which have ingested toxic dinoflagellates and which are subsequently eaten by man. The relationship between blooms of plankton and shellfish poisoning was perhaps first noted by Lamouroux (cited by Chevallier and Duchesne, 1851), who observed that during certain seasons the sea appeared as a yellowish " foam ", and that this foam was probably responsible for the poisonous properties of the shellfish. However, most early workers attributed the poisoning to other causes: copper salts, putrefactive processes, diseases of the shellfish, a " virus ", other marine organisms, contaminated water, industrial wastes or bacterial pathogens.

In 1888, Lindner also suggested a food-chain relationship for shellfish poisoning, and subsequently this hypothesis received more favorable consideration. In 1937, Sommer and his colleagues published the results of their intensive investigation of the problem on paralytic shellfish poisoning. They demonstrated a direct relationship between the number of *Gonyaulax catenella* Whedon et Kofoid in the sea water and the degree of toxicity in the mussel, *Mytilus californianus* Conrad. These workers also established methods for extracting and assaying the poison, and suggested an experimental and clinical approach to the problem that has served as a guide for subsequent workers in the field.

Table I lists the marine protista of the order Dinoflagellata and one member of the Chrysophyta (*Prymnesium*) which are known to be toxic.

One member of the order Rhaphidophyceae, *Hornellia marina* Subrahmanyan, has been implicated in the deaths of fishes and crustaceans (Subrahmanyan, 1954).

The molluscs and other marine animals that have been implicated in paralytic shellfish poisoning due to dinoflagellate toxin, and the clinical syndrome of the poisoning, are noted in Section VI.

B. *Chemistry*

Following the report of a mass poisoning from the eating of mussels, Salkowski (1885) made what was probably the first important study on the chemistry of shellfish poison. He prepared four alcoholic extracts from mussel tissues, concentrated them by evaporation and then reconstituted with water. When the reconstituted product was forced into alcohol a viscous precipitate formed. The filtrate from this precipitation contained the poison. In 1888, Brieger isolated a substance he called " mytilotoxin ", which produced effects similar to those provoked

TABLE I. NOXIOUS FLAGELLATES

Protista	*Distribution*	*Type of poisoning in man* *Potentially dangerous to man**	*Reference*
Gymnodiniidae			
Amphidinium sp.	Temperate waters	*	Hunter (1962)
Cochlodinium catenatum Okamura	Japan	*	Miyajima (1934)
Gymnodinium brevis Davis	Gulf of Mexico and coasts of Florida	Respiratory irritant	Wilson and Ray (1956) Woodcock (1948) Lackey and Hynes (1955)
Gymnodinium sp.	South Africa	Paralytic shellfish poisoning	Sapeika (1958)
Gymnodinium galatheanum Braarud	Southwest Africa	*	Braarud (1957)
Gymnodinium mikomotoi Miyaki et Kominani	Japan	*	Miyajima (1934)
Gymnodinium splendens Lebour	Washington and British Columbia	*	Nightingale (1936)
Gymnodinium veneficum Ballantine	English Channel	*	Ballantine and Abbott (1957)
Noctilucidae			
Noctiluca scintillans (Macartney) Ehrenb.	World-wide	*	Aiyar (1936)
Peridiniidae			
Gonyaulax catenella Whedon et Kofoid	Pacific coast of North America	Paralytic shellfish poisoning	Whedon and Kofoid (1936) Sommer *et al.* (1937)

Gonyaulax monilata Howell	Florida	*	Howell (1953)
Gonyaulax polyedra Stein	Southern California, Portugal, Australia	*	Kofoid (1911) Santos-Pinto (1949)
Gonyaulax tamarensis Lebour	Atlantic coast of North America	Paralytic shellfish poisoning	Sommer *et al.* (1937) Needler (1949)
Heterocapsa triquetra (Ehrenb.) Stein	Baltic Sea	*	Lindemann (1924) Brongersma-Sanders (1957)
Peridinium trochoideum (Stein) Lemm.	Brazil	*	Faria (1914)
Pyrodinium phoneus Conr. et Wolosz.	Belgium	Paralytic shellfish poisoning	Koch (1938)
Polykrikidae			
Polykrikos schwartzii Bütschli	Atlantic Ocean; North, Baltic and Mediterranean seas; California	*	Sommer and Clark (1946) Brongersma-Sanders (1957)
Prorocentridae			
Exuviaella baltica Lohm.	Angola, West Africa	*	Silva (1956)
Prorocentrum micans Ehrenb.	California	*	Brongersma-Sanders (1957)
Prorocentrum sp.	Brazil	*	Faria (1914)
Prymnesiidae			
Prymnesium parvum N. Carter	Mediterranean Sea	*	Liebert and Deerns (1920) Shilo and Aschner (1953)

* Associated with mass mortality of marine organisms, or experimental data indicates their potentiald anger as a source of poison.

following the ingestion of toxic mussels. Richet (1907) isolated a substance which caused signs not unlike those described for one of Brieger's toxic fractions. As the signs were similar to those he had previously noted following poisoning with a toxin from sea anemones (" congestine "), he called the new poison " mytilocongestine ". Ackermann (1922) identified a number of bases in extracts from mussels, including adenine, arginine, betaine, neosin, methylpyridylammonium hydroxide and crangonine.

Partial purification of the toxin was obtained by Müller (1935) who used permutit as an absorbent, eluted with saturated potassium chloride and separated the poison by extraction of the residue from evaporation with methanol. Sommer and associates (1948) decolorized with active charcoal, filtered through charcoal, removed the alcohol by evaporation, extracted the lipid impurities with ether, passed the residue through sodium permutit and eluted with 20% barium chloride. Treatment with absolute ethanol removed the barium, and on evaporation to dryness the product showed a lethality of 6–12 μg per mouse unit.

Schantz *et al.* (1957) obtained high yields of the pure toxin from California mussels and Alaska butter clams using chromatography on carboxylic acid exchange resins prior to chromatography on acid-washed alumina. Mold *et al.* (1956) found that distribution of the toxin in a solvent system of *n*-butanol, ethanol, 0·1M aqueous potassium carbonate and α-ethyl caproic acid in a volume ratio of 146 : 49 : 200 : 5, with the aqueous layer adjusted to pH 8, resulted in a separation of the poison into two components; one of which was slightly more toxic than the other. It was suggested that the poison existed in two tautomeric forms because upon standing in acid solution each of the components equilibrated to form the same mixture.

According to Schantz (1963) both clam and mussel toxins are basic in nature, forming salts with mineral acids. They are stable in acid solutions but labile in alkaline solutions when exposed to the air. The dihydrochloride salts are very soluble in water but much less so in methanol and ethanol, and are insoluble in all lipid solvents. They have a specific optical rotation of + 130°, show no absorption in the ultraviolet, and have the same infrared spectra with strong absorption at 3, 6 and 9 μ. The molecular formula is $C_{10}H_{17}N_7O_4.2HCl$, and the molecular weight is 372. The poison takes up one mole of hydrogen to form a non-toxic compound. The poison does not appear to be a quaternary ammonium substance. Benedict-Behre and Jaffe creatinine tests are given by the toxin.

Tests for free guanidinium groups, enols of 1,3 diketones, primary

and secondary alcohols, and the Benedict test for reducing sugars have all been negative.

Wintersteiner *et al.* (see Schantz, 1960) have shown that oxidation of the poison in a mild alkaline solution exposed to the air reduces the toxicity in direct proportion to the oxygen uptake. The mixture had a molecular extinction of 6000 to 7000 in ultraviolet at 235 and 333 mμ. Further studies indicated that the unsaturated bond is probably involved in the toxic structure of the poison and would appear to be the point at which oxidation of the poison occurs when exposed to air at pH values above 7. With strong oxidation, Sakaguchi-positive compounds are obtained. Guanidoproprionic acid, urea, ammonia, carbon dioxide and guanidine have been isolated as oxidation products.

A comparison of the properties of the poison from mussels and clams (Schantz *et al.*, 1957) with those from dinoflagellates indicated that the toxins had very similar properties. Subsequently, in chromatographic studies, Burke and associates (1960) demonstrated that the toxin from *Gonyaulax catenella* and that from mussels move in a similar manner. In 1962, Schantz and colleagues isolated the toxin in a purified form from G. *catenella*; they found the physical, chemical and gross physiopharmacological properties of the poison to be identical with those of *Mytilus californianus* and *Saxidomas giganteus*.

It has been suggested that the toxin might be formed by a bacterium with the protistan but a number of investigators have isolated the dinoflagellates free of bacteria and demonstrated that the organism is still capable of producing the poison. This finding indicates that the toxin is a metabolic product and not the result of a symbiotic effect of bacteria.

The evidence to date suggests that ring structures are present and that several of the nitrogen atoms are involved in a heterocyclic structure. It appears that there are no aromatic structures, and no conjugate unsaturation or isolated carbonyl groups. Titration suggests that one of the two basic groups may be a guanidine (pK_a 11·5) and the other an amine (pK_a 8·1).

In reviewing the chemistry of " prymnesin ", the toxin from *Prymnesium parvum* N. Carter (a flagellate found in fresh, brackish and marine waters), Parnas (1963) notes that the poison is nondializable, poorly soluble in water, insoluble in carbon tetrachloride, chloroform, benzene, ether, ethyl acetate and *n*-butanol. It absorbs on activated charcoal, kaolin and $Mg(OH)_2$; but does not absorb on anion and cation exchangers. It gives a positive reaction for carbohydrates and to ninhydrin. Prymnesin prepared in the dark has a typical absorption at 260 mμ, which following irradiation falls to 240 mμ. The biological

activity is destroyed on heating. When maintained at an alkaline pH there is a loss of both the hemolytic and ichthyotoxic activities. More recent studies by Reich and Spiegelatein indicate that a number of chrysomonad flagellates may be toxic (Parnas, personal communication, 1964).

C. *Toxicology*

Determinations of the lethal dose of the poison under varying conditions and in different animals have been carried out by a number of workers. These studies indicate that this poison is one of the most lethal biological toxins known. Sommer *et al.* (1937) found that the dry weight of 3000 *Gonyaulax* was approximately 15 μg (about 15% of the wet weight), and that this quantity yielded 1 μg of the toxin, or 1 mouse unit. A mouse unit, or average lethal dose, was defined as the amount of toxin that would kill a 20 g mouse in 15 min with signs of paralysis or respiratory failure (Prinzmetal *et al.*, 1932; Sommer and Meyer, 1937). Subsequently, various testing methods and assays were studied by Medcof *et al.* (1947), Meyer (1953), and McFarren *et al.* (1956). McFarren and associates found the oral LD_{50} per kg body weight to vary considerably with the animal used and with its strain and weight. Their figures would indicate that the human is twice as susceptible to the poison as the dog and approximately four times more susceptible than the mouse.

In 1955 a Canadian-United States Conference on Shellfish Toxicology adopted a bioassay based on the use of the purified toxin isolated by Schantz and his colleagues (1958). Studies utilizing the methods outlined by the Conference indicate that the intraperitoneal minimal lethal dose of the toxin for the mouse is approximately 9·0 μg/kg body weight. The intravenous minimal lethal dose for the rabbit is 3·0–4·0 μg/kg of body weight. The minimal lethal oral dose for man is thought to be between 1·0–4·0 mg. Wiberg and Stephenson (1960) demonstrated that the LD_{50} of the purified toxin in mice was:

Oral route	263 (251–267) μg/kg
Intravenous route	3·4 (3·2–3·6) μg/kg
Intraperitoneal route	10·0 (9·7–10·5) μg/kg

Female mice were more susceptible than males, and increases in the pH or addition of sodium ions to the injection medium reduced the lethal activity.

In 1932, Prinzmetal *et al.* demonstrated that the poison from *Mytilus californianus* was slowly absorbed from the gastrointestinal tract and rapidly excreted by the kidneys. It depressed respiration, the

cardioinhibitory and vasomotor centers, and conduction in the myocardium. The rabbit and mouse seemed more susceptible to the toxin than the dog; the frog appeared quite resistant. Using the same poison, Kellaway (1935) demonstrated further actions on the nervous and cardiovascular systems. He suggested that the venom had a direct effect on both the central nervous system, particularly the respiratory and cardiovascular centers, and on the peripheral nervous system, particularly the neuromuscular junction and the sensory nerve endings. The poison caused a rapid fall in systemic arterial pressure and a slowing of respiration. The latter Kellaway attributed to the central effects of the toxin.

Fingerman and associates (1953) found that in the frog the toxin had a marked effect on peripheral nerve and skeletal muscle. The " curare-like " action was attributed to some mechanism which prevented the muscle from responding to acetylcholine. Subsequently, Bolton *et al.* (1959) obtained somewhat similar results; they demonstrated a progressive diminution in the amplitude of the end plate potential of the frog nerve-muscle preparation exposed to the toxin. The toxin also modified the contraction of the directly stimulated muscle, a change which was in part reversible. Attempts to detect cytological or histochemical changes at the neuromuscular junction were unsuccessful.

On the basis of their observations, Pepler and Loubser (1960) concluded that the toxin had a very marked specific acetylcholinesterase inhibitory effect similar to the organo-phosphorus compounds. This point, however, is open to considerable question. Schantz (1960) indicated that the contraction of isolated muscle fibers in the presence of ATP and magnesium ions is not inhibited by the poison, nor does the toxin alter the rate of oxygen consumption in the respiring diaphragm of the mouse.

Murtha's work (1960) not only confirms many of the findings and impressions of the earlier workers but it gives us considerable insight into the various modes of action for the poison. The poison has a direct effect on the heart and its conduction system. It produces changes which range from a slight decrease in heart rate and contractile force with simple P-R interval prolongation or S-T segment change, to severe bradycardia and bundle-branch block or complete cardiac failure. These changes are not unlike those provoked by other marine toxins (Russell and van Harreveld, 1954; Russell and Emery, 1960; Saunders *et al.* 1962), although this should not be interpreted to imply that these toxins are indeed related. On the isolated cat papillary muscle the toxin provokes a prompt but reversible depression in contractility.

In those cases where spontaneous contractures are abolished, Murtha found that the muscle still responded to electrical stimulation, although contractile force had been reduced approximately 50%.

In the vagotomized dog, cardiac contractile force decreases 50% within the first minute following injection of the poison. There is a concomitant, precipitous fall in systemic arterial pressure. A fall in blood pressure was also observed by Murtha in both intact and partially eviscerated mammals, indicating that the mechanism proposed by Kellaway (changes in the splenic circulation) is not responsible for the cardiovascular crisis. The toxin does not produce vasodilatation in the vessels of the mammalian leg or kidney, nor does it affect the rate of blood flow in the isolated rabbit ear. In cervical cord-sectioned, bilaterally vagotomized mammals the immediate precipitous fall in arterial blood pressure was not observed by Murtha, although there was some subsequent decrease in blood pressure.

These findings, along with those observed with isolated heart preparations, indicate that the toxin has a direct effect on the heart, an effect which is in part responsible for the cardiovascular crisis; and while the poison may produce changes in the peripheral vascular system these changes are not of sufficient magnitude to precipitate deleterious changes in the systemic arterial blood pressure. It will be interesting to learn what alterations the toxin produces in pulmonary artery pressure and flow, since changes in the pulmonary vascular bed appear to be responsible for the precipitous fall in systemic arterial pressure sometimes provoked by certain animal venoms (Russell *et al.*, 1962; Halmagyi *et al.*, 1965), as well as by a number of other toxic substances. The work by Murtha also indicates that a significant part of the cardiovascular crisis is in some manner concerned with the direct action of the toxin on the central nervous system, although the experiments do not exclude the perhaps improbable conjecture that cerebral anoxia secondary to cardiac centered vascular failure may be a factor. Murtha suggests that the central nervous system effects may be mediated through the spinal cord.

Murtha's work on the phrenic nerve-diaphragm preparation also confirms earlier findings. The toxin depresses mammalian phrenic nerve potentials, suppresses the indirectly elicited contractions of the diaphragm and often reduces the directly stimulated contractions. In the anesthetized, artificially respired mammal the toxin suppresses contraction of the quadriceps muscle following reflex stimulation at a time when stimulation of the motor nerve produces muscular contraction. Recovery from the effects on nerve and reflex transmission usually occurred simultaneously. These and other studies indicate

that the effect of the poison is greater on reflex transmission than on the nerve, and that these changes occur, for the most part, independently of the changes in the cardiovascular system.

M. H. Evans (personal communication, 1965) has found that the toxin has no specific effect on the neuromuscular junction in frog and mammalian limb muscles. In mammals, nerve conduction and muscle contraction were almost equally sensitive to the toxin. C. Y. Kao (personal communication, 1965) states that in the frog sartorius muscle, 0·1 μg/ml of *saxitoxin* first increases the threshold for spike generation but subsequently blocks spikes. He feels that the toxin interferes with sodium conductance without altering potassium or chloride conductances.

Parnas (1963) notes that when the toxin of *Prymnesium parvum* is injected into mice or frogs it too produces respiratory paralysis. He suggested that this was provoked through the action on the central nervous system at the synaptic level. The toxin did not affect skeletal muscle but did inactivate the motor end plates. It caused smooth muscle to contract (Parnas suggests that this might occur through the release of acetylcholine); subsequently, it inhibited both this contraction and the response to acetylcholine, histamine, serotonin, bradykinin, nicotine and barium chloride. In a personal letter (1964), he states that the toxin acts as a non-depolarizing blocking agent at the post-synaptic membrane of the neuromuscular junction. Intracellular recordings from the frog's heart indicate that the toxin causes depolarization.

III. Porifera

Of the 5000 or so species of sponges, only a few tropical and subtropical forms are known to be dangerous to man. Our knowledge of the toxicity of these highly organized colonies of unicellular monads to other animals is very meager. Some sponges have been implicated in the food-chain relationship of icthyotoxism but the evidence so far presented is very circumstantial.

A. *Poisoning*

Some Porifera are known to eject substances which are toxic to certain of the animals in their environment. De Laubenfels (1932) states that when *Tedania toxicalis* de Laubenfels is placed in a bucket with fishes, crabs, molluscs and worms, in an hour or perhaps less, these animals will be found dead. The toxin appears to be located in the gemmules. The stimuli for the discharge of the toxin and the mechanism by which these animals release their poison are not known.

Where humans are involved, poisoning probably occurs through deposit of the toxin(s) in the superficial abrasions produced by the fine, sharp spicules of the sponge. It is well known that traumatic injury to the human skin can be produced by the spicules, and it is believed that in many cases of poisoning this occurs prior to the deposit of the toxin(s) on the skin. Certainly, an abraded skin is more likely to absorb a toxin than an uninjured one. However, there are clinical cases of poisoning by sponges in which no traumatic injury was reported. These cases indicated that the toxin may be absorbed directly through healthy skin.

The animal appears to be able to cause poisoning either in or out of the water. The most serious case of poisoning I have observed involved a 27-year-old skin diver who having abraded the skin of his hands while collecting stony, but not otherwise dangerous, coral decided to assist in the packing of freshly caught *Tedania nigrescens* (Schmidt) aboard the boat. After handling these animals for approximately 30 min, he complained of an intense burning sensation over the hands, pruritus and some malaise. When seen 1 hour later the patient presented systemic manifestations. This case demonstrates that sponge poisoning can occur on handling the animal after it has been removed from the water.

"Sponge fisherman's disease" of the eastern Mediterranean (maladie des pêcheurs d'éponges), described by Zervos (1934), is probably caused by the Actiniae frequently found with sponges. However, there is clinical evidence to support the contention that some sponges are capable of producing skin disorders in man. Such disorders are characterized by localized burning, itching, swelling and edema, and redness, in the less severe cases, and in addition, systemic manifestations such as malaise, weakness, sweating, nausea, syncope and parasthesias in the involved extremity in the more severe cases.

B. *Chemistry and toxicology*

Many sponges have an offensive odor and taste and are rarely, if ever, eaten by fishes or other marine forms. Some chemical, pharmacological and toxicological investigations have been carried out on extracts of the whole body of several of these sponges. Among the species studied to date are *Fibulia nolitangere* D. and M., *Suberites domunculus* (Olivi), *Pseudosuberites pseudos*, *Geodia cydonium* (Jameson), *Hippospongia equina* (Schmidt), *Calyx nicaeensis* (Risso), *Halichondria panicea* (Pallas), *Hymeniacidon perleve* (Montagu), *Tethya aurantium* (Pallas), and the fresh-water sponges *Ephydatia mülleri* (Lieberkühn), *Spongilla lacustris* (L.), and *S. fragilis* (Leidy).

In 1906, Richet precipitated a substance from extracts of the siliceous sponge *Suberites domunculus*, which when injected into the dog produced vomiting, diarrhea and dyspnea, and caused hemorrhages in the gastric and intestinal mucosa, peritoneum and endocardium. Arndt (1928) demonstrated that extracts from certain fresh-water sponges produced diarrhea, dyspnea, prostration and death when injected into homoiothermic animals. These same extracts had some hemolytic effect on sheep and pig erythrocytes, and blocked cardiac function in the isolated frog heart preparation. The extracts were heat stable and produced no deleterious effects when taken orally. Ackermann and his colleagues (1961), and others, have isolated or identified a number of substances from both marine and fresh-water sponges. These include:

ribonucleic acid
desoxyribonucleic acid
-β-D-arabofuranoside of thymine
-β-D-arabofuranoside of uracil
-β-D-ribofuranoside of 2-methoxyadenine
pentofuranoside of uracil
guanine
adenine
O-methyl purine
methyladenine
1-methyladenine
lysine
choline
acetylcholine
betaine
taurine
taurobetaine
histamine
dimethylhistamine
agmatine
guanidine derivatives
glycocyamine
putrescine
phosphocreatine
phosphoarginine
hippospongine
zooanemonine
herbipolin ($C_7H_5ON_5$)
eledonine
inositol
cholesterol
cholestanol
neospongosterol
clionasterol
poriferasterol
homarine

While some of these substances can provoke both local and systemic manifestations in lower animals, it is not known which, if any, of these compounds are responsible for the poisoning of man.

IV. Cnidaria

Venomous forms are found in all three classes of living cnidarians:

Hydrozoa, or hydroids and hydromedusae.
Scyphozoa, or true jellyfishes.
Anthozoa, or sea anemones, sea feathers and corals.

Of the 9000 or so species of this phylum, approximately seventy have been implicated in injuries to man, or are known to be capable of penetrating the human skin. Among those that have inflicted injuries on humans, or whose venom apparatus has been described in some detail are the following; and of these the sea-wasps (Cubomedusae) and Portuguese man-of-war (*Physalia*) are the most dangerous.

HYDROZOA

Medusae:	*Sarsia tubulosa* (M. Sars), *Pennaria tiarella* (Ayres), *Olindioides formosa* Goto.
Hydroids:	*Halecium beani* (Johnston), *Sertularia cupressina* (L.), *Lytocarpus philippinus* (Kirchenpauer).
Millepore corals:	*Millepora alcicornis* L., *M. complanata* Lamarck.
Siphonophores:	*Physalia physalis* (L.) (Portuguese man-of-war), *Rhinophora filiformis* (Lamarck), *Rhizophora eysenhardti* Gegenbaur.

SCYPHOZOA

CUBOMEDUSAE (Sea Wasps):	*Carybdea alata* Reynaud, *C. rastoni* Haacke, *Tamoya gargantua* Haeckel, *Chiropsalmus quadrigatus* Haeckel, *Chironex fleckeri* Southcott, *Tripedalia cystophora* Conant.
CORONATAE:	*Linuche unguiculata* (Schwartz), *Stephanoscyphus racemosus* (polyp of *Nausithoë punctata* Kölliker).
SEMAEOSTOMAE:	*Chrysaora quinquecirrha* (Desor), *Sanderia malayensis* Goette, *Cyanea capillata* (L.), *C. lamarcki* Pér. and Les., *Pelagia noctiluca* (Forskål), *P. colorata* Russell.
RHIZOSTOMEAE:	*Rhizostoma pulmo* (Macri), *Lobonema smithi* Mayer, *Cassiopea xamachana* R. P. Bigelow, *Acromitoides purpurus* (Mayer), *Catostylus mosaicus* (Quoy and Gaimard).

ANTHOZOA

ACTINIARIA (Anemones):	*Actinia equina* L., *Segartia elegans* Dalyell, *Actinothoë* (*Sagartia*) *longa* (Verrill), *Adamsia palliata* (Bohadsch), *Anemonia sulcata* (Pennant), *Diadumene cincta* Stephenson, *Aiptasiomorpha luciae* (Verrill), *Corynactis australis* Haddon and Duerden, *Bunodactis elegantissima* (Brandt), *Anthopleura xanthogrammica* (Brandt), *Rhodactis howesii* Saville Kent.
MADREPORARIA (Corals):	*Acropora palmata* (Lamarck).

A. *Venom apparatus*

The stinging unit of the cnidarian is the *nematocyst*, which is formed within an interstitial cell, the *cnidoblast*. All members of the

phylum have nematocysts, of which there are many different kinds, and individual animals may possess more than one kind of nematocyst. While all cnidaria are potentially dangerous, only a few have nematocysts capable of penetrating the skin and poisoning humans. The reader is referred to the fine works of Weill (1934), Hyman (1940) and F. S. Russell (1953) for a more complete study of the nematocyst.

The cnidoblasts are small rounded or ovoid cells which are widely distributed throughout the epidermis, except on the basal disc. Particularly abundant on the tentacles, they are used both as offensive and

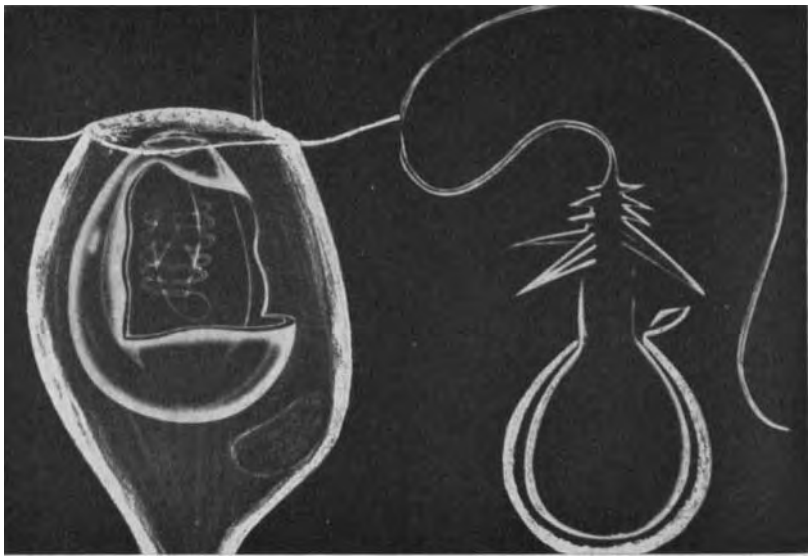


FIG. 2. Diagrammatic sketch of an undischarged (left) and discharged (right) nematocyst of a cnidarian. (Modified from Halstead, 1959.)

defensive weapons, as well as for aids in anchorage. The typical cnidoblast contains a basal nucleus, and a capsule, the nematocyst, containing a long coiled tube or hollow thread. In *Hydra* the thread of penetrant nematocysts (*stenoteles*) appears to be continuous with a " head " which may contain the venom (Chapman, 1961). A short, bristle-like projection, the *cnidocil*, is embedded in a crater on the discharge end of the cell (Fig. 2). In the stenoteles of *Hydra* an operculum covers the junction of the undischarged thread to the capsule. The thread may vary in length from 50 μ to 1 mm, while the nematocyst itself is

from 4 to 225 μ in length. The intracapsular space contains a clear fluid which according to some investigators contains the venom.

The cnidoblasts are produced within interstitial cells distant to their final site in the epithelium. None originate in the tentacles. They migrate to their final location in the ectoderm by ameboid activity and passive transport. The cnidoblast adjusts itself to a superficial position, with that part of the cell containing the nematocyst directed so that the thread can be discharged into the offending or stimulating organism. The cnidocils, when present, are receptor structures which receive and conduct stimuli to the cell. However, stimuli may be received and conducted by the cell membrane independent of cnidocils.

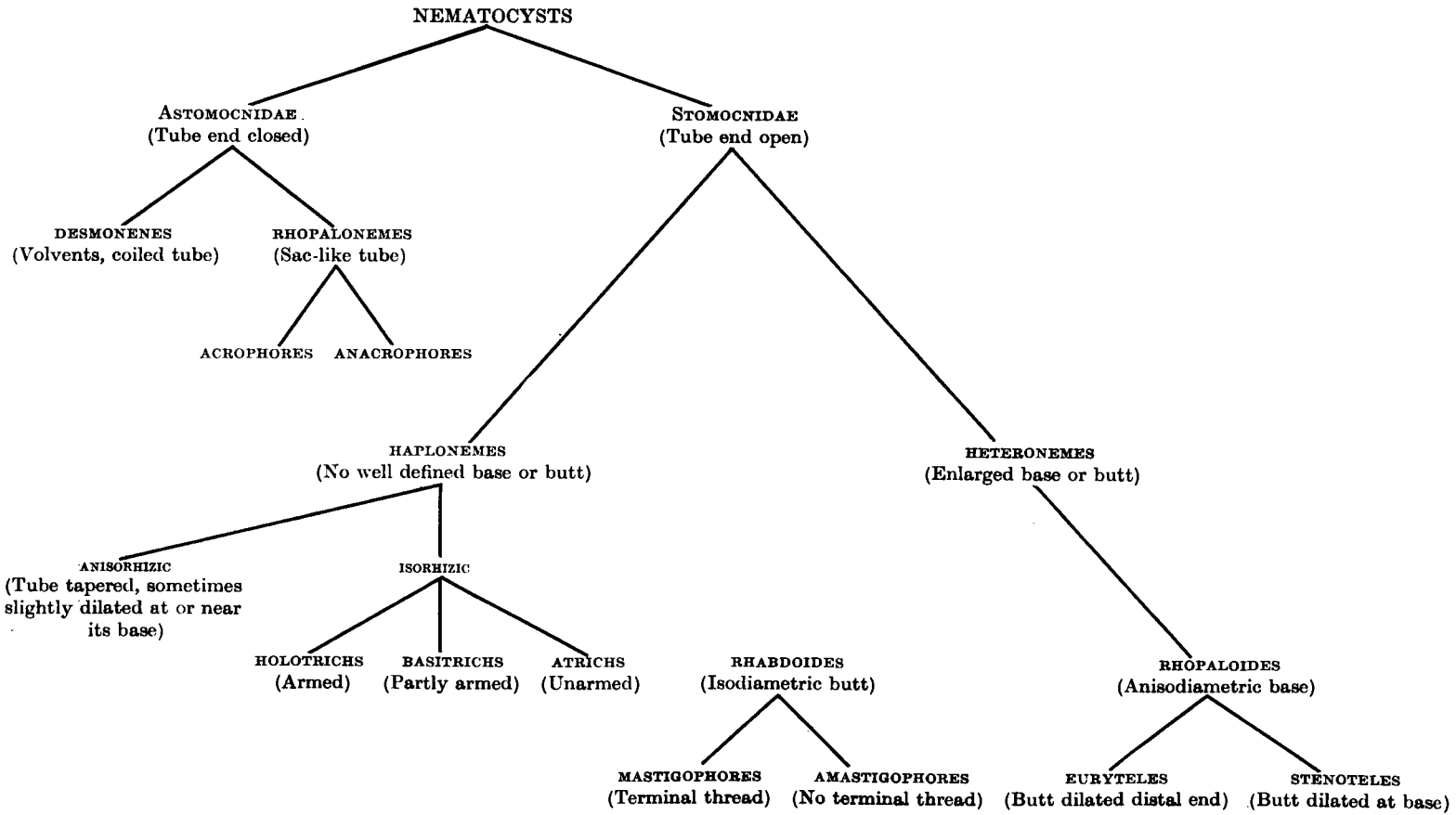
Under normal conditions, nematocysts discharge in a highly localized fashion in response to a specific localized stimulus (Pantin, 1942). There does not usually appear to be any coordinated response by the various parts of the animal. Although discharge may be elicited by direct mechanical stimulation, a sensitizing chemical stimulus will greatly reduce the threshold at which the discharge takes place. The cell appears, for example, to be particularly sensitive to lipoidal substances absorbed upon proteins. Nematocyst sensitization by various natural occurring and synthetic surface-active substances has been studied extensively by Yanagita (1960).

The mechanism for the discharge of nematocysts is an interesting, though controversial one (this problem is reviewed by Chapman and Tilney, 1958; see in particular Picken, 1953; Robson, 1953; and Picken and Skaer, 1965). In some instances it would appear to involve an increase in the permeability of the capsule wall following appropriate stimulation, with the result that either as a direct response to increased hydrostatic pressure, or a change in pH, or swelling of the colloidal substances within the capsule, the tube is forced out as the entire nematocyst " explodes ". The discharged nematocyst, which may appear pear-shaped (Fig. 2), consists of a bulb (the old capsule), and the tube or thread, commonly armed with spines about the base. The mechanism for transfer of the venom to the envenomized prey or victim is not thoroughly understood.

Nematocysts have been classified on the basis of structure, function and taxonomy, but until Weill proposed his elaborate nomenclature for these structures there was little common communication on their forms. Weill described seventeen categories of nematocysts and these have been adopted by many workers in the field.

The classification shown on the opposite page, based upon structure, is adopted from the discussion by Hand (1961).

From the functional standpoint, nematocysts have been divided



Classification of Nematocysts after Hand (1961).

into three types: the *volvent* type, in which the tube end is closed; the *penetrant* type, in which the tube end is opened; and the *glutinant* type, in which the tube is open and sticky. The volvent type is unarmed; its threads, when discharged, wrap around and entangle the bristles or fibers of the offending animal. The penetrant type is armed with spiralling rows of spines which serve in anchoring the thread to the object of attack. The point of the thread is capable of penetrating certain epithelial tissues, and venom may be discharged through its open end into the wound. The piercing ability of some penetrants is sufficient to puncture the chitinous cuticles of several marine animals. The glutinant type of nematocyst appears to respond only to mechanical stimuli and may be used by a cnidarian for anchoring its tentacles during locomotion.

B. *Chemistry and toxicology*

As with many of the earlier studies on venoms, the initiating investigations on the chemical and toxicological properties of cnidarian venom were carried out with crude saline or water extracts prepared from the whole animal, or from one or several of its parts. The findings from these studies were subject to considerable variation, and the reports in the literature of those years reflect the uncertainty of the chemical analyses. It is apparent that some of these early workers were studying normal constituents of the animal's tissues, some of which are limited to the lower phyla. While these constituents are not toxic to the animals of the lower phyla they may produce deleterious effects in higher animals. During more recent years it has been possible to isolate specific nematocysts from cnidarians so that at the present time most investigators are working with either a highly concentrated extract from nematocysts or with the material discharged from the nematocysts following electrical stimulation.

Just after the turn of this century, Richet (1906) and his colleagues separated three pharmacologically active fractions from the tentacles of *Actinia*, which when injected into dogs produced scratching, pulmonary edema or sedation. These fractions were named " thallasin ", " congestin " and " hypnotoxin ". [The phenomenon of anaphylaxis was discovered during certain of these studies on cnidarian toxins (Richet and Portier, 1936).] These substances were not specific compounds but rather mixtures having deleterious biological effects. Thallasin has been shown to induce the release of histamine in tissues, and following its intravenous administration in the cat causes the appearance of a " slow contracting substance " in the plasma (Jaques and Schachter, 1954).

In 1923, Ackermann *et al.* isolated tetramethyl ammonium hydroxide (" tetramine ") from *Actinia equina*. Subsequently, he and his co-workers isolated a number of other quaternary ammonium compounds from cnidarians, many of which have been studied for their zootoxicological properties. Among the nitrogenous bases so far studied are N-methylpyridinium hydroxide, homarine, trigonelline, γ-butyrobetaine and zooanemonin. Of this group, tetramine alone is associated with a curare-like activity, although it does not appear to have any deleterious effect on certain crustacean nerve-muscle preparations (Cowan and Ing, 1935). Also, the amount present in the animal hardly seems sufficient to produce the activity with which it has been implicated. Tetramine is a common constituent of several cnidarian tissues and has been suggested as a transmitter substance in cnidarian " nerve activity ". It is possible that while it may not be the substance directly responsible for the paralyzing effect of the toxin, it contributes in a significant way to the development of this activity. It must be concluded that although tetramine is present in the extracts from tentacles and other parts its exact role as a toxin in cnidarian venom has not yet been established.

5-Hydroxytryptamine (5-HT, serotonin) has been identified in a number of cnidarians (Welsh, 1960), and is a common constituent of many venoms (Erspamer, 1961). In Cnidaria it is found in the tentacles, body wall, acontia, and several other parts. There is always a particularly high concentration in those parts where nematocysts are concentrated. 5-HT is, of course, a potent pain-producing substance in man, although the mechanism by which it causes pain is not known. It may provoke changes in the permeability of sensory nerve endings, thereby altering the transfer of ions to receptor sites, or it may be concerned with the transport and distribution of certain ions about the nerve endings. As a potent vasoconstrictor it may effect circulation about the sensory nerve endings and thus induce changes leading to the development of pain. While it no doubt contributes to the pain-producing effect of the toxin it is not the only pain producer in the venom.

Finally, 5-HT may contribute directly to the mechanism through which certain of the local effects are produced. It may cause changes resulting in localized edema and itching, as well as the changes responsible for the vascular effects and hemorrhage. On the other hand, it is a potent histamine releaser and along with histamine and other histamine releasers (Uvnäs, 1960) in the toxin may contribute to the localized changes. It is absorbed very slowly from the skin and subcutaneous tissues. It is not an important factor in the lethal or para-

lyzing property of the toxin. The evidence to date indicates that the lethal and paralyzing effects of cnidarian toxin are due to proteins, probably of low molecular weight.

Injection of the highly labile crude toxin from nematocysts of *Physalia* produces paralysis in fish, frogs and mice. Animals killed following stingings by *Physalia* exhibit marked pulmonary edema, right cardiac dilatation with venous congestion of the larger vessels of the chest and portal circulations. Lane and Dodge (1958) suggest that the toxin affects the respiratory centers before producing changes in the voluntary muscles. It alters the permeability of the capillary wall but does not appear to produce hemolysis. It also causes changes in the isolated heart of the clam which resemble those provoked by acetylcholine.

Recently, Lane (1961) subjected lyophilized "crude" extracts of *Physalia* nematocysts to chromatography and obtained nine spots, four of which accounted for 95% of the total lethal activity in the crab *Uca pugilator*. By paper electrophoresis he separated the same extracts into four fractions, three of which contained the total lethality, the principal lethal portion being in two fractions. The crude toxin was lethal to mice at 1·7 mg/kg body weight. Lane suggested that *Physalia* toxin is a relatively simple protein consisting of only a few toxic peptides which are synthesized by gastrodermal cells and which pass through the mesoglea and then into the nematocyst during the morphogenesis of this structure.

Payne (1961) has shown that extracts of tentacles from *Chironex fleckeri* cause marked, prolonged contracture of rat uterine muscle. The active substance was heat labile. She feels this activity is similar to that which causes the respiratory distress in victims stung by this Cubomedusa.

The toxic principle of the sea anemone *Rhodactis howesii* appears to be a non-dialyzable protein, relatively stable between pH 4·5 and 10·0, and having an order of lethality (for the partially purified extract) of 2·6 mg/kg body weight (Farber and Lerke, 1963).

In conclusion, cnidarian toxin contains a number of quaternary ammonium compounds, of which tetramine is the more active toxicologically. It also contains 5-hydroxytryptamine, histamine and histamine releasers and several proteins of relatively low molecular weight. The lethal and paralyzing effect of the toxin appears to be caused, for the most part, by the protein(s) which may act directly on cholinergic neurons. The relationships between central and peripheral mechanisms for paralysis have not been clearly defined. Certain of the symptoms and signs of cnidarian poisoning—localized edema,

redness, itching, pain and the vascular changes—may be attributable to the 5-HT, histamine and histamine-releasing substances.

C. *Clinical problem*

It has long been known that the nematocysts of certain cnidarians can penetrate the human skin. Of such nematocysts, most are capable of penetrating only the thin membranes of the mouth, tongue or conjunctiva, but some possess sufficient force to pierce the skin of the inner sides of the arms and hands, and sometimes even the thicker surfaces of the body.

The cutaneous lesions produced by nematocysts may vary considerably. The fire, or stinging, corals (*Millepora*) produce small reddened, somewhat papular eruptions, which appear 1 to 10 h following the contact, and usually subside within 24 to 96 h. In severe cases the papules may proceed to pustular lesions and desquamation. The stinging is usually associated with some localized pain, generally of short duration. Pruritus is common.

The lesions produced following contact with the Portuguese man-of-war (*Physalia*) appear as small papular eruptions in one or several discontinuous lines which may sometimes encircle the extremity or injured part. Each papule may be surrounded by an erythematous zone. In some cases the papules may be very close together, indicating that multiple discharge of the nematocysts took place as the tentacle passed over the injured part. The papules develop rapidly and often increase in size during the first hour following the stinging. Pain is often present and in some cases may be very severe. While it usually tends to be localized, it may spread to involve the entire injured extremity as well as the adjacent lymph nodes. In some cases the papules may vesiculate and proceed to pustulation and desquamation. I have seen several cases in which multiple areas of hyperpigmentation could be found over the involved part several years following the stinging. Joint and muscle pains are not uncommon and may persist for a number of hours following the injury.

General systemic manifestations may also develop following *Physalia* envenomation. Weakness, nausea, headache, pain and spasms in the large muscle masses of the abdomen and back, lacrimation and nasal discharge, increased perspiration, and vertigo have been reported in many cases. Difficulty and pain on respiration, changes in pulse rate and severe muscle spasms have been reported in the more severe cases.

Contact with the true jellyfishes gives rise, in the less severe cases, to symptoms and findings similar to those noted above. In the more severe cases there is immediate, intense, burning pain. The lines of

contact appear as separate swollen wheals, sometimes purple, which may disappear in a few hours or may proceed to vesiculation and necrosis. The lesions usually heal by granulation and cicatrization. Localized edema is not uncommon. In addition, pain and difficulty in respiration, severe spasms of the abdomen and back, profuse lacrimation and nasal and bronchial secretions are sometimes reported. Vertigo, mental confusion, increased pulse rate and dilatation of the pupils may be reported. A number of fatalities have occurred following stingings by Scyphozoa, several within a matter of minutes. Hyperpigmentation and cheloid formation have been reported in a number of cases.

Initial treatment consists of the application of alcohol to the injured area. If this is not available, dry sand or flour should be sprinkled on the lesions and after 30 sec scraped off with a knife. Under no circumstances should the affected area be rubbed with wet sand or fresh water. Application of a topical analgesic-cortisone lotion is advised, and in severe pain the use of codeine or meperidine may be indicated.

According to J. H. Barnes (personal communication, 1965) the three most troublesome Cnidaria on the Australian coastline are the Irukandji carybdeid and the chirodropids *Chironex fleckeri* and *Chiropsalmus quadrigatus*. The Irukandji stinger causes only minor local effects, which, however, are followed some time later by backache, weakness, headache, painful spasms in the abdomen, thighs and chest, some dyspnea, increased perspiration, dryness of the mouth, and vomiting. The illness is non-fatal and responds well to intravenous pethidine. Stingings by the larger chirodropid *C. fleckeri* are usually more serious than those inflicted by *C. quadrigatus*. However, stings by both animals are painful, and give rise to whealing with a fringing flare of erythema. Edema is usually present, especially following envenomation by *C. fleckeri*. The injured skin is often discolored, at first brownish but later purple. The discoloration may persist for years, the affected area giving rise to intermittent itching. Exudation beneath the seared lines of tentacle contact raises bullae 5 mm or more in height. Necrosis deep into the subcutaneous tissues is not unusual, and is slow to heal. Permanent cicatricial scarring may result.

Barnes states that in severe stingings the pain is instantaneous and extremely severe. Collapse, if it occurs, may be sudden or preceded by violent twitching. Death may occur within minutes, and would appear to be due to cardiac arrest of either myocardial or central origin. More than fifty fatalities due to marine stings have been recorded for the Australian coastline, all from tropical waters and all during the warmer months of the year.

Stings by the stony corals (*Acropora*) give rise to immediate pain often followed by itching and the development of small wheals or blebs, which may ulcerate. According to Halstead (1959) " sponge fishermad's disease " is due to the actinian, *Sagartia elegans*. Small pieces of the coral may also break off in the skin giving rise to lesions which often become secondarily infected, particularly by *Staphylococcus albus*.

Occasionally, poisoning takes place during swimming following accidental ingestion of broken tentacles from the cnidarian. In these cases there may be marked systemic effects without any localized lesions, and severe nausea and vomiting.

Martin (1960) has reported fatal poisonings following ingestion of the sea anemone *Rhodactis howesii*.

V. Echinodermata

There are approximately 6000 species of echinoderms of which at least eighty are known to be venomous or poisonous. A number of members of the class Asteroidea have been implicated in injuries to humans, including the common starfish *Crossaster papposus* (L.) and several members of the genus *Echinaster*, and among others the many spined, multi-rayed starfish *Acanthaster planci* (L.).

In the class Echinoidea there are many venomous forms, including the following sea urchins, all of which have caused injuries in humans: *Toxopneustes pileolus* (Lamarck), *T. elegans* Döderlein, *Araeosoma thetidis* (H. L. Clark), *A. violaceum* Mortensen, *Sphaerechinus granularis* (Lamarck), *Asthenosoma ijimai* Yoshiwara, *A. varium* Grube, *Diadema setosum* (Leske), *D. savignyi* Michelin, *D. paucispinum* A. Agassiz, *D. antillarum* Philippi, *Echinus acutus* Lamarck, *Echinothrix calamaris* (Pallas), *E. diadema* (L.), *Paracentrotus lividus* (Lamarck), *Salmacis bicolor* L. Agassiz, and *Strongylocentrotus droebachiensis* (O. Fr. Müller). Ingestion of the ova of certain urchins gives rise to poisoning, although it is not as yet clear whether the poisoning is due to bacteria, a toxin or an allergy.

It has long been known that certain sea cucumbers produce a substance that is toxic to fishes and other marine animals, and perhaps to man (Cooper, 1880). In the Indo-Pacific area the tissue fluids of several holothurians, in particular *Holothuria atra* (Jaeger) and *H. argus* (Jaeger), are used by fishermen to deactivate fishes. Frey (1951) relates how natives on Guam cut up the common black sea cucumber and squeezed the contents of the animal into blocked crevices and pools. " Before long the water became noticeably turbid in appearance, and shortly thereafter fish began coming to the surface of the pool, exhibiting much the same type of behavior as in rotenone poisoning." I have seen

the same technique used in Saipan, where the fishermen wear goggles to protect their eyes from the irritating substances of these animals.

According to Nigrelli and Jakowska (1960) at least thirty species belonging to four of the five orders of Holothuroidea are toxic. Some toxic species [*Thelenota ananas* (Jaeger), *Stichopus variegatus* Semper, *Holothuria atra* (Jaeger) and *H. axiolaga* H. L. Clark] are highly esteemed as food in the Orient.

A. *Venom apparatus*

1. *Asteroidea*

The starfishes have simple thorny spines of calcium carbonate in the form of calcite intermingled with organic substances. The spines are held erect by a number of muscles. Specialized glandular tissue is embedded in the calcite and is capable of secreting a toxin which can be discharged into the water or perhaps directly into the skin. The stimulus for the discharge of the poison and the mechanism by which it is released are not known.

2. *Echinoidea*

In the regular sea urchins the body is globular and radially symmetrical. It is enclosed in a hard calcite shell from which calcareous spines and pedicellariae arise. The spines may be straight and pointed, curved, flat-topped, club-shaped (bearing poison glands), oar-shaped, umbrella-shaped, thorny, fan-shaped or hooked, and may vary in length from less than 1 mm to over 30 cm. Each spine is borne on a tubercle of the thecal plate, to the rim of which the spine muscles are attached. The spines serve in locomotion, protection, digging, feeding, producing currents and harboring larvae; certain of them bear " poison " glands.

" Poisonous spines " are most highly developed in the family Echinothuridae, particularly in the genus *Asthenosoma*. The smaller spines in *Asthenosoma varium* Grube terminate in specialized poison organs containing a gland, which empties its toxin through the hollow spine tip into the wound produced by the spine. In other echinothurids, such as in *Araeosoma thetidis* (H. L. Clark), these secondary spines also terminate in poison organs but they are much less specialized than in *Asthenosoma varium*. The poison organs may rupture as the spine enters the skin, thereby discharging their toxin directly into the wound. In *Asthenosoma* the spines of the aboral side are enclosed in a thickened, ringed and pigmented bag of skin which is thought to contain poison glands. In *Phormosoma* the primary spines on the oral side are also thought to contain poison glands (Mortensen, 1929). In Diadematidae

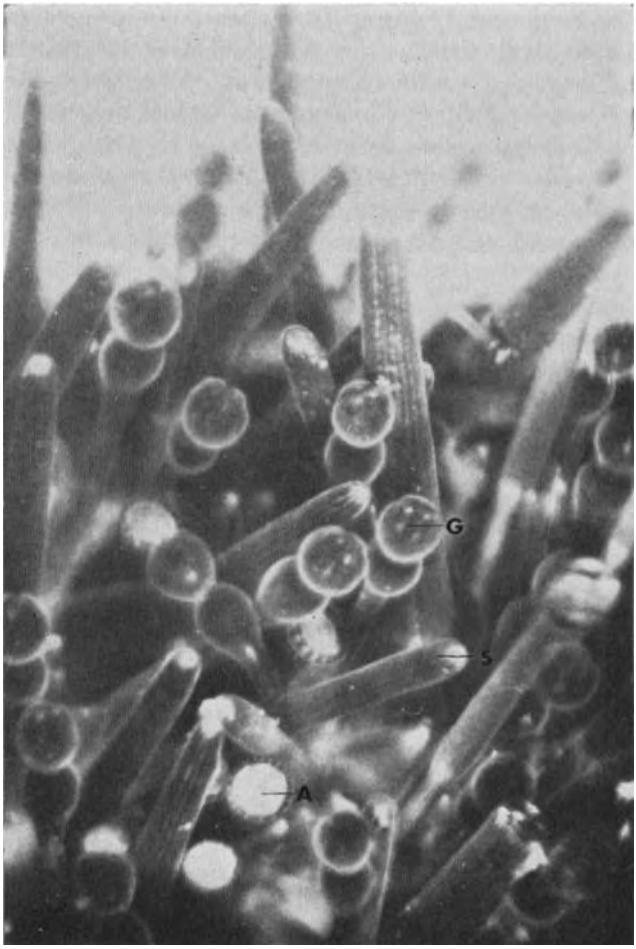


FIG. 3. Living *T. gratilla* showing spines (S), closed globiferous pedicellariae (G) and ambulacral tube feet (A). Note globous swelling between stalk and head of pedicellaria. (From Alender, Thesis, Univ. Hawaii, 1963.)

the spines may exceed a foot in length, and members of one particular genus, *Diadema*, are often implicated in injuries to humans. It should be noted that the data implicating certain spines with poisonous activity are limited to several anatomical studies on echinoderm spines. There does not appear to be any biochemical or toxicological evidence, at the present time, to indicate that these structures do indeed contain a poison.

In addition to the venomous spines, sea urchins and starfishes

are provided with small, pincer-like organs, the pedicellariae, which are distributed over their entire body surfaces between the spines. The pedicellariae are modified spines with flexible heads and serve in keeping larvae and minute creatures, as well as debris, from settling over the body of the urchin or starfish where they might obstruct the respiratory organs or the tube feet. Pedicellariae are most highly developed and always present in the sea urchins, where four main types may be found; there is considerable variation in form within these types.

The *glandular*, *gemmiform* or *globiferous* type pedicellaria serves as a venom organ. In most echinoids the so-called " head " of this type is composed of three calcareous jaws or valves, each having a large basal portion with a convex outer surface and a concave inner one, and each tapering into a delicate shaft curving sharply inward and terminating in a fine, rounded, tooth-like fang. The tip of the shaft is sometimes channelled, presenting the appearance of two lamellae which merge to form the tooth-like fang. The jaws are usually invested in a globose, fleshy and somewhat muscular sac which possesses a single or double gland over each valve. The venom glands discharge the toxin through ducts which open just proximal to the tooth-like fang. In the asteroids the poison glands are contained in the concave cavities of the valves and do not possess a muscular sheath. In Echinothuriidae is found a variant globiferous type pedicellaria, *dactylous*, which reaches its highest development in *Araeosoma*, where each of the four or five long jaws are topped by a disciform expansion.

According to Nichols (1962) the jaws of the pedicellaria open when touched on the outside, and they may open still further if touched on the sensory hairs inside the valves. If the appropriate chemical stimulus is applied to the body surrounding the pedicellaria the jaws will close and discharge the venom. If the stimulus is applied only to the sensory processes inside the valves the poison will be ejected while the valves remain open. Pedicellariae may become detached from the body of the urchin and still continue to inject venom into a wound.

In some sea urchins poison glands may not only lie in the " head " but may encircle the stalk. The stalk, or stem, is attached to the head either directly by muscles or by a flexible muscular neck of varying lengths.

3. *Holothuroidea*

Some holothurians possess special defense organs, the Cuvierian tubules, which arise from a common stem of the respiratory tree. When these animals are irritated they emit these organs through the

anus; the tubules become elongated by hydrostatic pressure so that once through the anus they become long, extremely sticky threads in which the attacking animal becomes ensnared. As noted by Endean (1957) the process of elongation may split the outer layer of covering cells thereby releasing a proteinaceous material which forms an amorphous mass having strong adhesive properties. These elongated threads, which may attain a length of several feet, separate from their attachment and are left behind as the holothurian crawls away. The autotomized parts are regenerated in time.

In some sea cucumbers, however, as in *Actinopyga agassizi* Selenka, the tubules do not become sticky, nor do they elongate, but they are eviscerated in a somewhat similar manner and discharge from certain highly developed structures filled with granules a toxin which is capable of killing fishes and other animals. Evisceration may be provoked by handling the animal, either in or out of the water, and by excessive changes in temperature, pH and oxygen balance. The relationship between evisceration and the discharge of the toxin is not known, but injections of the extract *holothurin* from these tubules, or the addition of the toxin into water containing live holothurians, elicits the process. In still other sea cucumbers, as in *Holothuria atra*, which do not possess Cuvierian tubules, the toxin may be discharged through the body wall.

B. *Chemistry and toxicology*

It is well known that many echinoderms secrete from their integument, or sometimes abundantly over their body surfaces, a mucus which appears to play an important role in the animals' defensive armament. Fontaine (1964) has demonstrated that in the brittle star, *Ophiocomina nigra* (Abildgaard), the massive multicellular glands, which for the most part are distributed over the external surfaces facing upwards or laterally, discharge a viscous substance on stimulation that is characterized as a highly sulphated acid mucopolysaccharide. The combined mucous secretions of this ophiuroid are acid mucopolysaccharides; one or more of the mucins contains amino sugars, sulphate esters, and occurs complexed to protein. The pH of the mucus is approximately 1, which probably makes it highly offensive to fishes and other marine animals. While the biological activities of these mucins have not been thoroughly investigated, Fontaine has shown that they do have some anti-coagulant activity.

A quaternary ammonium base, $C_7H_7NO_2$ (picolinic acid methyl betaine), has been isolated from certain starfishes, sea urchins, and

sea cucumbers. This substance, called *homarine*, is soluble in water and alcohol and characterized by an absorption minimum at 240 mμ and a maximum at 272 mμ. Although it has been found in many invertebrate marine forms it appears to be absent in fresh-water invertebrates. There is a tendency for the more complex marine invertebrates to have a higher concentration of homarine than the lower forms. Ultraviolet irradiation destroys the material but heating does not. The molar extinction coefficient is 6325 l/mole/cm. Homarine does not appear to have any significant neurohumoral effect (Gasteiger *et al.*, 1960), and the biological significance of its presence in these animals has not yet been determined.

Several phosphagens have been isolated from the Echinodermata (Thoai and Roche, 1960). Phosphoarginine has been found in all five classes, while phosphocreatine is found only in Ophiuroidea, Echinoidea and Holothuroidea. Phosphotaurocyamine, phosphoglycocyamine and phospholombricine have not been isolated from echinoderms. While the distribution of the phosphagens in marine forms may be explained by variations in metabolism, and their presence as different phosphagens in genital cells is specific, it has not yet been determined what role they contribute to the pharmacology of the animal.

A steroid, 7, 24(28)-ergostadien-3β-ol, has been isolated from the starfish *Pisaster ochraceus* (Brandt) (Fagerlund and Idler, 1959), while another steroid, possibly a Δ^7 sterol has been isolated from the slate pencil sea urchin (? *Heterocentrotus mammilatus*) (Bergmann and Domsky, 1960). Echinochrome *A* has been found in the body and spines of at least four species of sea urchins, while a naphthoquinone pigment has been identified with echinochrome *A* in *Diadema setosum* (Nishibori, 1959).

Pérès (1949) states that the injection of the thermostable extracts from macerated pedicellariae of *Sphaerechinus granularis*, and certain other species, will kill isopods, crabs, octopods, starfishes, lizards and rabbits, though it has little effect on frogs. He notes that the toxic factor can be found in all other tissues of the animal, although it is much more concentrated in the pedicellariae.

Recently, from the heads of the globiferous pedicellariae of *Tripneustes gratilla* (L.), Alender (personal correspondence) has separated a non-dialyzable, thermolabile, biuret positive substance which exhibits ultraviolet absorption at 278 mμ. In mammals this substance caused a marked fall in systemic arterial pressure and appeared to have a direct effect on the heart. It produced lysis of fish erythrocytes but had no effect on amphibian nerve or the neuromuscular junction. The extract contained no acetylcholine, histamine or 5-hydroxy-

tryptamine. It was separable into 7 bands by polyacrylamide gel electrophoresis.

When a mouse is stung by pedicellariae from a venomous sea urchin it displays symptoms of respiratory distress and shows a significant fall in body temperature. The toxin has an inhibitory effect on the oyster heart.

In 1952, Nigrelli named the toxic substance(s) of the Bahamian sea cucumber, *Actinopyga agassizi*, *holothurin*. It is composed of 60% glycosides and pigment, 30% salts, polypeptides and free amino acids, 5 to 10% insoluble protein and 1% cholesterol. A cholestrol-precipitated fraction known as holothurin *A*, which represents 40% of the crude holothurin, has been given the empirical formula C_{50-52} H_{81-85} O_{25-26} S Na (Chanley *et al.*, 1959). It appears to consist of at least four steroid aglycones bound individually to four molecules of monosaccharides; it shows no absorption in the ultraviolet region. It is probably a mixture of several related sulfate ester glycosides, each containing a steroid aglycone of approximately 26–28 carbon and 4–5 oxygen atoms, one molecule each of four different sugars and one molecule of sulphuric acid as a sodium salt (Chanley *et al.*, 1960). It resembles digitonin and other saponins in both its chemical and biological activities. Holothurin has a deleterious effect on some sharks and has been suggested as a shark repellent.

In 10 parts per million, holothurin is lethal to *Hydra*, the mollusc *Planorbis*, and the annelid *Tubifex tubifex*. It has slightly greater hemolytic action than saponin, and stimulates hemopoiesis in the bone marrow of winterized frogs. It also appears to have some antimetabolic activity (Nigrelli and Jakowska, 1960). In the mammalian phrenic nerve-diaphragm preparation, holothurin *A* produces a contracture of the muscle, followed by some relaxation, and a gradual decrease in the recorded amplitude of both the directly and indirectly elicited contractions, the latter decreasing at a slightly greater rate than the former. The intravenous LD_{50} in mice is approximately 9 mg/kg body weight (Friess *et al.*, 1960). In experiments in frogs, Thron and his colleagues (1963) have demonstrated that holothurin *A* produces an irreversible block and destruction of excitability on the single node of Ranvier in the sciatic nerve. The toxin does not produce any observable damage to the axonal walls or sheath. It is possible that the principal deleterious neurotoxic effect is directed toward the nodal membrane. The poison does not exert a blocking action on the *in vitro* AChE-ACh system.

Rio and associates (1963) have extracted a saponin-like substance from the sunburst starfish, *Petasometra helianthoides* A. H. Clark,

which differs principally from holothurin in its sugar moieties. The toxin is lethal to *Fundulus heteroclitus* in solutions of 1 ppm. The Atlantic starfish, *Asterias forbesi* (Desor), which also contains a holothurin-like toxic substance, is resistant to the sunburst starfish toxin.

C. *Clinical problem*

Traumatic injuries of the skin by the spines of sea urchins without envenomation are well known. *Tripneustes ventricosus* (Lamarck), the white sea urchin of the West Indies, has been implicated in numerous injuries to humans and must be handled with considerable care. The spines of this species, as well as many others, are extremely brittle. They may break off in a puncture wound causing considerable local reaction, and if not removed often give rise to infection. Some spines are absorbed within 16 to 48 h, whereas others may need to be removed surgically. Both the venomous and nonvenomous spines can give rise to granulomatous nodular lesions.

Injuries by pedicellariae, and certain of the spines of sea urchins, give rise to immediate intense pain, localized swelling and redness about the wound, an aching sensation in the involved part, nausea and syncopy; and in the more severe cases, difficulties in respiration, parasthesia about the mouth with some atonia of the muscles of the lips, tongue, larynx and eyelids, and sometimes the muscles of the limbs. In the most severe cases these symptoms and signs develop more rapidly. Complete atonia and ataxia may occur, and the victim may experience severe respiratory distress. The agonal period last 1 to 14 h, usually without subsequent complications.

Poisonings by Holothuroidea are rare. Occasional, acute gastric distress with nausea and vomiting have been reported following the ingestion of certain toxic sea cucumbers. The symptoms are usually of short duration and without serious sequelae. Pruritus with mild swelling and redness of the hands has been reported following the handling of some holothurians. Acute conjunctivitis has been observed in persons who have swum in waters polluted with the tissue extracts of toxic sea cucumbers.

VI. Mollusca

There are approximately 80 000 species of molluscs, of which about eighty-five have been implicated in poisoning to man or are known to be toxic under certain conditions. A number of other molluscan species are potentially dangerous, or are suspected of being venomous or poisonous to man or other animals. The majority of the poisonous or venomous species are found in three of the five families

of molluscs: Gastropoda, Pelecypoda and Cephalopoda. While it is not within the confines of this review to discuss all of these animals and their toxins, attention is given to the more dangerous species.

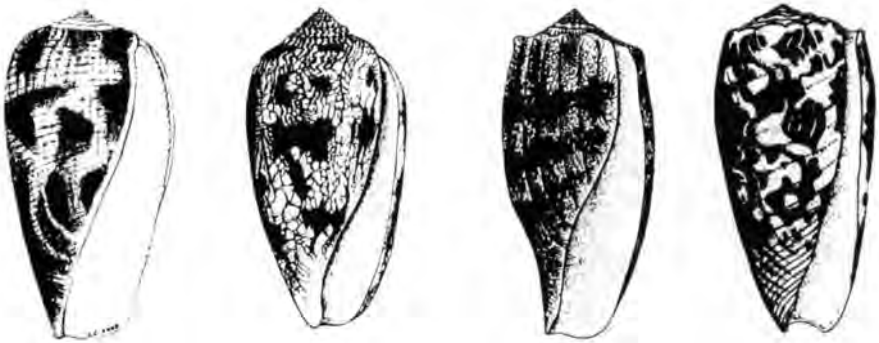


FIG. 4. Left to right: *Conus tulipa*, *Conus textilis*, *Conus geographus* and *Conus striatus*.

A. *Venomous*

1. *Gastropoda*

Most of the venomous species of molluscs are found in the families Gastropoda and Cephalopoda. The most dangerous gastropods are members of the genus *Conus*. In Table II I have attempted to divide the venomous cones into three types as suggested by Kohn (1959): piscivorous, molluscivorous and vermivorous. It should be emphasized that this table is based on the present state of our knowledge, which for some of these animals is very limited.

In the table on p. 292 some species of *Conus* have been omitted because of insufficient information on their feeding habits. *C. californicus* Hinds is known to feed on both gastropods and polychaetes, and has been observed to sting a fish (Saunders, personal correspondence, 1964). Several other species, *C. eburneus*, *C. figulinus*, *C. tessulatus*, *C. distans* Hwass, *C. coronatus* Gmelin, *C. striatus* and even *C. textilis* are equally as difficult to classify. Endean and Rudkin (1965) suggest that it may be possible to distinguish between the piscivorous, molluscivorous and vermivorous Conidae on the basis of the differences in the structure of their radular teeth. Their several studies indicate the importance of this possibility.

It should be further noted in assessing the table that the reports of envenomations in humans by *C. aulicus*, *C. marmoreus*, *C. omaria* and *C. textilis* have been subject to considerable question. The evidence to date would seem to indicate that only the piscivorous species pose a serious threat to man, although as shown by Endean and Rudkin

TABLE II. SOME VENOMOUS SPECIES OF *Conus*

Species	*Toxic to Man*	*Mice*	*Paralysis in Fishes*	*Molluscs*	*Polychaetes*
PISCIVOROUS					
C. catus Hwass	X	X	X	O	O
C. geographus L.	X	X	X	O	O
C. obscurus Sowerby	X				
C. magnus L.		X	X	+	O
C. stercusmuscarius L.		X	X	+	O
C. striatus L.	X	X	X	+	O
C. tulipa L.	X	X	X	O	O
MOLLUSCIVOROUS					
C. ammiralis L.		O	O	X	?
C. aulicus L.	X?	X?	O	X	O
C. episcopus Hwass		O	O	X	O
**C. marmoreus* L.	X?	O	O	X	O
C. omaria Hwass	X?	O	O	X	O
C. textilis L.	X?	X	X	X	X
**C. tigrinus* Sowerby		O	O	X	O
VERMIVOROUS					
C. arenatus Hwass		O	N	O	X
C. eburneus Bruguière		N	N +	X	X
C. emaciatus Reeve			O	O	X
C. flavidus Lamarck		O	O	O	X
C. figulinus L.		O	O	+	X
C. imperialis L.	X	O	O	O	X
C. leopardus (Röding)	X		O	O	X
C. lividus Hwass	X	N	O	O	X
C. miles L.			O	O	X
C. millepunctatus Lamarck			O	O	X
C. omaria Hwass			O	O	X
C. planorbis Born		O	+	?	X
C. pulicarius Hwass	X	N	O	O	X
C. quercinus Solander	X	N	O	O	X
C. rattus Hwass		O	X	O	X
C. sponsalis Hwass	X	O	O	+	X
C. tessulatus Born		N	X	+	?
C. virgo L.		O	X	+	X

* Synonymous.
X Experimental or clinical evidence indicates toxicity.
\+ May be lethal but does not produce paralysis.
? Possibly toxic, or evidence questioned or conflicting.
N Produces localized necrosis.
O Non-toxic, insofar as is known.

(1965) the venoms of some vermivorous species provoke hemorrhage and necrosis in mammals, and have been implicated in injuries to man.

Other gastropods known to be venomous or poisonous are *Neptunea antiqua* (L.), *N. arthritica* Bernardi, *N. intersculpta* Sowerby, *Buccinum leucostoma* Lischke, *Fusitriton oregonensis* (Redfield), *Cassis tuberosa* L., *Aplysia californica* Cooper and *A. vaccaria* Winkler.

2. *Cephalopoda*

Cephalopods secrete a venom in their salivary glands that has a deleterious effect, and in particular a paralytic effect, on certain animals and occasionally on man. The toxin is used to immobilize the animal's prey; it also appears to have a digestive function. It plays a lesser role as a defensive weapon. Among the cephalopods that have been implicated in bites on humans or are known to be venomous are: *Octopus vulgaris* Cuvier (? = *O. rugosus*), *O. appollyon* (Berry), *O. bimaculatus* Verrill, *O. macropus* Risso, *O. rubescens* [= *E. cirrosa* (Lamarck)], *O. fitchi* (Raf.), *O. flindersi* Cotton, *Eledone moschata* (Lamarck), *E. aldrovandi* and *Sepia officinalis* L. In addition, some octopods are known to acquire *Physalia* tentacles and employ them as offensive and defensive weapons (Jones, 1963).

B. *Paralytic shellfish poisoning*

Paralytic shellfish poisoning is caused by certain molluscs which have ingested toxic dinoflagellates and which are subsequently eaten by man. Data concerning the organisms responsible for the poisoning, the chemistry and toxicology of the poison, and related problems are found in Section II. The present discussion is limited to the mechanism of the intoxication and a review of the shellfish most often implicated in the poisonings.

It is not known how the poison accumulates or concentrates in the mollusc; it can be stored in certain organs of the animal without deleterious effect. The site of concentration of the toxin may vary with the different species of shellfish, with the different seasons of the year and with certain other factors. In most instances the poison is concentrated in the digestive glands of the mollusc. In several clams the toxin may accumulate in the gills, while in at least one clam, *Saxidomus*, it is found in the siphons. Table III lists most of the molluscan species which have been reported to transvect dinoflagellate poison.

In addition, paralytic shellfish poisoning may be caused by at least one echinoderm, *Pisaster ochraceus* (Alaska to California), and one arthropod, *Emerita analoga* (Stimpson) (Oregon to South America). A non-paralytic type of shellfish poisoning following the ingestion of

certain clams, oysters and gastropods has been reported in Japan. It appears that this type of poisoning may also be traced to toxic plankton.

TABLE III. MOLLUSCA IMPLICATED IN PARALYTIC SHELLFISH POISONINGS

Species	*Distribution*
Mopalia muscosa Gould	Pacific coast of North America
Acmaea pelta Eschscholtz	Pacific coast of North America
Murex brandaris L.	Mediterranean Sea and west coast Africa
Arca noae L.	Mediterranean Sea
Cardium edule L.	European seas
Donax denticulatus L.	West Indies
Mactra (= *Spisula*) *solidissima* Dillwyn	Atlantic coast of North America
Schizothaerus nuttalli Conrad	Pacific coast of North America
Mya arenaria L.	Atlantic coast of North America, coasts of Greenland, Great Britain, Scandinavia, Japan, Alaska, British Columbia, Oregon and northern California
Mytilus californianus Conrad	Aleutian Islands east and south to Socorro Islands
Mytilus edulis L.	World-wide
Mytilus planulatus (Lamarck)	Victoria and Tasmania
Modiolus areolatus Gould	New South Wales
Modiolus demissus (Dillwyn)	Virginia to Florida
Crassostrea gigas (Thunberg)	Japan, Pacific northwest
Ostrea edulis L.	Atlantic coast of Europe
Placopecten magellanicus (Gmelin)	Labrador to North Carolina
Penitella penita Conrad	Pacific coast of North America
Ensis directus (Conrad)	Canada to Florida
Siliqua patula Dixon	Alaska to central California
Spondylus americanus Hermann	North Carolina to West Indies and Gulf of Mexico
Spondylus buccalis Röding	Philippines to Indonesia, Micronesia, New Guinea
Macoma nasuta Conrad	Kodiak Island to Baja California
Macoma secta Conrad	British Columbia to Baja California
Protothaca staminea Conrad	Aleutian Islands to Baja California
Saxidomus giganteus Deshayes	Alaska to central California
Saxidomus nuttalli (Conrad)	Northern California to Baja Calinia
Tivela stultorum Mawe	Central California to Baja California

C. *Venom apparatus*

1. *Conus*

The venom apparatus of *Conus* is thought to be homologous with the unpaired gland of Leiblein of certain of the higher gastropods. It serves as an offensive weapon for the gaining of food and, to a much lesser extent, as a defensive weapon against predators. It consists of a muscular *bulb*, a long coiled *venom duct*, the *radula* (the *radula sheath*), and the *radula teeth*. The muscular *pharynx* and extensible *proboscis* are considered to be accessory organs (Fig. 5).

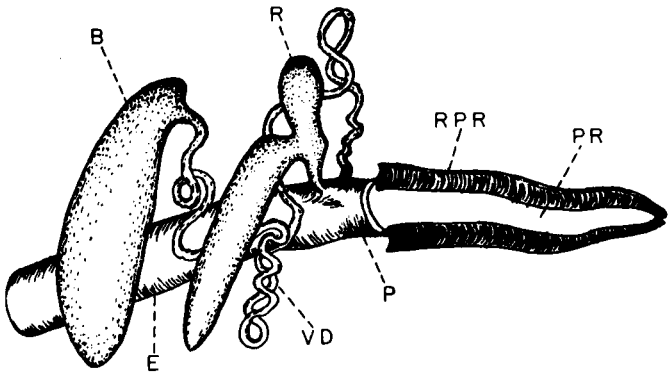


FIG. 5. Diagrammatic sketch of the venom apparatus of a *Conus*. B, bulb; E, esophagus; P, pharynx; PR, proboscis; RPR, reflected proboscis; R, radula; VD, venom duct.

The venom is thought to be secreted in the venom duct, a long convoluted tube extending from the bulb to the pharynx. According to Kohn and associates (1960), the length of the duct may be fifteen times the straight line distance between its origin and site of insertion. These investigators believe that the extreme length of this structure is best explained as an adaption that increases the secretory surface, and hence the volume of venom produced. The " venom " bulb lies against the posterior body wall, and while earlier workers assumed that this structure was a poison gland, more recent studies (Hermitte, 1946; Hinegardner, 1958) indicate that it probably secretes a mucoid substance which on contraction of the bulb forces the venom from the duct. Examination of the lumina of bulbs from several different species of *Conus* indicates that they are free of venom, although a small amount may sometimes be found in the area immediately proximal to the duct. In freshly prepared bulbs from *C. geographus* we have found a thin coat of mucus over the layer of cuboidal cells lining the lumen. This material was not toxic to mice. While the

bulb is generally considered unimportant in the production of the toxin, it probably plays some role in the venom apparatus. In a recent letter (5 May, 1964), Endean states that he has good evidence that the venom of *Conus* is produced in both the bulb and the duct.

The radula is a Y-shaped organ lying anterior to the esophagus-stomach and opening into the pharynx just anterior to the entrance of the venom duct. It produces the radula teeth. The organ is divided into three sections. The largest section, which overlies the esophagus-stomach, may contain as many as thirty teeth in various stages of development. The short arm of the gland attaches to the pharynx and contains most of the mature teeth. The third section is known as the ligament sac. The radula teeth are needle-like, from 1–10 mm in length and almost transparent. They vary in size and shape depending on the species involved.

A ligament is attached to the base of each tooth and serves as a means of fixation while the tooth is in the radula sheath. The teeth are moved from the radula into the pharynx and thence into the proboscis. They are then thrust by the proboscis into the prey during the stinging act. It is not known whether this is done by a sling shot-like mechanism, or by hydrostatic pressure or by some other means. In some species the tooth is held forcibly by the proboscis during the stinging act, while in others it is freed into the victim. When and how the venom gets into the radula teeth is not known. Some investigators have suggested that this occurs as the teeth are being transported through the pharynx or proboscis, while others feel that filling of the tooth and envenomation of the prey do not occur until the tooth is fired, or even until after it is fired, into the tissues of the victim.

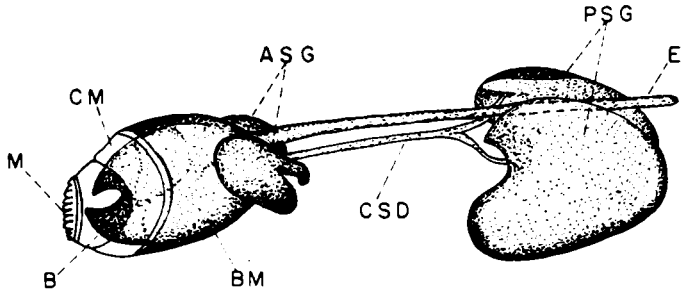


FIG. 6. Diagrammatic sketch of the venom apparatus of an octopus. ASG, anterior salivary glands; B, beak; BM, buccal mass; CM, circular muscle; CSD, common salivary duct; E, esophagus; M, mouth; PSG, posterior salivary glands.

2. *Octopus*

The venom apparatus of the octopus consists of the paired *posterior salivary glands*, the two *short* (salivary) *ducts* which join them with the *common salivary duct*, the paired *anterior salivary glands* and their *ducts*, the *buccal mass* and the *mandibles*, or *beak* (Fig. 6). The two paired salivary glands differ markedly in their size, structure and function (Arvy, 1960; Gennaro *et al.*, 1962). The common salivary duct opens into the sub-radular organ anterior to the tongue. The paired ducts from the anterior salivary glands open into the posterior pharynx. The buccal mass, or pharynx, is a muscular complex with two powerful horny jaws like an inverted parrot's beak. The exact role played by the two paired salivary glands in the preparation of the venom has not yet been fully established, although the posterior glands are known to be by far the more important as venom-producing organs. Further consideration of these structures in the production of the toxin will be found in the discourse on the chemisty and toxicology of molluscan poisons.

D. *Chemistry and toxicology*

1. *Gastropoda*

The venom of *Conus* is white, grey, yellow or black depending on the species involved; it is viscous and contains proteins and carbohydrates. Its pH varies from 7·8 to 8·1. On microscopic examination, granules of various sizes and shapes may be seen. In *C. striatus* some granules are almost round and measure 2–3 μ in diameter, while others are more oval in shape and measure approximately 7 μ in length and 3 μ in width. According to Kohn *et al.* (1960), these granules stain yellow with iodine, are insoluble in alcohol and resist boiling with concentrated HCl, but will dissolve in an aqueous solution of 20% KOH. The active principle is non-dialyzable. Toxicity is reduced but not lost on heating and incubation with trypsin. In *C. textile*, according to McColm and Endean (Endean, personal correspondence, 1964), the venom is contained in sausage-shaped or ellipsoidal granules varying in length from 3–7 μ and in width from 3–4 μ at their widest point. Indoles can be detected histochemically in the granules, each of which possesses a sheath of lipoprotein.

The lethal component of *Conus* venom has not been identified. Among the substances that have been found in extracts of the ducts or from the ducts are: homarine, gamma-butyrobetaine, N-methylpyridinium, several amines, possible indole amines, 5-hydroxytryptamine, lipoproteins and carbohydrates. Kohn and associates (1960)

found that as little as one thousandth of the total venom from one specimen of *C. textile* was lethal to *C. californicus*. In one species of fish, 0·2 mm^3 of the venom duct contents of *C. striatus* was lethal, while in mice the lethal dose was approximately 0·2 mg of venom duct content.

The lethal dose varies not only with the species involved but also with the area of the duct from which the extract is prepared. The contents of the posterior half of the duct in most species so far examined appear to be far more toxic than the contents from the anterior half. In mice, Endean and Rudkin (1965) found that *C. geographus* and *C. magus* venoms were lethal at 0·2–1·3 mg/kg body weight, while the minimal lethal dose for *C. striatus* venom was 21 mg/kg, and for *C. stercusmuscarius* venom, in excess of 200 mg/kg. In blennies they found the LD_{50} for the venom from the posterior duct of *C. striatus* to be 0·4 mg/kg, while the minimum lethal dose for *C. stercusmuscarius* venom was approximately 1·0 mg/kg. The minimal lethal dose of anterior duct venom from the latter cone was 3·0–28·0 mg/kg of blenny.

When *Conus* venoms toxic to mice are injected a syndrome develops which indicates that the venom has a particular effect on this animal's nervous system. Such signs as muscular weakness, changes in the deep reflexes, tremors, convulsions, ataxia, and paralysis of the skeletal muscles are sometimes seen. Respiratory rate is usually affected and complete cessation of respirations leading to death occurs in the more seriously poisoned animals. Increased parasympathetic activity may sometimes be observed following the injection of some *Conus* venoms. Signs related to the gradual development of cardiac failure may be evident in the more seriously poisoned mice.

Fishes injected with certain *Conus* venoms display radical alterations in their stance and swimming movements, erection of their fins, blanching, respiratory changes and muscular paralysis. Gastropods poisoned by the toxin often retract, sometimes violently, into their shells. Subsequently, when they are withdrawn, they appear to be paralyzed. Some may try to climb a wall of their tank only to fall to the bottom when paralysis occurs.

It would appear that the principal activity of the venom is directed toward the peripheral nervous system, although there is good evidence of some direct central nervous system involvement. Whyte and Endean (1962) have shown that venom from the posterior half of ducts of *C. geographus* blocks conduction in the mammalian phrenic nerve-diaphragm preparation, and causes paralysis of isolated skeletal muscle. *C. textile* venom did not produce these changes.

Extracts lethal to mammals have been prepared from the venom

duct of *C. californicus*. The lethal component was non-dialyzable, heat labile, and at pH 8 it was stable for 1 day at 5°C and for several weeks at −20°C. No lethal activity could be demonstrated with extracts from the bulb. In mice the LD_{50} was approximately 2·4 mg protein per kg body weight; this was an amount equivalent to the contents of 1·6 venom ducts. In mammals, small amounts of the venom produced a decrease in systemic arterial pressure and cardiac rate, and an increase in respirations. Larger amounts caused more profound cardiovascular changes, and respiratory arrest. Positive pressure breathing did not prevent death in those cases where severe cardiac changes and cessation of respirations occurred (Whysner and Saunders, 1963).

Asano and Itoh (1960) have demonstrated that the salivary poison of the gastropod *Neptunea arthritica*, which is sometimes eaten in Japan, is tetramine. They suggest that histamine, choline and choline ester also found in the salivary glands of this mollusc act synergistically with the tetramine in producing the poisoning. Fänge (1960) notes that in *N. antiqua*, the tetramine is probably responsible for almost all of the biological activity of the salivary gland extract. He found that approximately 1% of the gland consists of tetramine.

The role of pharmacologically active choline esters in certain Muricidae hypobranchial glands is not known (Whittaker, 1960). While the hypobranchial gland is thought by some to be a venom organ, this has not been established. It is difficult to propose, on the basis of the substances identified from the gland, the role of this structure in Muricidae. Extracts of the gland contain urocanylcholine, which combines the ganglion-stimulating properties of acetylcholine with a strong neuromuscular blocking action (Erspamer and Glässer, 1957).

The digestive glands of the sea hares, *Aplysia vaccaria* and *A. californica*, contain a toxin which is water and acetone soluble, and which produces muscular weakness and death in mice and chicks. Frogs are more resistant to the poison (Winkler, 1961).

Homarine appears to be widely distributed in mollusca, particularly in the squid *Loligo* and the welk *Busycon*, although its significance in these animals is not understood.

2. *Cephalopoda*

An impressive number of substances have been isolated from or identified in the salivary glands of various cephalopods since the early contributions of Bert (1867) and Lo Bianco (1888). Many of these substances have known biological activities, although these activities are not always apparent in the physiopharmacological effect of the whole toxin; and some substances either do not have a significant

biological activity or else our present state of knowledge does not indicate what activity is present. Finally, the amounts of the various components of the salivary secretions of cephalopods are subject to such variations that it is most difficult to determine whether or not a particularly toxic substance is present in a sufficient amount to be deleterious to the envenomated victim. The importance of the synergistic effects of several of the toxic components, and of the autopharmacological response, further complicate the consideration of the chemistry and toxicology of this venom.

With this admittedly fragile apology in mind, I shall note a few of the substances and biological activities that have been identified with the salivary glands of cephalopods: tyramine, octopine, agmatine, adrenaline, noradrenaline, 5-hydroxytryptamine, L-*p*-hydroxyphenylethanolamine, histamine, dopamine, tryptophan, and certain of the 11-hydroxysteroids, polyphenols, phenolamines, indoleamines and guanidine bases.

The L-*p*-hydroxyphenylethanolamine was first described by Erspamer in 1940. It was found in extracts of the posterior salivary glands of *Octopus vulgaris* and identified with an adrenaline-like activity. It is thought to be the precursor of hydroxyoctopamine, or L-*nor*-adrenaline (Erspamer, 1952). Hartman *et al.* (1960), showed that the content of the posterior salivary glands of *O. apollyon* or *O. bimaculatus* decarboxylated L-3,4-dihydroxyphenylalanine (DOPA), DL-5-hydroxytryptophan, DL-*erythro*-3,4-dihydroxyphenylserine, DL-*erythro-p*-hydroxyphenylserine, DL-*m*-tyrosine, DL-*erythro-m*-hydroxyphenylserine, histidine, L-histidine, DL-*erythro*-phenylserine, 3,4-dihydroxyphenylserine, tyrosine and *m*-tyrosine.

Among the activities that have been demonstrated for the salivary glands of cephalopods are those shown in Table IV. In general, the salivary glands of cephalopods contain little or no proteolytic enzymes, amylases or lipases; hyaluronidase may be present in some secretions.

Ghiretti (1959) purified a protein, *cephalotoxin*, from the posterior salivary glands of *Sepia officinalis* which he suggested was the biologically active component of the toxin. It gave positive biuret and ninhydrin reactions, and had maximum ultra-violet absorption at 276–278 mμ. Four bands migrating towards the cathode are seen on starch gel electrophoresis at pH 8·5. Further purification was obtained by absorption on calcium phosphate gel at neutral pH, and three bands were obtained on electrophoresis. Treatment with trypsin at 37°C and at neutral pH resulted in the complete loss of activity. The toxin contained no cholinesterase or aminoxidase activity. Analysis of cephalotoxin from the posterior salivary gland of *Octopus vulgaris*

TABLE IV. ACTIVITIES OF SALIVARY GLANDS OF *Octopus vulgaris*

Activity	*Salivary gland* Posterior	Anterior	*Reference*
Tyramine oxidase	X		Blaschko *et al.* (1952–53)
Tryptamine oxidase	X		Blaschko *et al.* (1952–53)
5-Hydroxytryptamine oxidase	X		Blaschko *et al.* (1952–53)
Proteolytic	X		Ghiretti (1953)
Hyaluronidase	X	X	Romanini (1954)
Mucinolytic	X	X	Romanini (1954)
Dopa decarboxylase	X		Hartman *et al.* (1960)
Histamine oxidase	X	weak	Arvy (1960)
Succinic dehydrogenase	X	X	Arvy (1960)
Phosphatase	X	weak	Arvy (1960)
Adenosine-triphosphatase	X	weak	Arvy (1960)
Butyrylthiocholinesterase	X	O	Arvy (1960)
Acetylthiocholinesterase	X	O	Arvy (1960)
Acetylnaphtholesterase	X	X	Arvy (1960)
Alpha naphtholase		weak	Arvy (1960)

showed: protein 74·05% (N determination), 64·25% (biuret reagent); carbohydrates, 4·71% and hexosamines, 5·80% (Ghiretti, 1960).

In 1949, Erspamer observed that the posterior salivary glands of *Eledone moschata* and *E. aldrovandi* contained a substance which when injected into mammals caused marked vasodilatation, and produced hypotension and stimulation of certain extravascular smooth muscles. The substance was first called *moschatin* but was later renamed *eledoisin*. It is an endecapeptide having the following amino acid sequence:

Pyr - Pro - Ser - Lys - Asp (OH), - Ala -
Phe - Ileu - Gly - Leu - Met - NH_2

Subsequent studies showed that the eledoisin was fifty times more potent than acetylcholine, histamine or bradykinin in its ability to provoke hypotension in the dog. It produces an increase in the permeability of the peripheral vessels, stimulates the smooth muscles of the gastrointestinal tract, and causes an increase, which is atropine resistant, in salivary secretions. It is easily distinguishable from the kinins and substance P (Erspamer and Anastasi, 1962). In spite of its marked pharmacological activities, the role and significance of this substance in the salivary glands of *Eledone* is not clear. It is not found in the salivary glands of *Octopus vulgaris* or *O. macropus*, which might indicate that it is not a necessary component of the cephalopod toxin. However, its biological activities may be duplicated by some related,

but as yet undescribed, polypeptide in the other cephalopod toxins. It would appear that eledoisin plays some part in protein synthesis, and it is quite possible that its role in the salivary gland is limited for the most part to this activity.

E. *Clinical problem*

1. *Gastropoda*

The following cones have been implicated in injuries to man: *Conus geographus*, *C. tulipa*, *C. catus*, *C. striatus*, *C. obscurus*, *C. textilis*, *C. imperialis*, *C. aulicus*, *C. marmoreus*, *C. pulicarius*, *C. quercinus*, *C. litteratus*, *C. lividus*, *C. sponsalis* and *C. omaria*. The first five appear to be the most dangerous, although *C. textilis* cannot be discounted (Kohn, 1963).

The sting gives rise to immediate, sometimes intense, localized pain at the site of the injury. Within 5 min the victim usually notes some numbness and ischemia about the wound, although in a case seen by the author the affected area was red and tender rather than ischemic. A tingling or numbing sensation may develop about the mouth, lips and tongue, and over the peripheral parts of the extremities. Other symptoms and signs may develop during the first 30 min following the injury. These include: hypertonicity, tremor, muscle fasciculations, nausea and vomiting, dizziness, increased lacrimation and salivation, weakness, and pain in the chest which increases with deep inspiration. The numbness about the wound may spread to involve a good part of the extremity or injured portion. In the more severe cases, respiratory distress with chest pain, difficulties in swallowing and phonation, marked dizziness, blurring of vision and an inability to focus, ataxia, and generalized pruritus have been reported. In fatal cases " respiratory paralysis " precedes death.

2. *Cephalopoda*

Bites by octopods are very rare, and their effects are usually limited to localized signs and symptoms. Halstead (1949), Berry and Halstead (1954), and Flecker and Cotton (1955) have recorded several interesting cases and the reader is directed to their works for a more descriptive account of poisonings in man.

The bite of the octopus results in two small puncture wounds; they appear to bleed more freely than one would expect from a similar non-envenomized traumatic wound. Pain is minimal, and in the two cases seen by the author it was described as no greater than that which would have been produced by a sharp pin. Tingling or numbness about the wound site are not uncommon complaints. Swelling is

usually minimal immediately following the injury but may develop 6–12 h later. Localized pruritus sometimes occurs over the edematous area. " Light-headedness " of several hours duration was reported in both cases observed by us; there were no other systemic symptoms or signs in these two cases. The wounds healed slowly.

In the case reported by Flecker and Cotton (1955), the patient complained of dryness in the mouth and difficulty in breathing following the bite, but no localized or generalized pain. Subsequently, breathing became more labored, swallowing became difficult and the patient began to vomit. Severe respiratory distress and cyanosis developed, and the victim expired. The findings at autopsy were negative.

3. *Shellfish poisoning*

Three types of shellfish poisoning are recognized. *Gastrointestinal shellfish poisoning* is characterized by nausea, vomiting, abdominal pain, weakness and diarrhea. The onset of symptoms generally occurs 8–12 h following ingestion of the offending mollusc. This type of intoxication is caused by bacterial pathogens, and is usually limited to gastrointestinal signs and symptoms. It rarely persists for more than 48 h.

Halstead (1965–66) calls the second type *Erythematous shellfish poisoning*. It is more commonly known as allergic shellfish poisoning. It is characterized by an allergic response, which may vary from one individual to another. The onset of symptoms and signs occurs 30 min to 6 h after ingestion of the mollusc to which the individual is sensitive. The usual presenting signs and symptoms are diffuse erythema, swelling, urticaria and pruritus involving the head and neck, and then spreading to the body. Headache, flushing, epigastric distress and nausea are occasional complaints. In the more severe cases, generalized edema, severe pruritus, swelling of the tongue and throat, respiratory distress and vomiting sometimes occur. Death is rare but persons with a known sensitivity to shellfish should avoid eating all molluscs. The sensitizing protein appears more capable of provoking a serious autopharmacological response than most known sensitizing proteins.

Paralytic shellfish poisoning is known variously as gonyaulax poisoning, paresthetic shellfish poisoning, mussel poisoning, or mytilointoxication. Pathognomonic symptoms develop within the first 30 min following ingestion of the offending mollusc. Parasthesia, described as tingling, burning or numbness is noted first about the mouth, lips and tongue; it then spreads over the face, scalp and neck, and to the finger tips and toes. Sensory perception and proprioception

are affected to the point that the individual moves incoordinately, and in a manner similar to that seen in another more common form of intoxication. Ataxia, incoherent speech or aphonia are prominent signs in severe poisonings.

The patient complains of dizziness, tightness of the throat and chest and some pain on deep inspiration. Weakness, malaise, headache, increased salivation and perspiration, thirst, and nausea and vomiting may be present. The pulse is usually thready and rapid; the superficial reflexes are often absent and the deep reflexes may be hypoactive. If muscular weakness and respiratory distress grow progressively more severe during the first 8 h, death may ensue. If the victim survives the first 10–12 h the prognosis is good. Death is usually attributed to " respiratory paralysis ". The case fatality rate varies from 1 to 10%.

VII. Poisonous Fishes

Approximately 500 species of marine fishes are known to be toxic, or may on ingestion be poisonous to man. This number does not include those fishes which have caused a poisoning traceable to bacterial pathogens. Most, but by no means all, of these species are found in the coral-reef belt. As a whole their distribution is spotty, even in a particular part of the ocean or around an island. They tend to occur in greater numbers around islands than along continental shores. Most species are non-migratory reef fishes; a few predaceous species might be considered migratory, although certainly not in the strict sense of the word. They may be either herbivores or carnivores. Some poisonous species have tissues which are toxic at all times; other species are poisonous only at certain periods, or in certain areas, while still others have only specific organs which are toxic, and the toxicity of these tissues may vary with time and location.

Although early workers were inclined to attribute fish poisoning to a single common cause, it is now obvious that several etiological factors may be involved. Among those that have been suspected or suggested, at one time or another, of being implicated in ichthyotoxism are:

1. Feeding on toxic or non-toxic marine plants. In the latter case it has been suggested that the metabolic processes of the fish were able to alter certain plant components into a toxic form.
2. Feeding on toxic protista, particularly dinoflagellates.
3. Feeding on shoreline terrestrial plants whose berries or leaves have been swept into the sea.

4. Pollution of the ocean by industrial poisons.
5. The dumping of large quantities of metals, particularly copper, into the ocean, such as occurred in the Pacific at the end of World War II.
6. Epidemics in fishes, giving rise to toxic endogenous substances.
7. Spawning activities.
8. Feeding on corals.
9. Feeding on jellyfishes.
10. Feeding on palolo worms.
11. Feeding on toxic molluscs.
12. Feeding on zooanthellae in corals.
13. Feeding on other toxic fishes.
14. The dumping of radioactive materials into the ocean.
15. Climatic changes.
16. Leaving the fish exposed to moonlight.

The history of fish poisoning dates back at least to the Fifth Dynasty of the Egyptians. Figures in the tomb of the pharoah Ti indicate that *Tetraodon* was known in that period (2500 B.C.), and evidence indicates that these people knew the fish to be poisonous. According to Autenrieth (1833), Galen observed that the moray could be poisonous. Tani (1945) states that the earliest record of poisoning following ingestion of tetraodontoid fishes in the Orient is that from the Han Dynasty (202 B.C.–A.D. 220). He cites a passage noting the death of a man following ingestion of the liver of one of the puffers.

The first historian of the West Indies, Martyr, described the first known incident of fish poisoning in that area in 1555. A few years later, de Landa wrote of the lethal properties of the puffers off Yucatan (Baughman, 1952). Quiros (1606) speaks of a fish called "Pargos" which poisoned his entire crew in the New Hebrides.

Kaempfer, a physician to the Dutch Embassy in Japan during the seventeenth century, describes the poisoning produced by several species of "Furube" (puffers). He also notes the use of the fish by "people that by some long and tedious sickness are grown weary of their lives, or are otherwise under miserable circumstances, frequently choose this poisonous fish, instead of a knife or halter to make away with themselves" (Kaempfer, 1690–1692). The use of this fish as an agent for poisoning man, and other animals, has a long and fascinating history which extends down to the present. It is the animal poison most commonly employed by characters in fiction for the doing away with rivals or enemies. In a recent Ian Fleming novel it was used to poison the British secret agent, James Bond, who almost went to eternal rest

from its effects. Only the prompt administration of artificial respiration saved the sleuth.

Perhaps the most interesting description, and certainly one of the most lucid, is that of Captain James Cook:

7th September, 1774.
" This afternoon a fish being struck by one of the natives near the watering-place, my clerk purchased it, and sent it to me after my return on board. It was of a new species, something like a sunfish, with a large, long, ugly head. Having no suspicion of its being of a poisonous nature, we ordered it to be dressed for supper; but, very luckily, the operation of drawing and describing took up so much time, that it was too late, so that only the liver and row were dressed, of which the two Mr. Forsters and myself did but taste. About three o'clock in the morning we found ourselves seized with an extraordinary weakness and numbness all over our limbs. I had almost lost the sense of feeling; nor could I distinguish between light and heavy bodies, of such as I had strength to move; a quart pot, full of water, and a feather being the same in my hand. We each of us took an emetic, and after that a sweat, which gave us much relief. In the morning, one of the pigs, which had eaten the entrails was found dead."

Aboriginal peoples sought to differentiate poisonous from non-poisonous fishes on the basis of their colors, the condition of their gills, the positions of scales, and the staining effects of various organs on metals. In some areas of the world the fish was often fed to some animal. If the animal remained asymptomatic for a number of hours the fish was considered safe to eat.

More recent testing methods have included the " silver coin test ", the " ant test ", the " fly test ", and the " copper test ". None of these, however, are satisfactory. A number of field tests are being studied by several investigators at the present time, and it is hoped that a more reliable testing method for fish toxins will be developed within the next several years.

More recent reviews on fish poisoning have been prepared by Hiyama (1943), Yudkin (1944), Lee and Pang (1945), Russell (1952), Fish and Cobb (1954), Mills (1956), Halstead (1958), Randall (1958), Banner *et al.* (1963a, b), Halstead (1964).

A. *Ichthyosarcotoxic fishes*

Ichthyosarcotoxism is caused by the ingestion of fishes containing a poison within their musculature, viscera or skin. It is generally identified with the kind of fish involved: *ciguatera, tetraodon, scombroid, clupeoid, cyclostome* or *elasmobranch. Hallucinatory fish poisoning* is also identified with this type of poisoning.

1. *Ciguatera*

The word ciguatera was perhaps first applied to a poisoning caused by the ingestion of the marine snail *Livona pica* (" cigua "), a staple seafood found throughout the Caribbean (Poey, 1866). The word is now commonly used to indicate that type of fish poisoning characterized by certain gastrointestinal-neurological manifestations. It may occur following the ingestion of certain tropical reef and semi-pelagic marine species such as the barracudas, groupers, sea basses, snappers, surgeonfishes, parrotfishes, jacks, wrasses, and perhaps certain gastropods. Randall (1958) suggests that *gymnothorax* poisoning should not be separated from ciguatera poisoning. Dr. Paul Scheuer of the Hawaii Marine Laboratory reports that ciguatera toxin and moray eel (*Gymnothorax javanicus*) toxin are the same. Homogeneity has been established by thin-layer chromatography, paper electrophoresis, and by counter-current distribution (Helfrich, personal communication, 1965).

Approximately 300 species of marine fishes have been implicated in ciguatera poisoning. Among the most often incriminated are those listed in Table V. Since almost all of these fishes are normally edible, and many are valuable food fishes in some parts of the world, ciguatera poisoning is not only the most common but also the most treacherous form of ichthyotoxism.

TABLE V. SOME FISHES RESPONSIBLE FOR CIGUATERA POISONING*

Species	*Distribution*
Acanthuridae, surgeonfishes, 15 species†	
Acanthrus bleekeri Günther	East Indies, Polynesia
A. hepatus (L.)	African and American Atlantic coasts
A. lineatus (L.)	Philippine Islands, East Indies, Polynesia
A. nigrofuscus (Forskål)	Red Sea to Polynesia, Formosa
A. olivaceus Bloch and Schneider	Pacific Islands
A. triostegus (L.)	Johnston Island
Ctenochaetus strigosus (Bennett)	Red Sea to tropical Pacific, Formosa

* Compiled from the studies of Hiyama (1943), Fish and Cobb (1954), Halstead and Russell (1964), and Russell and Halstead (1965).

† Number of species given by Halstead (1956), and adjusted on the basis of more recent findings.

TABLE V—*continued*

Species	*Distribution*
Acanthuridae, surgeonfishes, 15 species—*continued*	
Naso unicornis (Forskål)	Tropical Pacific
Zebrasoma flavescens (Bennett)	Johnston Island
Z. veliferum (Bloch)	Oceania
Aluteridae, filefishes, 17 species	
Alutera monoceros (L.)	West Indies, tropical Pacific north to China and Japan
A. schoepfi (Walbaum)	Florida
A. scripta (Osbeck)	East and West Indies, tropical Pacific
Amanses sandwichiensis (Quoy and Gaimard)	Philippine Islands, tropical Pacific
Anacanthus barbatus Gray	Philippine Islands
Monacanthus chinensis (Osbeck)	East Indies to Ryuku Islands
Oxymonacanthus longirostris (Bloch and Schneider)	Philippine and Gilbert Islands
Pervagor melanocephalus (Bleeker)	Philippine Islands
Pseudomonacanthus macrurus (Bleeker)	Philippine Islands
Stephanolepis hispidus (L.)	Florida
S. setifer (Bennett)	Indian Ocean, Japan, tropical Atlantic
Balistidae, triggerfishes, 20 species	
Abalistes stellaris (Bloch and Schneider)	Indo-Pacific, Philippine Islands
Balistapus undulatus (Mungo Park)	Indo-Pacific, Kenya
Balistes capriscus Gmelin	Atlantic Ocean, Mediterranean Sea
Balistoides viridescens (Bloch)	Red Sea to East Indies, Philippine Islands
B. niger (Bonnaterra)	Indo-Pacific to Marshall Islands
Canthidermis sobaco (Poey)	Florida, Cuba
Melichthys ringens (Osbeck)	India, Philippine Islands
Odonus niger (Ruppell)	Indo-Pacific, Philippine Islands
Pseudobalistes fuscus (Bloch)	Philippine Islands
Rhinecanthus aculeatus (L.)	Kenya, Viet-Nam, Oceania
R. verrucosus (L.)	Philippine Islands
Sufflamen chrysoptera (Bloch)	Eastern Pacific
Carangidae, jacks (pompanos), 25 species	
Caranx ascensionis (Osbeck)	Japan, Oceania, Caribbean Sea
C. bartholomaei Cuvier and Valenciennes	Caribbean Sea
C. carangus (Bloch)	West Indies
C. crumenopthalmus (Bloch)	Red Sea to Polynesia
C. crysos (Mitchill)	Tropical seas

TABLE V—*continued*

Species	*Distribution*
Carangidae, jacks (pompanos), 25 species—*continued*	
C. hippos (L.)	Orient, East and West Indies
C. ignobilis (Forskål)	Line Islands
C. melampygus Cuvier	Indo-Pacific to Hawaii, Japan
C. ruber (Bloch)	West Indies, Tropical seas
C. sexfasciatus Quoy and Gaimard	Indo-Pacific, Taiwan
Elagatis bipinnulatus (Quoy and Gaimard)	Caribbean Sea
Scomberoides sancti-petri (Cuvier)	Malaya
Seriola fasciatus (Bloch)	Caribbean Sea
Trachurus trachurus (L.)	Atlantic coast of Europe
Zonichthys falcatus (Cuvier and Valenciennes)	Cuba
Chaetodontidae, butterflyfishes, 10 species	
Chaetodon auriga Forskål	Johnston Island
C. ephippium Cuvier	Johnston Island, Phoenix Islands
C. reticulatus Cuvier	Malaysia
Heniochus acuminatus (L.)	Indian Ocean
Holacanthus imperator (Bloch)	Malaysia, tropical seas
Pygoplites diacanthus (Boddaert)	Indian Ocean to Hawaii, Japan
Labridae, wrasses, 7 species	
Cheilinus fasciatus (Bloch)	East Indies, Japan, tropical Pacific
C. rhodochrous Gunther	Johnston Island
Coris gaimard (Quoy and Gaimard)	East Indies, tropical Pacific to Hawaii
C. julis (L.)	European seas
Epibulus insidiator (Pallas)	Indian Ocean to Hawaii, Japan
Lachnolaimus maximus (Walbaum)	Florida, Caribbean Sea
Lethrinidae, porgies, 6 species	
Lethrinus miniatus (Forster)	Oceania
L. opercularis Cuvier and Valenciennes	Tropical Pacific
L. variegatus Cuvier and Valenciennes	Philippine Islands, Oceania
Monotaxis grandoculis (Forskål)	Red Sea, Indo-Pacific to Hawaii
Lutjanidae, snappers, 28 species	
Aprion virescens Cuvier and Valenciennes	Japan, tropical Pacific to Hawaii
Bodianus rufus (L.)	Cuba
Gnathodentex aurolineatus (Lacépède)	Oceania
Lutjanus apodus (Walbaum)	West Indies

TABLE V—*continued*

Species	*Distribution*
Lutjanidae, snappers, 28 species—*continued*	
L. argentimaculatus (Forskål)	Red Sea, Indian Ocean
L. aya (Bloch)	Caribbean Sea
L. blackfordii Good and Bean	Caribbean Sea
L. bohar (Forskål)	Red Sea, Indo-Pacific, Japan
L. coatesi Whitley	South Pacific, Australia
L. cyanopterus (Cuvier and Valenciennes)	Polynesia
L. fulviflamma (Forskål)	Red Sea, Indo-Pacific
L. gibbus (Forskål)	Australia
L. janthinuropterus (Bleeker)	Indo-Pacific
L. monostigma (Cuvier and Valenciennes)	Samoa
L. semicinctus (Quoy and Gaimard)	Indo-Pacific
L. vaigiensis (Quoy and Gaimard)	Indian and western Pacific oceans
Ocyurus chrysurus (Block)	South seas
Paradicichthys venenatus Whitley	Australia
Muraenidae, morays, 19 species	
Echidna nebulosa (Ahl)	Baltic, North Mediterranean and tropical seas
Enchelycore nigricans (Bonnaterra)	Yugoslavia
Gymnothorax buroensis (Bleeker)	Johnston Island
G. concolor (Abbott)	Cuba
G. flavimarginatus (Rüppell)	East Africa, Japan, Oceania
G. funebris Ranzani	Cuba
G. javanicus (Bleeker)	Johnston Island
G. meleagris (Shaw and Nodder)	East Africa, Japan, Oceania
G. moringa (Cuvier)	Pacific and tropical Atlantic oceans, Gulf of Mexico
G. pictus (Ahl)	East Indies, tropical Pacific
G. undulatus (Lacépède)	Tropical Pacific, Hawaii
Muraena helena (L.)	Mediterranean Sea
M. insularum Jordan and Davis	Pacific Ocean, Gulf of Mexico
M. lentiginosa Jenyns	Pacific Ocean, Gulf of Mexico
M. tile (Hamilton-Buchanan)	Tropical Indian Ocean
Scaridae, parrotfishes, 15 species	
Scarus blochi (Cuvier and Valenciennes)	Malaysia
S. caeruleus (Bloch)	Mediterranean Sea, Africa, West Indies
S. ghobban Forskål	Red Sea, Indian Ocean, China, West Indies
S. jonesi (Streets)	Phoenix Islands
S. microrhinos (Bleeker)	Japan to tropical Pacific
S. perspicillatus Steindachner	Johnston Island
S. vetula (Bloch and Schneider)	West Indies

TABLE V—*continued*

Species	*Distribution*
Serranidae, sea basses, 26 species	
Cephalopolis fulvus punctatus (L.)	West Indies
Epinephelus adsensionis (Osbeck)	West Indies, tropical seas
E. argus (Bloch and Schneider)	Red Sea, Indo-Pacific to Australia and Hawaii
E. cabrilla (L.)	Gulf of Mexico
E. fario (Thunberg)	West Indies, tropical seas
E. fuscoguttatus (Forskål)	Red Sea, Japan, Indo-Pacific, Oceania
E. maculatus (Bloch)	Indian and Pacific oceans, West Indies
E. merra (Bloch)	Oceania
E. morio (Cuvier and Valenciennes)	Caribbean Sea
Mycteroperca tigris (Cuvier and Valenciennes)	West Indies, tropical seas
Plectropomus leopardus	Tuamotu Islands
P. oligacanthus Bleeker	Okinawa, Indo-Pacific
P. truncatus Fowler	Okinawa to western tropical Pacific
Trisotropis bonaci (Poey)	Cuba
T. venenosus (L.)	West Indies, Florida
T. v. apua (Bloch)	West Indies, Florida, tropical seas
Variola louti (Forskål)	Oceania, Philippine Islands
Sphyraenidae, barracudas, 7 species	
Sphyraena barracuda (Walbaum)	Japan to tropical Pacific
S. forsteri Cuvier and Valenciennes	Indian and Pacific oceans
S. guachancho Cuvier and Valenciennes	Gulf of Mexico
S. jello Cuvier and Valenciennes	Africa, Red Sea, Indian Ocean
S. picuda (Bloch and Schneider)	Indo-Pacific, Brazil, West Indies
S. sphyraena (L.)	West Indies

Most fishes involved in ciguatera poisoning are reef or shore species; a few are open-water forms. Almost all species are found between latitude 35°N and 34°S. They are usually bottom-dwellers, although seldom found below depths of 200 feet, and are more likely to be found in lagoons than along seaward reefs. Most toxic species are either carnivorous or benthonic algae feeders. According to Randall (1958), none appear to be plankton-feeders as adults, and among the carnivorous species there appears to be a positive correlation between the amount

of fish in the diet and the degree of toxicity. There is also a tendency for the larger fish of a species to be more toxic than the smaller fish of the same species. In most cases the flesh is less toxic than the viscera. The liver is usually the most poisonous part of the fish, although the testes may be equally as toxic.

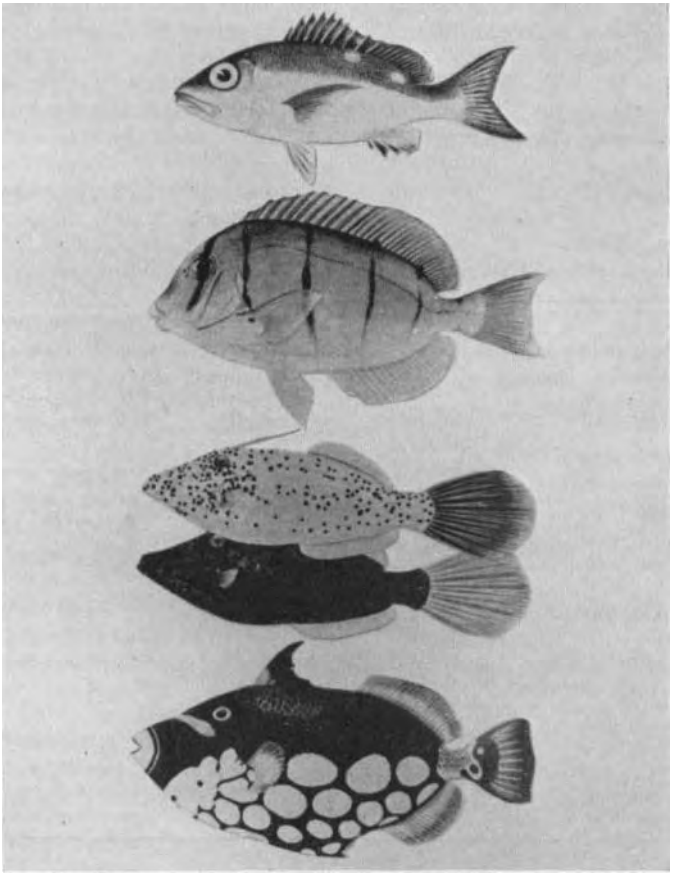


FIG. 7. Some marine fishes responsible for ciguatera poisoning. Top to bottom: *Lutjanus bohar*, red snapper; *Acanthurus triostegus*, surgeonfish; *Alutera scripta*, filefish; *Balistoides niger*, triggerfish. (From Hiyama, 1943.)

It appears certain that this form of poisoning is associated with the food-chain relationship of the fish. It is suspected that the poison originates in a benthonic organism, from which it is transferred directly to herbivorous fishes and indirectly to carnivorous. While both herbi-

vorous and carnivorous fishes can cause ciguatera poisoning, the latter fishes are the more toxic, and in some areas are the only fishes sufficiently toxic to cause poisoning in man. This would indicate that the more predaceous species feed upon the toxic herbivorous species, and

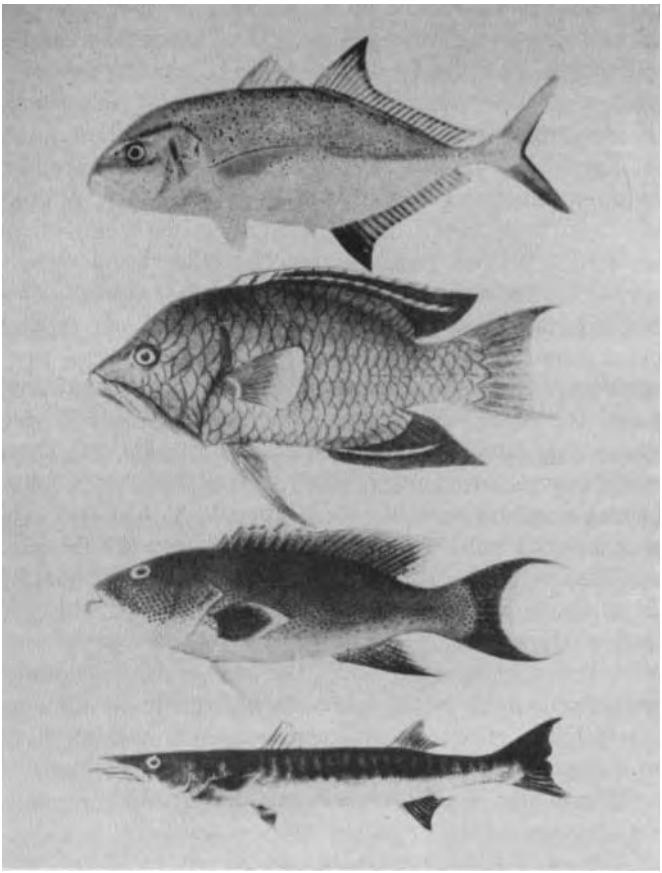


FIG. 8. Some marine fishes responsible for ciguatera poisoning. Top to bottom: *Caranx melampygus*, jack; *Epibulus insidiator*, wrasse; *Variola louti*, sea bass; *Sphyraena barracuda*, barracuda. (From Hiyama, 1943.)

that they in some manner accumulate the toxin without deleterious effects. The herbivorous forms may feed on a toxic alga, fungus or protistan, but the present evidence favors the blue-green algae (Cyanophyta) as the most probable common source for the ciguatera toxin. Randall (1958) has discussed the possible role of these algae

in the poisoning, and the reader should consult his work for a more thorough review. Banner *et al.* (1960) have presented some interesting data on the possible role of algae of the genus *Lyngbya* as agents in the poisoning.

More recently, Banner *et al.* (1963b) have studied the stomach contents of toxic *L. bohar* and have found the principal constituent to be reef fishes, particularly acanthurids. As many of the Acanthuridae on which *L. bohar* feeds are toxic it was suggested that this family of fishes may be the chief dietary source of the toxin. Acanthurids, in turn, appear to feed on many kinds of algae. One of the blue-green algae, *Plectonema terebrans* (Borinet and Flahautt), was found growing epiphytically on most of the algae eaten by the common acanthurids in Christmas Island; this alga has been suspected of being associated with fish poisoning in the Gilbert Islands. Helfrich and Banner (1963) have shown that several non-toxic fish fed on a diet of toxic *L. bohar* may become toxic.

(*a*) *Chemistry.* Regrettably, most of the investigations on the chemistry and toxicology of ciguatera toxin prior to 1955 contributed very little to our present knowledge on this poison. Indeed, these studies sometimes led investigators into rather unfruitful areas. Among other problems there was considerable confusion about the solubility of the toxin, and it was not until the works of Hashimoto (1956), Banner and Boroughs (1958) and McFarren and Bartsch (1959) that it became apparent that the more lethal portions of the poison could not initially be extracted with water. This finding helped to clarify some of the discrepancies previously reported. By the end of 1959, ciguatera poison was described as being heat stable, stable to drying, soluble in ether, petroleum ether, chloroform, acetone, methanol and 90% ethanol, and insoluble in water and dilute acids. It was thought to be a lipid. It was also described as being dialyzable.

In 1960, Banner *et al.* reported that a partially purified extract of the toxin from *L. bohar* could be obtained by extraction of the dried tissues of the fish with 95% ethanol, followed by concentration, dilution with distilled water to an alcoholic solution of approximately 25%, extraction with diethyl ether and evaporation of the ethereal extract to dryness. In a peanut oil vehicle the extract was found to be lethal at 200 mg/kg body weight when injected intraperitoneally into mice. Further tests indicated that Soxhlet extraction with alcohol was a superior method for separating the toxin. A more lethal fraction was subsequently obtained by column chromatography using solvents of varying polarity.

Hessel *et al.* (1960) found the toxin from *L. bohar* to be soluble in ethyl acetate, isopropyl alcohol, methylene chloride, methyl ethyl ketone and carbon tetrachloride. Further experiments indicated that the active portion probably did not contain acid or basic groups. He prepared an acetone extraction of the toxic musculature, concentrated the residue, extracted with ether, evaporated the ether and extracted the residue with acetone. The light yellow, mobile oil was then emulsified into frog Ringer's solution and the material assayed on the frog sciatic nerve. It was found that the toxin had an inhibitory effect on the action potential, and that this effect could be correlated with the oral toxicity test in cats.

Subsequently, Hessel (1961) prepared seven fractions of the toxin by various methods of fractionation. A partially purified product was obtained by dissolving the toxin in warm methanol and precipitating the non-toxic contaminants by cooling to −20°C. The toxin was recovered from the methanol by evaporation and subsequent fractionation was carried out by silicic acid column chromatography. Four fractions were collected. These were assayed by feeding experiments with cats, by intraperitoneal injections of aqueous emulsions into mice, and by studies on the action potential of excised frog sciatic nerve preparations. The studies indicated that the toxin could be carried through the seven extraction-fractionation processes. However, the last two fractions were the only fractions that showed appreciable toxicity. From this work Hessel concluded that the toxic component of ciguatera poison probably contains more than one substance, and that these substances possess polar characteristics, and that they are probably not phospholipids.

The most recent discussion of extraction and separation procedures for obtaining ciguatera toxin from *L. bohar* is that by Banner *et al.* (1963b). The method is outlined in Fig. 9. The 4 kg of dried fish, which does not include the viscera, is equivalent to approximately 16 kg of whole fresh fish.

According to Li (1965) the toxic portion of ciguatera poison is an anticholinesterase which causes death through asphyxiation. Protopam chloride with atropine was found to be an effective antidote.

(*b*) *Toxicology.* When semi-purified preparations of the toxin (Banner *et al.*, 1960) are injected intravenously into rabbits they produce an immediate fall in blood pressure with a simultaneous increase in respiratory rate and depth. As the blood pressure returns toward normal, respiratory rate decreases and becomes irregular. Temporary changes are noted in the electrocardiogram during the period of

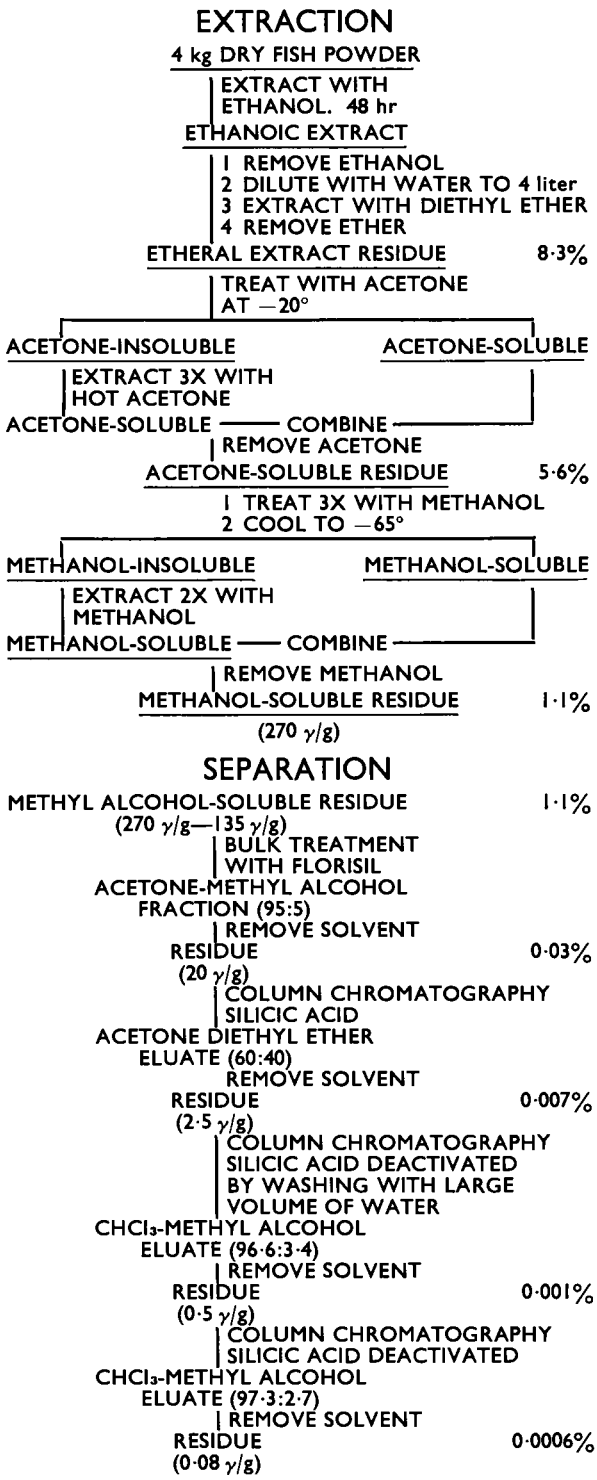


FIG. 9. Procedures used by Banner *et al.* (1963b) for extraction and separation of ciguatera toxin. Yields expressed as percentages of original dry weight. Lethality shown in parenthesis as amount per gram in mice injected intraperitoneally.

hypotension (Banner *et al.*, 1963a). In cats, similar changes have been observed by us following administration of the toxin. In small doses the poison produces a fall in systemic arterial pressure concomitant with an increase in respiratory rate, and transient changes in the electrocardiogram and electroencephalogram. With large doses there is a more precipitous fall in systemic arterial pressure and severe respiratory distress, which may sometimes lead to cessation of respirations. The electrocardiogram may reflect changes varying from prolongation of the PR interval or ST, T segment changes to a third degree block. The changes in the electroencephalogram are indicative of a decreased blood supply to the brain. The toxin did not appear to have a direct, deleterious effect on those areas of the brain where the conventional lead electrodes were placed.

Further studies by Banner and his colleagues (1963b) indicated that the toxin appeared to have an effect on mammalian neuromuscular transmission, or at least on the junction and/or the nerve. Direct stimulation of the muscle produced contractions which were not significantly altered by the toxin. In the toad sciatic nerve-sartorius muscle preparation these investigators found that the normal twitch and tetanus response elicited through the nerve were lost following prolonged exposure to the toxin; the muscle retained its contractility when stimulated directly. Observations from a single experiment performed in our own laboratory appeared to indicate that the toxin had a direct effect on the post-synaptic membrane, similar to that of certain non-depolarizing, blocking substances. It has not yet been possible to evaluate this single observation.

Mammals which ingest fish containing the ciguatera poison develop muscular weakness, particularly in the hind legs first, muscular incoordination and ataxia, vomiting, diarrhea, and increased parasympathetic activity. If lethal amounts are consumed, marked respiratory distress, vomiting, salivation, cyanosis and prostration are often seen prior to death. Deep reflexes are usually hypoactive, and in the more severe cases of poisoning the animal loses its righting reflex.

(*c*) *Clinical problem.* In 1871, Garnier wrote that in New Caledonia fish poisoning occurred so frequently that " some apprehensive people no longer dare to eat fish ". " The islanders attribute these illnesses to evil spirits who will conceal themselves in the bodies of fish to torment them." According to Banner *et al.* (1963a), replies from questionnaires sent out by the South Pacific Commission indicated that fish poisoning was known in practically every island in the region. The number of

cases of ichthyotoxism reported during recent years and the numerous findings of toxic fish by biologists and public health workers indicate that ichthyosarcotoxism is an important medical entity within a broad circumglobal area. The clinical significance of the entity has been the subject of numerous reports (von Fraenkel and Krick, 1945; Lee and Pang, 1945; Cohen *et al.*, 1946; Halstead and Lively, 1954; Halstead, 1958; Banner *et al.*, 1963a).

The first symptoms and signs of ciguatera poisoning are usually evident within 4 h following ingestion of the offending fish. The presenting symptoms are nausea and parasthesia (described as numbness or tingling) about the mouth, tongue and throat, or sometimes over the face and distal parts of the fingers and toes. Weakness, abdominal pain, vomiting, diarrhea and chills are often experienced. If the poisoning is severe the victim may also complain of severe malaise, muscular weakness and incoordination, chills and fever, restlessness, insomnia and dyspnea. Increased sweating and hypotension are sometimes observed during the acute illness. Other symptoms and signs sometimes seen during the agonal period are headache, dizziness, dilation of the pupils, strabismus, ptosis and severe pain in the back and thighs. The superficial and deep reflexes are usually hypoactive or absent. The victim often experiences parasthesia which is almost classical in type: hot objects feel cold and cold objects feel hot.

In fatal poisonings the above findings progressively worsen, severe muscular weakness and muscular incoordination develop and walking becomes difficult. Pruritus, often limited to the palms of the hands and soles of the feet becomes intense, breathing becomes labored and cyanosis may develop. Convulsions have been reported. There is no evidence to indicate that a case of ciguatera poisoning imparts immunity. A second poisoning in a patient previously stricken within 6 months, or perhaps even a year, is more likely to be more serious than the same poisoning in a patient not previously exposed to the toxin.

The symptoms and signs of ciguatera poisoning appear to be very similar to those of paralytic shellfish poisoning, and it is possible that the components of the two toxins are related.

2. *Tetraodon*

Tetraodon, puffer or fugu poisoning may occur following the ingestion of certain puffers, ocean sunfishes or porcupinefishes. The puffers, or puffer-like fishes, appear to be the only fishes universally regarded as poisonous. They are known in various parts of the world as globefishes, blowfishes, balloonfishes, swellfishes, toadfishes,

blasers or tambores. These names stem from the fish's ability to inflate itself by taking in large quantities of air or water. Of the approximately 100 species of these fishes, over 50 have been involved in poisonings to man or are known to be toxic under certain conditions. Among the most often incriminated are those listed in Table VI.

TABLE VI. SOME FISHES RESPONSIBLE FOR TETRAODON POISONING

Species	*Distribution*
Diodontidae, porcupinefishes, 10 species	
Diodon holocanthus L.	East Indies, Japan, tropical Pacific, Hawaii
D. hystrix L.	Red Sea, Indo-Pacific, Japan, Philippine Islands, Oceania
Molidae, sunfishes, 1 species	
Mola mola (L.)	Japan, Australia
Tetraodontidae, puffers, 40 species	
Canthigaster bennetti (Bleeker)	East Indies, Philippine Islands, Australia, south Pacific
Sphoeroides alboplumbeus (Richardson)	Japan
S. annulatus (Jenyns)	Baja California
S. florealis (Cope)	India, Philippine Islands, New Hebrides, Hawaii
S. hamiltoni (Richardson)	Cape Africa, Australia, Oceania
S. honckeni (Bloch)	Cape Africa, east coast of Africa, China
S. lobatus (Steindachner)	Baja California
S. lunaris (Bloch)	Red Sea, east coast of Africa, Indo-Pacific, China, Japan, Australia
S. maculatus (Bloch)	South Africa, Indian Ocean, Atlantic coast of North America
S. oblongus (Bloch)	Indo-Pacific, China, Japan, Philippines, Australia, tropical Pacific
S. rubripes (Temminck and Schlegel)	Viet-Nam, Japan, Australia
S. sceleratus (Forster)	East coast of Africa, Indo-Pacific, Japan, Australia, Oceania, Caribbean Sea
S. spengleri (Bloch)	Florida, Caribbean, Brazil
S. testudineus (L.)	Caribbean, Brazil
S. vermicularis (Temminck and Schegel)	China, Korea, Japan
Tetraodon basilevskianus (Baselewsky)	East China Sea, Japan
T. cutcutia (Hamilton)	Gulf of Mexico, Baja California

TABLE VI—*continued*

Species	*Distribution*
Tetraodontidae, puffers, 40 species—*continued*	
T. firmanentus (Temminck and Schlegel)	Japan
T. fluviatilis (Hamilton)	Indo-China, East Indies
T. hispidus (L.)	Red Sea, east coast of Africa, Indo-Pacific, Japan, China, Australia, Oceania
T. immaculatus (Bloch and Schneider)	Red Sea, east coast of Africa, Indo-Pacific, China, Philippine Islands, south Pacific
T. lineatus L.	Mediterranean Sea, coast of East Africa, East Indies
T. melagris (Lacépède)	East Indies, Polynesia
T. nigropunctatus Bloch and Schneider	Red Sea, east coast of Africa, Indo-Pacific, Japan, Philippine Islands, Australia, Oceania
T. niphobles (Jordan and Snyder)	Japan
T. patoca (Hamilton)	India, East Indies, Philippine Islands
T. pelambangensis Bleeker	Viet-Nam
T. pseudommus (Chu)	Japan
T. psittacus (Bloch and Schneider)	Brazil, Guiana
T. reticularis Bloch and Schneider	Indo-Pacific, China, Philippine Islands, Guam, tropical Pacific
T. stellatus Bloch and Schneider	Red Sea, east coast of Africa, Indo-Pacific, China, Korea, Japan, Oceania

Puffers are chiefly tropical fishes, although some species do extend into temperate zones. They sometimes present a public health problem in Japan, China, the Philippine Islands, the East Indies, and certain parts of Oceania. Their toxin is the most lethal of all the ichthyosarcotoxic types. It is concentrated for the most part in the ovaries or testes, the liver, and the intestines. Lesser amounts are found in the skin; the body musculature is usually free of the poison. The appearance and amount of toxin in the fish is related to the reproductive cycle, and appears to be greatest just prior to spawning, which usually occurs in the late spring or early summer. Toxicity is considerably lower during the late fall and winter periods.

(*a*) *Chemistry.* Attempts to purify *tetrodotoxin* and study its chemistry were initiated by Japanese workers over 70 years ago. The

first significant works were those of Tahara (1894, 1910). He isolated several substances, one a white, hygroscopic powder to which he assigned the formula $C_{16}H_{31}NO_{16}$. It is now apparent that his " tetrodotoxin " contained less than 1% of the pure toxin. Ishihara (1924)

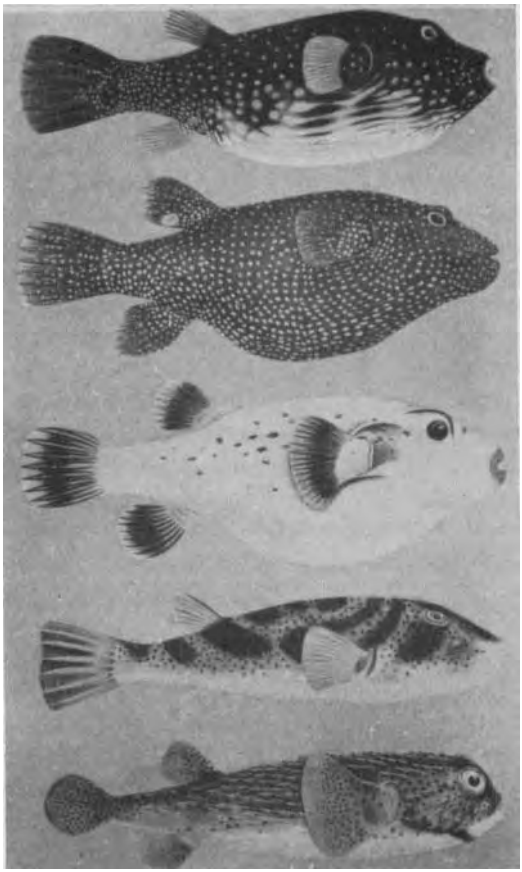


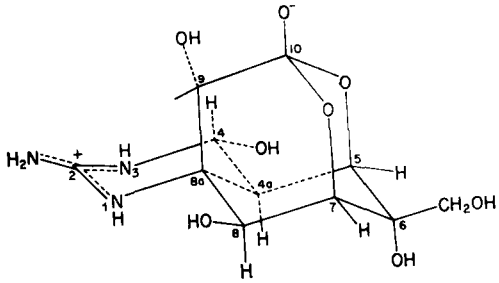
FIG. 10. Some tetraodontoid fishes implicated in puffer poisoning. Top to bottom: *Tetraodon hispidus*, death puffer; *T. meleagris*, white-spotted puffer; *T. nigropunctatus*, black-spotted puffer; *Sphoeroides annulatus*, Gulf puffer; *Diodon hystrix*, porcupinefish. (From Halstead, 1959.)

observed that the reducing properties of the poison increased on hydrolysis, and concluded that an integral part of the toxin was an ester of glucose. This was challenged by Nagai and Ito (1939) who found that " a reducing carbohydrate " which might have been considered the poisonous substance by Ishihara, " is removed by means

of $AgNO_3$ and seems to have no toxic value." They concluded that all of the nitrogen of the toxin existed as amino nitrogen, and that the toxin might well be an acyclic compound. It is obvious that these investigators were working with very impure extracts of the poison.

Further studies on the crude toxin have been carried out by a number of workers, but it was not until 1950 that Yokoo reported on the isolation of a very pure crystalline toxin, *spheroidine*, from the ovaries of *Sphoeroides rubripes*. This toxin was given the formula $C_4H_7O_3N$ (molecular weight 116), but the formula was subsequently revised to $C_{12}H_{17}O_{10}N_3$ on the basis of further analyses and a cryoscopically determined molecular weight of 335 (Yokoo, 1952). Subsequently, Yokoo and Morosawa (1955) demonstrated the existence of two forms of the toxin which differed in toxicity but which gave identical infrared spectrograms and paper chromatograms. The spectrograms were almost identical to those obtained by Tsuda and Kawamura (1953), who had isolated tetrodotoxin from the same source, and who on the basis of analytical data proposed the formula $C_{12}H_{19}O_8N_3$.

During the past several years a number of excellent studies on the structure of this poison have been carried out by Tsuda *et al.* (1963), Tomie *et al.* (1963), Goto *et al.* (1963a, b) and Mosher *et al.* (1964). These studies indicate that the formula for the toxin is probably $C_{11}H_{17}O_8N_3$, and that in acid solution the poison exists as a zwitterion represented by the structure:



(*b*) *Toxicology*. The toxicological properties of puffer toxin have been the object of considerable study (Osawa, 1885; Ishihara, 1924; Iwakawa and Kimura, 1922; Katagi, 1927; Yano, 1938; Nagayosi, 1941; Yokoo, 1950; Yudkin, 1945; Matsumura and Yamamoto, 1954). The results from these various studies indicated that the poison had a deleterious effect on neuromuscular transmission, on conduction in somatic motor and sensory nerves, and on the sympathetic fibers. The toxin also had a direct effect on the medullary centers, on skeletal

muscle (reducing its excitability), and on the contractile force of the heart. It was said to have no cholinesterase activity.

In 1942, Kuros presented evidence which appeared to indicate that tetrodotoxin has little effect on the acetylcholine contracture of the perfused gastrocnemius muscle of the toad. He proposed that the toxin had no curariform-like activity. Subsequently, Furukawa *et al.* (1959) concluded, on the basis of their studies on the frog sartorius nerve-muscle preparation, that while tetrodotoxin has a potent narcotic effect on nerve and muscle it does not depolarize them. The poison did not suppress the sensitivity of the end-plate to acetylcholine, even at concentrations greater than those necessary for the narcosis of the nerve and muscle. All of the above studies were conducted with relatively crude preparations of the toxin; nevertheless, some of the findings are supported by more recent investigations.

When the toxin is ingested by mammals, lethargy, muscular weakness and incoordination develop rapidly. Ataxia occurs and paralysis is observed, first in the hind limbs and later in the fore limbs. Retching and vomiting are often severe. Deep reflexes are lost and respirations become labored. Cyanosis may be apparent and convulsions sometimes occur. In cats the oral LD_{50} of the toxin is in excess of 0·20 mg/kg body weight. The minimum lethal dose in mice is approximately 8·0 μg/kg.

Murtha and colleagues (1958) extracted the toxin from three species of *Sphoeroides* with acidified methanol, purified by precipitation with acetone, by adsorption on CS-101 and XE-89 resins and by final precipitation with A-20 resin and solid sodium carbonate. The product was assayed as a crystalline hydrochloride, and in cats found to have an intraperitoneal LD_{50} of less than 10 μg/kg body weight. When 5 μg/kg was injected intravenously into cats, there was a precipitous fall in blood pressure and rapid cessation of respirations. In bilaterally vagotomized cats with transected cervical cords, similar changes were seen. The electrocardiograms were unremarkable, except for decreases in heart rate. However, studies on the heart in open-chest dogs showed that the toxin had a deleterious effect on contractile force. Li (1963) was unable to demonstrate a change in cardiac output following injection of his preparation of the toxin. He suggested that the hypotensive crisis was due exclusively to changes in the peripheral blood vessels.

Murtha and colleagues (1958) also found that the toxin had a more depressant effect on respiration when it was given through the carotid artery than when given through a vein. They interpreted this fact to indicate that the poison had a direct effect on the brain. The finding

that retching and vomiting occurred in unanesthetized cats and not in anesthetized ones was also interpreted to indicate that the toxin had a direct effect on the central nervous system. These interpretations must certainly be questioned, but it is not wholly unlikely that the toxin does have a direct effect on the brain. Li (1963) suggested that the cause of death following a lethal dose of the poison is respiratory arrest from the action on the brain stem respiratory centers. Again, this may in part be so, although the arguments put forth by Li are not wholly convincing.

Intravenous or close intra-arterial injections of 4–10 μg/kg body weight of the poison caused a block in skeletal muscle's response to 30-per-second motor nerve excitation. The toxin also depressed the response of the muscle to direct stimulation, although the depression developed more gradually. The order of recovery was: return of muscle response to direct stimulation, return of response to repetitive nerve stimulation, and finally, return of response to slow stimulation of the nerve. Further studies indicated that paralysis occurs in the hind limbs at a time when the diaphragm is affected only partially, and the forelimbs not at all (Murtha and colleagues, 1958). These findings illustrated the ascending type of paralysis noted previously by Japanese workers.

Tetrodotoxin appears to have no effect on the resting potential (Russell *et al.*, 1961), although, as suggested by Kao and Fuhrman (1963) in their excellent report on *tarichatoxin*, the normal increase in sodium and potassium conductances associated with activity is probably markedly reduced. Since tetrodotoxin and tarichatoxin are identical substances, some of the findings reported by Kao and Fuhrman on the latter toxin are included here. The poison has no effect on oxidative metabolism, on extrusion of sodium from the nerve, or on the cholinesterase system. It has little effect on the postsynaptic cells in the autonomic ganglion. These workers feel that all of the systemic effects (of tarichatoxin) might be explained by its particular action on the preganglionic cholinergic and somatic motor nerves. The postganglionic adrenergic nerves are probably affected more slowly and to a lesser degree. They suggest a block in the preganglionic cholinergic and somatic motor nerves might interfere with impulses regulating vasomotor tone, and thus cause the hypotension, although as previously noted there is some evidence that the toxin might have a direct effect on the heart (and this writer suspects that some part of the hypotensive crisis might be due to changes in the pulmonary circulation). They further suggest that the deleterious effects on neural stimulation would lead to the changes in the skeletal muscles. Tachycardia might occur

from vagal escape, and normal reflex slowing of the heart in the presence of an increasing blood pressure might not be observed. While these suggested activities would certainly explain and simplify the syndrome of the poisoning, this writer suspects that the mechanisms may not be quite so simple. Nevertheless, further pharmacological studies with these activities in mind should be most fruitful.

As previously noted (Russell *et al.*, 1961; Mosher *et al.*, 1964) tetrodotoxin possesses pharmacological properties very similar to those of certain local anesthetics, though far more potent. As pointed out by the latter authors, the toxin appears to differ from procaine and cocaine in that it acts selectively to prevent or reduce the usual increase in permeability to the sodium ion without seeming to affect the permeability to potassium (Narahashi *et al.*, 1964).

In summary, the toxin has a particular effect on the axons of the preganglionic cholinergic and somatic motor nerves. It blocks the excitability of directly stimulated muscle fibers. In the frog it blocks the action potential at concentrations of approximately 1μg/l of bathing solution. The toxin provokes hypotension either through its effect on vasomotor tone through the preganglionic cholinergic fibers, or through its action on the heart directly. It depresses respiration by its effect on the nerve, neuromuscular junction and muscle, and perhaps by its direct effect on the respiratory centers. It may also depress the respiratory centers indirectly through the hypotensive crisis, which no doubt precipitates a cerebral and cerebellar anemia. It might be concluded that while these various mechanisms are not yet firmly established, our present state of knowledge on this, one of the most toxic non-proteins known, is far more gratifying than on any other of the fish poisons.

(*c*) *Clinical problem.* Tetraodon poisoning is the most dangerous form of ichthyosarcotoxism. According to Halstead (1964), Japanese statistics show a mortality rate of 61·5% for this type of poisoning. Approximately twenty persons a year die of the poisoning in Japan. Deaths are also reported from other endemic areas. The clinical case is characterized by the rapid onset (5–30 min) of weakness, dizziness, pallor and paresthesia about the lips, tongue and throat. The paresthesia is usually described as "tingling or pricking sensations", and is often noted in the limbs, particularly the fingers and toes, as the illness develops. While tetrodotoxin is considered by most workers to be a potent emetic, and nausea is often one of the presenting symptoms, vomiting does not occur in most cases. In those cases in which it does, the vomiting develops during the first hour of the illness.

In a severe poisoning the victim may complain of " numbness all over ", giving rise to a feeling of " floating in air ". Such victims usually have decreased or absent superficial and deep reflexes, as well as severe changes in proprioception. Other symptoms and signs often present during the acute stages of the poisoning include: sweating, increased salivation, pain on inspiration, changes in oral temperature and in the pupils, and hypotension with a weak but increased heart rate. In the more severe cases the muscular weakness increases, muscular fasciculations may be seen, and respiratory distress, chest pain and cyanosis are usually present. Petechial hemorrhages may develop. Paralysis involving the body musculature, the larynx and the extra ocular muscles is found in the most severe cases. Convulsions occasionally occur. Death is attributed to " respiratory paralysis ", and occurs 6–24 h following ingestion of the toxic fish.

3. *Scombroid*

Certain of the mackerel-like fishes, the tunas, skipjacks and bonitos are occasionally involved in poisonings to man. The symptomatology of these poisonings is quite different from that provoked by ciguatera toxin, although some of these fish may also be implicated in ciguatera poisoning. If scombroids are inadequately preserved, a toxic substance is formed within the body musculature. This substance was once thought to be histamine, formed by the action of enzymes and bacteria (Markov, 1943) or released by bacterial action on the death of the fish (Geiger *et al.*, 1944). However, more recent evidence would seem to indicate that the toxic component may not be histamine (Kawabata *et al.*, 1955a), although it does resemble this substance in certain of its properties (Kawabata *et al.*, 1955b). Kawabata has given the toxic substance the name " saurine".

Following ingestion of the offending fish the victim usually complains of nausea, vomiting, diarrhea and epigastric distress, flushing of the face, headache, and burning of the throat, sometimes followed by numbness, thirst, and generalized urticaria. These signs and symptoms usually appear within 2 h of the meal and subside within 16 h. In the more severe cases there may be some muscular weakness and pain. The poisoning is rarely serious. The offending fish is often said to have a " peppery taste ".

The general symptomatology is certainly similar to that of histamine poisoning, and the fact that antihistamines do lessen the distress would appear to indicate that a histamine-like substance or a substance that releases histamine in the victim may be involved in the poisoning.

4. *Clupeoid*

This form of poisoning sometimes occurs following ingestion of certain herring-like fishes of the tropical Pacific. According to Halstead (1956) seventeen species of Clupeidae have been found to be toxic at one time or another. Among these implicated have been *Arengus neopilchardus* (Steindachner), *Clupanodon thrissa* (L.), *Dussumieria acuta* Valenciennes, *Sardinella fimbriata* (Valenciennes) and *S. sindensis* (Day).

Like ciguatera poisoning this form occurs sporadically and over an extensive area. While the symptoms and signs provoked by the toxin are slightly different from those in ciguatera poisoning, it has not yet been definitely established that the toxin is indeed different from that responsible for ciguatera poisoning. Chemical and toxicological data on clupeoid poison are lacking.

5. *Cyclostome*

The slime and flesh of certain lampreys and hagfishes appears to contain a toxin which may produce gastrointestinal signs and symptoms (Coutière, 1899; Engelsen, 1922). The chemical and toxicological nature of the toxin is unknown.

6. *Elasmobranch*

Consumption of the musculature of the Greenland shark *Somniosus microcephalus* (Bloch and Schneider) has caused poisonings in both human and dogs (Jensen, 1914, 1948). The livers of several species of tropical sharks have caused severe poisonings and even deaths (Coutière, 1899; Phisalix, 1922; Halstead, 1959). Species reported to be poisonous at times include: *Carcharhinus melanopterus* (Quoy and Gaimard), *Heptranchias perlo* Bonnaterre, *Hexanchus grisseus* (Bonnaterre), *Carcharodon carcharias* (L.) and *Sphyrna zygaena* L.

Poisoning from the eating of toxic shark liver is characterized by nausea, vomiting, abdominal pain, diarrhea, headache and parasthesia about the mouth. These manifestations are sometimes evident within 30 min following ingestion of the organ. Malaise, weakness, muscle cramps and respiratory distress may develop. The pulse is usually weak and rapid; reflexes are hypoactive and the victim complains of visual disturbances and a sensation of " heaviness ". Ataxia, severe respiratory distress and coma usually precede death. In most cases complete recovery requires 5–20 days. The case fatality rate is not known. It has been suggested that hypervitaminosis A might be responsible for the poisoning. It has also been suggested that elasmobranch poisoning should be classified as a form of ciguatera poisoning.

7. *Hallucinatory fish poisoning*

This type of poisoning is characterized by central nervous system signs and symptoms, and by the lack of gastrointestinal manifestations. It has occurred following the ingestion of certain mullet and surmullet (goatfish). Among the species reported to have caused this poisoning are: *Mugil cephalus* L., *Neomyxus chaptalli* (Eydoux and Souleyet), *Paraupeneus chryserydros* (Lacépède), and *Upeneus arge* Jordan and Evermann. Reports of poisonings have been filed in the tropical Pacific and Hawaii (Jordan *et al.*, 1927; Fish and Cobb, 1954; Helfrich and Banner, 1960).

These fishes appear to be toxic only in certain specific areas and only during certain times of the year, usually June, July and August. Unfortunately a systematized collecting program of these fishes from an endemic area does not appear to have been made, thus it is difficult to predict just when the fish will become toxic. Helfrich and Banner (1963) were unable to produce the poisoning with seventy-five of the above fishes taken on Kauai and Molokai during July and August of 1959. Our own group failed to find any toxin in 123 specimens of *Upeneus arge* taken near Hanalei Bay on Kauai during August and September of 1961. One hundred additional specimens collected between Haena and Anahola on the same island during 1961 also proved to be non-poisonous.

In 1961, several fishermen on Kauai informed me that only the head of the goatfish was toxic, and that the body could be eaten with impunity. However, other fishermen stated that the body was equally as toxic. The former contention appears to have been handed down from the reports of Dr. Nils Larsen (Jordan *et al.*, 1927) of Queen's Hospital in Honolulu, who fed the brain of a goatfish, " weke pahala " to a cat which " at once went crazy, but recovered, as in fact, all cases soon recover." This story is well known among fishermen in the Hawaiian Archipelago and no doubt has influenced some reports on this problem. As noted by Helfrich and Banner (1963), boiling, frying or steaming does not appear to affect the poison.

Nothing is known of the chemistry or toxicology of this poison. However, the symptomatology in the human cases so far reported would seem to indicate that the offending substance is different from that responsible for ciguatera poisoning. The onset of symptoms occurs 10–90 min following ingestion of the toxic fish. The victim complains of light-headedness or dizziness, weakness, muscular incoordination and sometimes ataxia, hallucinations and depression. In the severe cases, there may be parasthesia about the mouth, and some muscular paralysis and dyspnea. The agonal period is usually of short duration,

2–24 h, and few cases are serious enough to bring the victim to the doctor. If the victim goes to sleep immediately following the poisoning he may have violent nightmares. This complaint accounts for the term " nightmare weke " being given to the causative fish *U. arge.* Reports of similar intoxications have been noted by Smith (1950) and Van Pel (1959).

B. *Ichthyootoxic fishes*

A number of fresh-water fishes and a few marine species produce a toxin which appears to be restricted to their gonads. In these fishes the body musculature and even the gastrointestinal organs are edible. Poisoning occurs following ingestion of the roe, or gonads and roe (Autenrieth, 1833; Knox, 1888; Coutière, 1899; Pawlowsky, 1927; Hubbs and Wick, 1951; Halstead, 1964). The eggs of *Scorpaenichthys marmoratus* Girard appear to be avoided by fish-eating and scavenging birds, as well as by mink and racoon (Pillsbury, 1957).

The poisoning is characterized by the rapid onset of nausea, vomiting and epigastric distress. Diarrhea, dryness in the mouth, thrist, tinnitus and malaise sometimes occur. In the more severe cases, syncopy, respiratory distress, chest pain, convulsions and coma may ensue. Complete recovery usually occurs within a few days.

C. *Ichthyohemotoxic fishes*

A toxic substance has been found in the blood of many species of fishes, although the principal contributions to our knowledge of the toxin have come to us through studies on the bloods of the eels *Anguilla* and *Muraena.* Poisonings from the ingestion of fresh blood are extremely rare. The few cases reported have occurred in persons who of their own volition have drunk quantities sufficient to cause symptoms; most of these have occurred following the ingestion of blood from the European fresh-water eels, or *Muraena helena.* In these cases there is some nausea, vomiting, epigastric distress, increased salivation, urticaria and generalized weakness. Parasthesia about the mouth, respiratory distress, paralysis and death have been reported. If eel blood is permitted to stand in contact with mucous membranes for 10–20 min, a severe inflammatory reaction may develop (Autenrieth, 1833; Pennavaria, 1888; Mosso, 1889; Springfield, 1889; Steindorff, 1914).

The several earlier works on the chemistry and physiopharmacology of eel sera have been reviewed by Courville *et al.* (1958), and by Ghiretti and Rocca (1963), and the reader is referred to these papers for a more detailed consideration. The latter authors fractionated eel serum as

shown in Fig. 11, and obtained a solution which on injection into rabbits produced increased salivation, respiratory distress, clonic convulsions, paralysis and death. The solution also hemolized rabbit red blood cells.

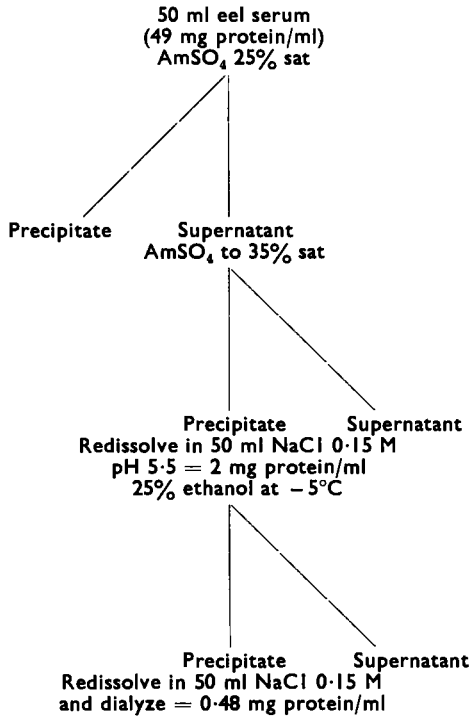


FIG. 11. Fractionation method for eel serum used by Ghiretti and Rocca (1963)

More recently, Rocca and Ghiretti (1964) fractionated the serum proteins on a DEAE cellulose column and demonstrated that the lethal activity was associated with a single fraction. Several fractions were obtained by linear gradient elution from 0·02M at pH 7·5 to 0·2M at pH 6·5 Tris-phosphate buffer. The fractions collected between pH 7·3 and 7·0 were found to contain 30 to 40% of the total proteins, and the entire lethal activity.

VIII. VENOMOUS FISHES

Fifteen years ago Dr. Halstead and I sat down one Monday afternoon in the old " Fish Museum " of the School of Tropical Medicine at Loma Linda and examined some notes I had prepared for a seminar at

Caltech. I recall we discussed the taxonomy, ecology, venom apparatus and the toxins of the venomous fishes. We also found time to consider the clinical problem of envenomation by fishes, and no doubt we mused over expeditions and experiences in the field of marine toxinology. In looking over my notes of that afternoon I find that a great deal of our knowledge on the venomous fishes at that time came from less than a dozen published works. If it became necessary to repeat that seminar today, I would need to read scarcely less than 500 articles, most of which have been written during these past 15 years. As in most areas of the biological sciences, contributions in marine toxinology continue to be made at an ever increasing, and sometimes alarming, rate. It would not be possible in this short review to present all of the

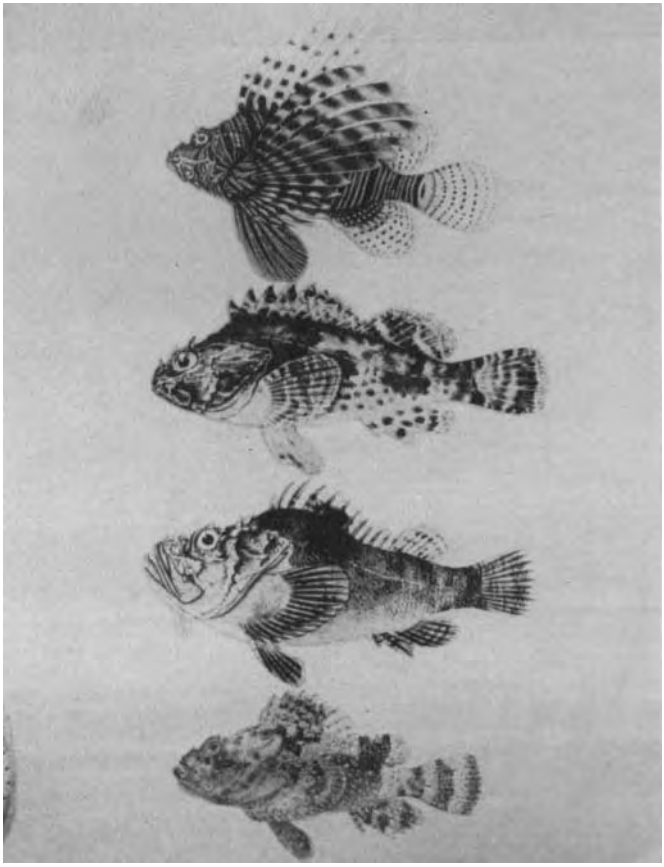


FIG. 12. Some venomous fishes. Top to bottom: *Pterois volitans*, zebrafish; *Scorpaena guttata*, sculpin; *Scorpaenopsis diabolus*, scorpionfish; *Scorpaena plumieri*, scorpionfish. (From Evermann and Seale, 1907; Hiyama, 1943; and Halstead, 1959.)

material on venomous fishes that has been published during this past decade. I have attempted to choose what I believe to be some of the more representative and important papers within this discipline. The selection will give the reader a general understanding of some of the problems in the field, and will, I trust, provide him with an adequate background for further study on this fascinating topic.

Well over 200 species of marine fishes, including the stingrays, scorpionfishes, zebrafishes, stonefishes, weevers, toadfishes, stargazers and certain of the sharks, ratfishes, catfishes and surgeonfishes, are known or thought to be venomous. For the greater part, venomous fishes are shallow-water reef or inshore fishes. They are most frequently

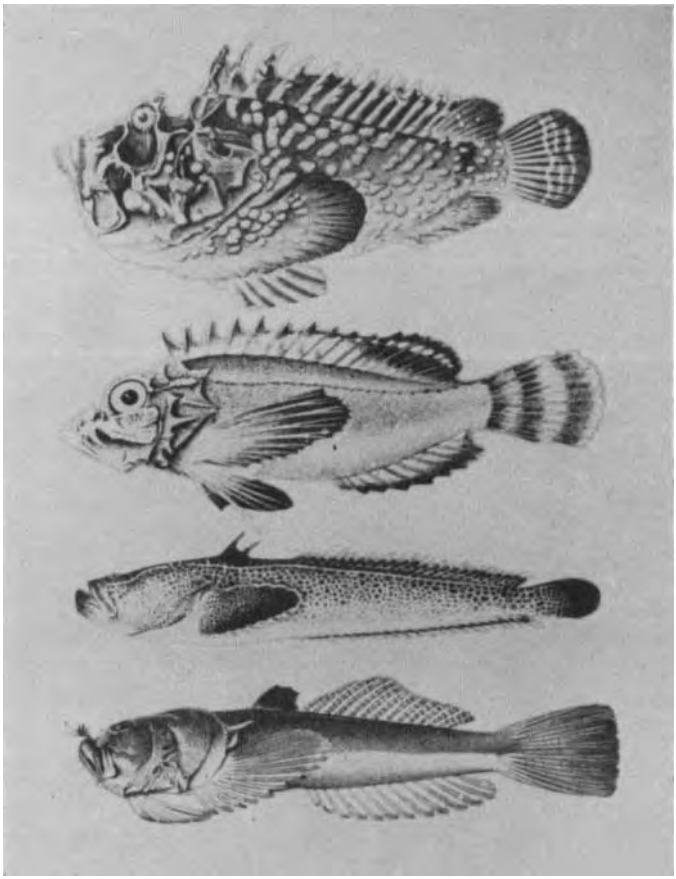


FIG. 13. Some venomous fishes. Top to bottom: *Synanceja horrida*, stonefish; *Minous monodactylus*, hime-okozo; *Thalassophryne reticulata*, toadfish; *Uranoscopus scaber*, star-gazer. (From Halstead, 1959; and Smithsonian Institute.)

found in the Pacific area. Some families, such as the stingrays, weevers and stargazers are chiefly benthonic, and may sometimes be found in quite deep waters. The great majority of venomous piscines are non-migratory and slow swimming. They tend to live in a protected habitat in or around rocks, corals, kelp beds, or they spend much of their time buried in the sand. Most species use their venom apparatus as a defensive weapon, chiefly against other fishes. Halstead and Mitchell (1963) state that less than 5% of the venom organs of these piscines have been described.

The venoms of these fishes differ markedly in their chemical and toxicological properties from the toxins of the poisonous fishes, and from the toxins of the other venomous animals. The most common characteristic of the toxins of the venomous fishes is their relative instability. Few of them are stable at room temperatures, and toxicity appears to be lost or markedly reduced even on lyophilization of freshly prepared crude extracts. In many cases the spines or structures containing the venom need to be stored at temperatures below —20°C if stability must be assured. While no basic structure for the toxin of any venomous fish has yet been established, or even proposed with any degree of fervor, this author has suggested that there is sufficient similarity in the zootoxicological properties of the venoms of the stingrays, weevers, scorpionfishes, and some of the others so far studied, to indicate that their venoms may be similar, or at least have some similar chemical components.

TABLE VII. SOME VENOMOUS FISHES

Species	*Distribution*
Acanthuridae, surgeonfishes	
Acanthurus bariene Lesson . .	Celebes, New Guinea, Philippine Islands
A. hepatus (Cuvier and Valenciennes) .	Mauritius, East Indies, Bismarck Archipelago, Gilbert Islands
A. lineatus (L.)	Zanzibar to Guam, Marshall and Marquesas Islands, Samoa
A. triostegus (L.)	East Africa, Indo-Pacific east to Revillagigedos, north to Japan and south to Australia
Ctenochaetus striatus (Quoy and Gaimard)	Red Sea to tropical Indo-Pacific
Xesurus scalprum (Cuvier and Valenciennes)	Japan and Ryukyu Islands

TABLE VII—*continued*

Species	*Distribution*
Batrachoididae, toadfishes	
Batrachus didactylus (Bloch)	Portugal
Batrachus grunniens (Bloch)	India to East Indies, Philippine Islands
Thalassophryne amazonica Steindachner	Brazil
T. dowi (Jordan and Gilbert)	Pacific coast of Central America
T. maculosa Günther	West Indies, tropical America
T. reticulata Günther	Pacific coast of Central America, West Indies, Brazil
Chimaeridae, ratfishes	
Chimaera affinis Capello	Atlantic coast of Canada
C. monstrosa L.	North Sea, Mediterranean Sea, Australia
Hydrolagus colliei (Lay and Bennett)	Pacific coast of North America
Dasyatidae, stingrays	
Dasyatis americana (Hildebrand and Schroeder)	Brazil, West Indies, Gulf of Mexico to Chesapeake Bay
D. akajei (Müller and Henle)	Yellow Sea, Korea, Japan
D. brevicaudatus (Hutton)	Indo-Pacific, Australia
D. brevis (Garman)	Baja California to Galapagoes Islands, Gulf of California
D. brucco (Bonaparte)	Yugoslavia
D. centroura (Mitchill)	North Carolina to New England
D. dipterurus (Jordan and Gilbert)	British Columbia to Central America
D. gerrardi (Gray)	India, Indonesia to Samoa
D. granulatus (Macleay)	Indonesia, Melanesia, Australia
D. guttatus (Bloch and Schneider)	Guiana
D. imbricatus (Bloch and Schneider)	Tropical Indian Ocean to western Pacific, China, Philippine Islands
D. kuhlii (Müller and Henle)	Indo-Pacific to Japan, New South Wales
D. latus (Garman)	Australia, Hawaiian Islands
D. pastinacus (L.)	Eastern Atlantic, Mediterranean to Natal, Indian Ocean
D. ponapensis (Günther)	Caroline Islands
D. sabinus (Lesueur)	Guiana to North Carolina
D. say (Lesueur)	Brazil, Guiana, West Indies, Gulf of Mexico to New Jersey
D. schmardae (Werner)	Guiana
D. sephen (Forskål)	Red Sea, Indo-Pacific, Micronesia, Melanesia, Philippine Islands, Australia

TABLE VII—*continued*

Species	*Distribution*
Dasyatidae, stingrays—*continued*	
D. uarnak (Forskål)	Tropical Indian Ocean, East Indies north to China, Australia
D. violaceus (Bonaparte)	Mediterranean
D. zugei (Müller and Henle)	Indian Ocean, East Indies, China, Japan
Gymnuridae, butterflyrays	
Gymnura altavela (L.)	Temperate latitudes both sides of Atlantic, Portugal, Mediterranean, Brazil to Massachusetts
G. japonica (Temminck and Schlegel)	China, Korea, Japan
G. marmorata (Cooper)	Southern California to Mazatlan, Mexico
G. poecilura (Shaw)	Red Sea, Indo-Pacific, Polynesia, China, Japan, Philippine Islands
Taeniura lymma (Forskål)	Red Sea to Fiji
Urogymnus africanus (Bloch and Schneider)	Red Sea, East Africa to Philippine Islands, Gilbert Islands to Queensland
Myliobatidae, bat stingrays	
Aetobatus narinari (Euphrasen)	Most tropical seas
Aetomylaeus nichofii (Bloch and Schneider)	Indian Ocean to Australia and western Pacific
Myliobatis aquila (L.)	Atlantic Ocean, Mediterranean, North Sea, Australia
M. bovina (Saint-Hilaire)	African warm waters to Delagoa Bay, Mediterranean
M. californicus (Gill)	Oregon to Baja California
M. cervus (Smith)	South Africa, Agulhas to Natal
M. peruvianus (Garman)	China to Peru
M. tobijei (Bleeker)	China Sea, Japan, Korea
Urolophidae, round stingrays	
Urolophus armatus (Müller and Henle)	New Ireland
U. aurantiacus (Müller and Henle)	Japan, Korea
U. halleri Cooper	Central California to Panama Bay
U. testaceus (Müller and Henle)	Australia
Urogymnus africanus (Bloch and Schneider)	Red Sea, East Africa to Philippine Islands, Gilbert Islands to Queensland

TABLE VII—*continued*

Species	*Distribution*
Heterodontidae	
Heterodontus francisci (Girard)	Central California to Baja California, Gulf of California
H. japonicus (Duméril)	Japan
H. philippi (Schneider)	Australia, Tasmania, New Zealand
H. zebra (Gray)	East India to east China Sea
Scorpaenidae	
Apistus carinatus (Bloch and Schneider)	Indian Ocean, Indonesia, Philippine Islands, China, Japan, Australia
A. cottoides Cuvier	Indonesia, China, New Zealand
A. evolans (Jordan and Starks)	Japan
Centropogon australis (Shaw)	East coast Australia, Queensland
Choridactylus multibarbis Richardson	India, Polynesia, China Sea
Dendrochirus brachypterus (Cuvier and Valenciennes)	East coast of Africa to Philippine Islands, Australia, Hawaiian and Samoan Islands
D. zebra (Quoy and Gaimard)	Indo-Pacific, Polynesia
Helicolenus dactylopterus (De la Roche)	Spain, Mediterranean
Inimucus barbatus (De Vis)	Queensland
I. didactylus (Pallas)	Indonesia, Melanesia, Philippine Islands
I. japonicus Cuvier and Valenciennes	Philippine Islands, East Indies, Japan
Minous inermis Alcock	India to Philippine Islands and Japan
M. monodactylus (Bloch and Schneider)	Indo-Pacific, China, Japan
Notesthes robusta (Günther)	East Indies, Australia
Pterois antennata (Bloch)	Tropical Indo-Pacific, Polynesia, China, Philippine Islands, Guam
P. lunulata Schlegel	Malaya, Philippine Islands, Japan
P. miles (Bennett)	Red Sea, Indian Ocean, Melanesia
P. radiata Cuvier	Red Sea, Indian Ocean, Melanesia, Polynesia
P. russellii Bennett	East coast Africa to India, Indonesia, Philippine Islands, Australia
P. volitans (L.)	Red Sea, east coast Africa, Indo-Pacific, Japan, Australia, Marshall, Society and Marquesas islands
Scorpaena guttata Girard	Central California to Gulf of California

TABLE VII—*continued*

Species	*Distribution*
Scorpaenidae—*continued*	
S. mystes Jordan and Starks	Pacific coast of Mexico, Central America
S. neglecta Temminck and Schlegel	Japan
S. porcus (L.)	Warm seas
Scorpaenodes guamensis (Quoy and Gaimard)	Red Sea to Philippine Islands, Australia, Guam, south Pacific
S. scabra (Ramsey and Ogilby)	Indo-Pacific
Scorpaenopsis cirrhosa (Thunberg)	Red Sea, Natal to Society Islands, Philippine Islands, Japan
S. gibbosa (Bleeker)	Indo-Pacific, Polynesia, Philippine Islands
Sebasticus marmoratus (Cuvier and Valenciennes)	Hong Kong to Japan
Synanceja erosa Langsdorf	Japan
S. horrida (L.)	India, Indonesia, China, Philippine Islands
S. trachynis Richardson	Australia
S. verrucosa Bloch and Schneider	Red Sea, Indian Ocean, Indo-Pacific, Micronesia, Philippine Islands, Australia
Trachinidae	
Trachinus araneus (Cuvier and Valenciennes)	Mediterranean, Portugal
T. draco (L.)	Mediterranean, Adriatic and Black Seas, Atlantic coast North Africa, North Sea to Norway
T. radiatus Cuvier and Valenciennes	Mediterranean
T. vipera Cuvier and Valenciennes	Mediterranean to North Sea
Uranoscopidae	
Uranoscopus bicinctus Schlegel	East Indies to Japan and China
U. duvali (Bottard)	Mediterranean, Indo-Pacific
U. japonicus (Houttuyn)	Malaya, Japan, China, Philippine Islands
U. scaber L.	South Africa to Indo-Pacific, Philippine Islands, Japan, Australia

Table VII shows some of the more common venomous fishes, or those fishes whose venom apparatus or venom has been studied to some extent. A number of other marine fishes are thought to be venomous but supportive morphological or toxicological data for most of these

species is wanting. Certain of the anglerfishes, butterflyfishes, jacks or pompanos, rabbitfishes, sea basses, snappers, spadefishes, squirrelfishes and surgeonfishes have been implicated in poisonings to man, but for one reason or another it would seem best at this time to defer treatment on their " venomousness".

A. *Stingray*

These elasmobranchs have long been known to be venomous (Plin y A.D. 23–79). They are often depicted as " demons of the sea ", " denizens of the deep " or " devilfishes ". Popular and scientific descriptions of their habits and feats, true and untrue, would fill this volume several times over. One interesting aboriginal story comes to us from Tasmania:

" Two women were bathing . . . the women were sulky, they were sad; their husbands were faithless, they had gone with two girls . . . they were swimming in the water, they were diving for crayfish. A stingray lay concealed in the hollow of a rock. The stingray was large, he had a very long spear . . . he pierced them with his spear . . . he killed them . . . he carried them away . . . he came close to shore . . . with him were the women, they were fast on his spear . . . they were dead.

Two black men fought the stingray; they slew him with their spears . . . the women were dead. The two men made a fire . . . On either side they laid a woman. The two black men sought some ants, some large blue ants. They placed them on the bosoms of the women. Severely, intensely were they bitten. The women revived . . . they lived once more."

An early stingray victim was Captain John Smith. Walter Russell, " gentleman, doctor of physicke ", who accompanied Captain Smith as he and a company of fourteen explored Chesapeake Bay in June, 1608, described the encounter (Mumford, 1903):

" . . . but our boate by reason of the ebbe, chansing to grownd upon a many shoules lying in the entrances, we spyed many fishes lurking in the reedes: our Captaine sporting himselfe by nayling them to the grownd with his sword, set us all a fishing in that manner; thus we tooke more in one houre then we could eate in a day.

But it chansed our Captaine taking a fish from his sword (not knowing her condition) being much of the fashion of a Thornback, but a long tayle like a ryding rodde, whereon the middest is a most poysoned sting, of two or three inches long, bearded like a saw on each side, which she strucke into the wrist of his arme neare an inch and a halfe: no blood nor wound was seene, but a little blew spot, but the torment was instantly so extreme, that in foure houres had so swolen

his hand, arme and shoulder, we all with much sorrow concluded his funerall, and prepared his grave in an Island by, as himselfe directed: yet it pleased God by a precious oyle Doctor Russell at the first applyed to it when he sounded it with probe, (ere night) his tormenting paine was so well asswaged that he eate of the fish to his supper, which gave no lesse joy and content to us than ease to himselfe. For which we called the island *Stingray* Isle after the name of the fish."

The suborder Myliobatoidea includes the families Dasyatidae, whiprays or stingrays; Urolophidae, round stingrays; Myliobatidae, bat- or eagle-rays; Gymnuridae, butterflyrays; and Potamotrygonidae,

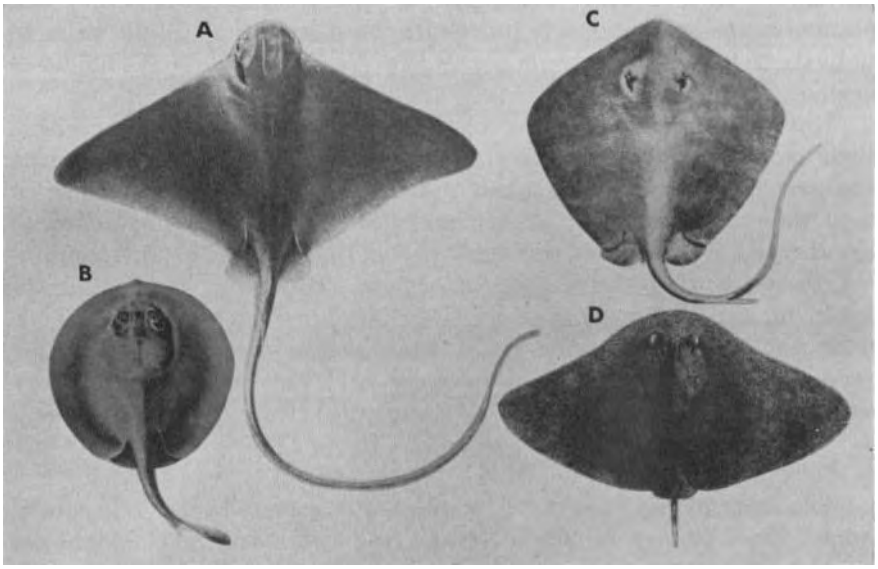


FIG. 14. Stingrays. A. *Myliobatis californicus*, bat stingray; B. *Urolophus halleri* round stingray; C. *Dasyatis diperurus*, diamond stingray; D. *Gymnura marmorata*, butterfly stingray. (From Walford, 1935; Hiyama, 1943; and Halstead, 1959.)

river rays. These elasmobranchs range in size from several inches in diameter to over 14 ft in length. Members of the families Dasyatidae and Urolophidae are for the most part shallow water fishes, and are usually found lying half buried in the ocean surf, or in the mud flats of a bay or slough. They are non-migratory, but most of the species move from shallow to deeper waters during the late summer or early fall months. They are rarely taken at depths greater than 15 fathoms. Of 1000 specimens of *Urolophus halleri* studied by us between 1951 and 1958, only fourteen were retaken at a distance greater than 15 miles from their original point of capture; 807 were recaptured within 3 miles of the point at which they were tagged; none were found at depths

over 15 fathoms, or more than 2 miles from shore. Myliobatidae are free swimming rays, and are less likely to be found on the bottom, although they do sometimes venture into very shallow waters to feed on bottom invertebrates (Gudger, 1937; Russell, 1953a; Russell, 1955).

The caudal spine, or sting, of the stingray is used by the animal as a purely defensive weapon, chiefly against those marine creatures which feed upon it. Stingrays do not " attack " other marine animals, nor man. The very structure of the venom apparatus including its innervation, the relationships of the muscles employed in the stinging act, the nature of the venom, and the rather obvious fact that this elasmobranch cannot reach its mouth with its sting, would seem to indicate that this structure is not used as part of the animal's offensive armament.

Not all stingrays possess significant amounts of venom within their caudal spines. In examining over 10,000 of these fish during the past 15 years we have found that approximately 30% had lost or torn their integumentary sheath and part of the tissues attached to the sheath. A number of these fish probably lost these venom containing tissues during the seining operation. However, some stingrays taken on set-lines had also lost their integumentary sheaths. Thus it is not difficult to understand why certain discrepancies on the venom, and on the symptomatology and treatment for stingray envenomation are found in the literature (Russell *et al.*, 1958a).

1. *Venom apparatus*

The *sting* of the stingray is a bilaterally serrated, dentinal caudal spine located on the dorsum of the animal's tail. Within this dentinal structure are numerous canals containing loose reticular connective tissues and small, thin-walled blood vessels. A thin layer of compact matrix is seen at the surface of the spine. There may be one or several of these spines. In adult *U. halleri* the sting is approximately 4 cm in length, while in larger species it may reach 30 cm in length. The sharp serrations are curved cephalically and as such are responsible for the lacerating effect as the sting is withdrawn from the victim's flesh. The spine is encased in an *integumentary sheath*. The venom is contained within the *ventrolateral grooves*. The relationships between these various structures are shown in Fig. 15.

In *U. halleri* the integumentary sheath is composed of a variable layer of loose areolar connective tissue covered by epithelium. The areolar connective tissue is rich in thin-walled blood vessels. Occasionally, it projects into the epidermis, forming delicate papillae which carry nutritive blood vessels.

Frequent, flattened, heavily pigmented cells are seen immediately adjacent to the basal layer of the epithelium. In places these cells form a distinct pigmented layer. The inner layer of basal cells is perpendicularly oriented (stratified columnar of Ocampo *et al.*, 1953). As the basal cells mature their nuclei become smaller and stain more

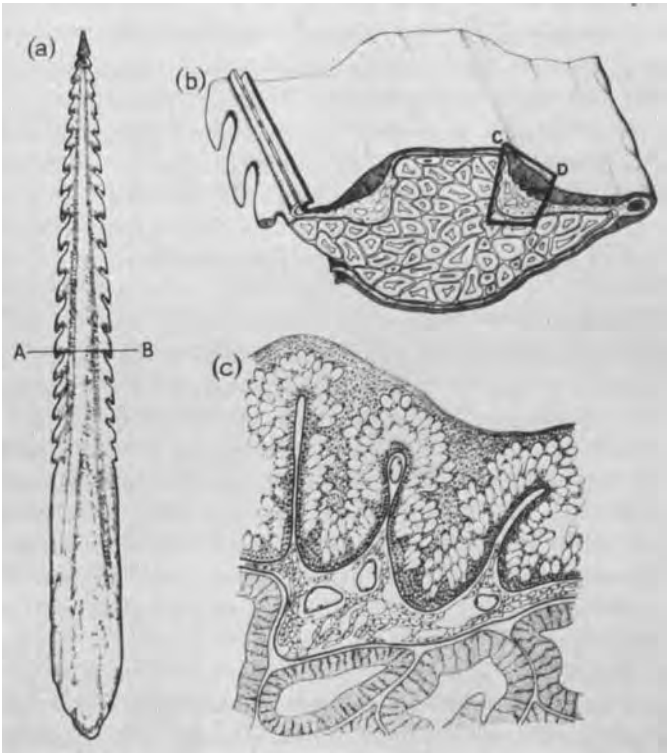


FIG. 15. Venom apparatus of the round stingray, *Urolophus halleri*. (a), sting; (b), cross section through A-B with integumentary sheath cut away to show serrations; (c), square CD showing dentinal sting, areolar connective tissue with blood vessels, and epithelium containing venom-producing cells. (From Russell, 1963.)

intensely. A fairly thick layer of oval, vacuolated (secretory) cells having a nucleus compressed at one end and eosinophilic cytoplasm, overlies the basal epithelium. These cells have been called glandular by Evans (1916) and Halstead and Bunker (1953). They are believed to contain the venom. Occasionally, narrow columns of modified squamous cells connect the basal layers of the epidermis with the outer layers. An outer layer of partially cornified cells with small

dark staining nuclei covers the vacuolated layer. On the surface is a thin layer of dense, cornified material in which no cell or nuclear detail remains (Russell and Lewis, 1956).

Fleury (1950) states that in *Myliobatis aquila* the venom is evacuated through one or two " excretory canals or their ramifications in the interdental space ". We have been unable to reach the same conclusions after studies on the venom apparatus of *Urolophus halleri* and *Myliobatis californicus*. The " ducts " described by Fleury are very similar to those we have noted as blood vessels. Many of our sections show blood cells in these structures. It is not particularly clear from Fleury's paper (which has been called to my attention by Dr. M. Castex of Argentina, to whom I am indebted), from whence these ducts arise and where they terminate. However, we have been preparing serial sections from the full length of the stings from several species of stingrays in order to evaluate Fleury's contention.

2. *Chemistry and toxicology*

The freshly prepared water extract of crude venom is clear, colorless, or faintly gray in color. It has a fishy taste and ammoniacal odor. Its pH is 6·76. The crude extract loses its toxicity within 4–18 h on standing at room temperatures. It is more stable at lower temperatures or in 20 to 40% glycerol. Most of the toxicity is lost on lyophilization. Total protein was found to be approximately 30%, total nitrogen 3% and total carbohydrate 3%. Ten amino acids were identified by paper chromatography. Several unidentified amino acids were also present. It was suggested that the crude toxin was a protein of average molecular weight (Russell *et al.*, 1958b).

Using disc electrophoresis we have recently identified 15 fractions in extracts from the venom-containing tissues of *Urolophus halleri*. Extracts prepared from sponges, which had been stabbed with fresh stings, contained 10 fractions. Further studies on these extracts, using gel filtration (Sephadex G 100 and G 200), suggest that the toxic protein(s) may have a molecular weight in excess of 100,000. The fraction showing the greatest lethal activity was found to have two or three distinct bands when subjected to disc electrophoresis. Further studies showed that the crude extracts contained serotonin, 5-nucleotidase and phosphodiesterase. There was no protease or phospholipase activity.

The venom is known to exert a deleterious effect on the mammalian cardiovascular system. Low concentrations of the venom give rise to simple peripheral vasodilatation or vasoconstriction. The most consistent change seen in the electrocardiographic pattern of cats when

small amounts of the venom are injected is bradycardia with an increase in the PR interval giving a first, second or third degree atrioventricular block. The second degree block is usually followed by sinus arrest. Reversal of the small dose effect occurs within 30 sec following the end of the injection. Cats receiving larger amounts of the venom show, in addition to the PR interval change, almost immediate ST, T wave change indicative of ischemia and, in some animals, true muscle injury. High concentrations cause marked vasoconstriction of the large arteries and veins as well as the arterioles. Much more serious is the direct effect on the heart muscle. The venom produces changes in heart rate and amplitude of systole, and may cause complete, often irreversible, cardiac standstill. It is apparent that the venom affects the normal pacemaker. The new rhythm evoked following cardiac standstill is often irregular and appears to be elaborated outside the sino-atrial node (Russell and van Harreveld, 1954; Russell *et al.*, 1957).

The venom depresses respiration. While part of this depression is secondary to the cardiovascular changes, the venom may have a direct effect on the respiratory centers of the medulla. The toxin produces many changes in the behaviour of animals. Some of these changes can be attributed to the direct effects of the venom on the central nervous system. In mammals the venom occasionally produces convulsive seizures. The mechanism of these seizures is not clear. They may be due in part to cardiovascular failure. Seizure patterns were not seen in electroencephalograms from anesthetized animals (Russell *et al.*, 1958a).

The venom does not appear to have a deleterious effect on neuromuscular transmission (Russell and Long, 1960; Russell and Bohr, 1962). When injected into the lateral ventricles of mammals it produced some slight apathy, astasia and licking motions, all of which were transient (Russell and Bohr, 1962). The LD_{99} has been calculated as 28·0 mg dried crude venom per kg mice (Russell *et al.*, 1958b).

Mice injected with a lethal dose of *U. halleri* venom develop hyperkinesis, prostration, marked dyspnea, blanching of the ears and retina, and exophthalmos. These are followed by complete atonia, gasping respiratory movements, coma and death. In cats the same syndrome is seen. Ataxia, dilated pupils, increased salivation, micturation, defecation, marked atonia, cyanosis and hypoactive or absent deep and superficial reflexes are also found. In monkeys a similar pattern is observed. In one monkey we observed a tonic-clonic generalized motor seizure accompanied by increased salivation, twitching of the head and marked dilation of the pupils (Russell *et al.*, 1958a).

3. *Clinical problem*

Injuries inflicted by stingrays are common in many areas of the world. Approximately 750 people a year are stung by these animals along the North American coasts (Russell, 1959). I have seen many similar envenomations in various parts of the Pacific, Indo-Pacific, Mediterranean and south Atlantic.

Of 1097 stingray injuries reported over a 5-year period in the United States, 232 were seen by a physician at some time during the course of the recovery of the victim. Sixty-two patients were hospitalized; the majority of these required surgical closure of their

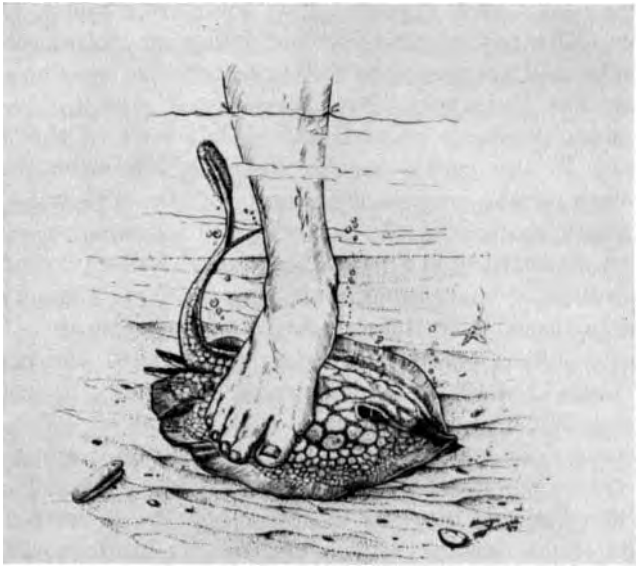


FIG. 16. Stinging action of the round stingray. (Russell and Lewis, 1956.)

wounds or treatment for secondary infection, or both. At least ten of the sixty-two victims were hospitalized for treatment because of overexuberant first aid care (the use of potassium permanganate, ammonia, formaldehyde or ice water). Only eight patients were hospitalized for the treatment of the systemic effects produced by the venom. There were two fatalities (Russell, 1953a; Russell *et al.*, 1958a; Russell, 1959).

Considerable care should be exercised when wading in shallow waters known to be inhabited by stingrays. Stingray injuries usually occur when the unwary victim treads upon the fish while wading in the ocean surf or mud flats of a bay, slough, or river. The fish often buries

itself in the sandy or muddy bottom and may remain motionless until stepped upon. The pressure of the foot on the dorsum of the fish provokes him to thrust his tail upward and forward, driving his sting into the foot or leg of the victim (Fig. 16). As the sting enters the flesh, the integumentary sheath surrounding the spine is ruptured and the venom escapes into the victim's tissues. In the withdrawal of the spine, the integumentary sheath may be torn free and remain in the wound.

Unlike the injuries inflicted by many venomous animals, wounds produced by the stingray may be large and severely lacerated, requiring extensive debridement and surgical closure. A sting no wider than 5 mm may produce a wound 3·5 cm long and larger stings may produce wounds 7 inches long. The sting itself is rarely broken off in the wound.

The stinging is followed by the immediate onset of intense pain, out of proportion to that which might be produced by a similar non-venomous injury. While the onset of pain is usually limited to the area of injury, it rapidly spreads, and usually becomes more severe during the first 30 min following the accident. In most cases the pain reaches its greatest intensity in less than 90 min and often persists (if untreated), though gradually diminishing in severity, for 6–48 h.

For the most part, the symptoms and signs of the poisoning are localized to the injured area. However, syncope, weakness, nausea and anxiety are common complaints and may be attributed, in part, to peripheral vasodilatation (Russell and van Harreveld, 1954, 1956), and in part to the reflex phenomenon precipitated by the severe pain. Vomiting, diarrhea, sweating, fasciculations in the muscles of the affected extremity, generalized cramps, inguinal or axillary pain, and respiratory distress are less frequently reported. Arrhythmias, paresthesia, and convulsions may occur. True paralysis is extremely rare, if it occurs at all. All of the " paralyses " seen by the author following severe stingings were muscle contractures, probably initiated as flexion reflexes stimulated by the intense pain. These contractures were relieved with drugs which alleviated pain, and which have no effect on true paralysis. Deaths are very rare following stingray injuries (Russell *et al.*, 1958a).

Examination reveals either a puncture or a lacerating wound, usually the latter, jagged, bleeding freely, and often contaminated with parts of the stingray's integumentary sheath. The edges of the wound may be discolored, though the discoloration is not usually marked immediately following the injury. However, within 2 h the discoloration may extend several cm from the wound. Subsequent necrosis of this area occasionally occurs in untreated cases.

A word might be added regarding the treatment of these injuries since they occur more frequently than injuries by any of the other venomous fish. A treatment to be successful must be instituted early and vigorously, and should be initiated by the victim. It must be directed towards alleviating the pain, preventing complications that may be evoked by the venom, and preventing secondary infections (Russell, 1953a).

The standard procedure for treatment of stingray injuries is well established. Injuries to an extremity should be irrigated with the salt water at hand, since much of the venom can be washed from the wound by this step. An attempt should be made to remove the integumentary sheath if it can be seen in the wound. If a properly qualified person is available, he may apply a constriction band directly above the wound site. The extremity should then be submerged in hot water at as high a temperature as the patient can tolerate without injury for 30–90 min. The addition of sodium chloride or magnesium sulfate to the hot water is optional. The wound should then be further examined for evidence of the integumentary sheath, debrided, sutured if necessary, and the appropriate antitetanus agents administered. While infections of these wounds are rare in properly treated cases, some physicians routinely give antibiotics. Elevation of the injured extremity is advised. Further medical advice can be obtained elsewhere (Russell *et al.*, 1958a).

We have studied 1725 cases of stingray injury during the past 15 years, and have found that in almost every case where the above therapeutic measures have been used there has been a degree of success ranging from complete alleviation of symptoms to " some improvement " of symptoms. We have also had the opportunity to clinically evaluate the use of potassium permanganate, ammonia and cryotherapy. We have found these measures to be of no value in the treatment of this entity.

B. *Weeverfish*

The weevers, members of the piscine family Trachinidae, are small marine fishes which are confined to the eastern Atlantic and Mediterranean coasts. The name " weever " is probably derived from a corruption of the Anglo-Saxon " wivre", meaning viper. The lesser weever, *Trachinus vipera* (Fig. 17), reaches a total length of approximately 11 cm. These fish are found in large numbers in the shallow waters of certain off-shore sandy grounds along the southeast English coast, in the continental southern North Sea, and along the coasts of the English Channel and Mediterranean Sea. They are often taken by inshore

vessels engaged in shrimping. However, as they are of little commercial value no figures are maintained on the yearly catch, or on the numbers landed at various ports.

The greater weever, *Trachinus draco* (Fig. 17), reaches a total length of approximately 45 cm, although Maretić (1957) reports that

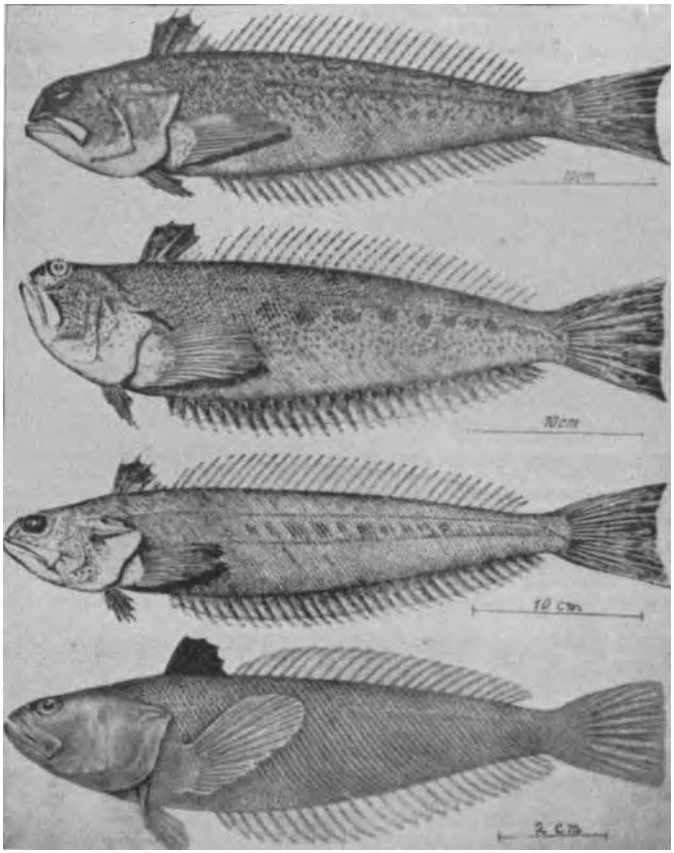


FIG. 17. Top to bottom: *Trachinus araneus*, *Trachinus radiatus*, *Trachinus draco*, *Trachinus vipera*. (From Smithsonian Institute, and Halstead, 1959.)

these fish may reach 50 cm in length. They are found in deeper waters than the lesser weever, and are usually taken by deep water trawlers and coastal cutters. When the catch is large enough to warrant marketing these fish command a good price. They are considered a delicacy by many fishermen I have met in North Sea ports. The practice of removing the venomous dorsal and opercular spines before

marketing does not appear to be well established in England, France or Holland at the present time (Russell and Emery, 1960).

1. *Venom apparatus*

The venom apparatus of these fishes consist of two *opercular spines*, five to eight *dorsal spines*, and the tissues contained within the *integumentary sheaths* surrounding the spines. The two dentinal opercular spines extend caudally and very slightly downward from near the superior margin of each operculum. Each is firmly attached to

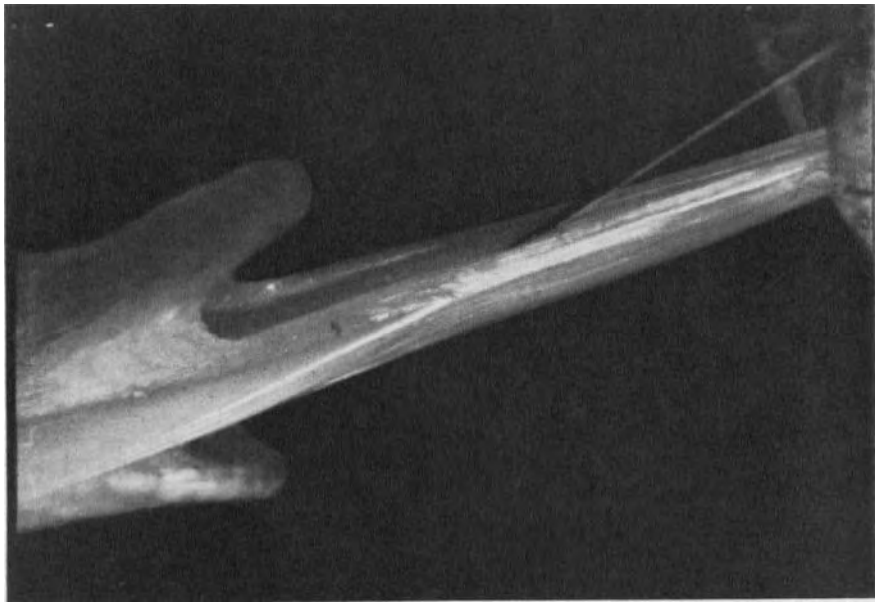


FIG. 18. Opercular spine of *T. draco*, showing pin inserted into superior groove. Note the conic cavity at the base of the groove. (Russell and Emery, 1960.)

the operculum for the proximal one third of its length, and lies free and superficial to the posterior portion for the distal two thirds.

Figure 18 shows a pin inserted into the mid-portion of the deep groove along the superior margin of the left opercular spine of *T. draco*. A similar groove exists along the inferior margin. Within the superior and inferior grooves, and in the two conic cavities into which they enter at the base of the spine, lies most of the spongy glandular tissue that produces the venom. The spine is covered by an integumentary sheath which, when ruptured, allows the toxin to escape from the venom-laden cells (Russell and Emery, 1960).

The five to eight dorsal spines are enclosed within individual integumentary sheaths connected by their interspinous membranes. The first spine curves slightly caudally from its articulated base to the tip. It has a prominent anteromedian ridge flanked by grooves. The other dorsal spines vary slightly from the first, and contain grooves of various depths. The venom is contained within the various grooves. The spines have been described elsewhere (Russell and Emery, 1960), and the microscopic anatomy of the tissues associated with the opercular and dorsal spines has been discussed by Byerley (1849), Schmidt (1874), Gressin (1884), Parker (1888), Borley (1907), Halstead and Modglin (1958), and Skeie (1962c).

2. *Chemistry and toxicology*

The first important study on the toxicological properties of weever-fish venom was that of Schmidt (1874). While some of his work was carried out with preserved stings, several experiments were conducted with fresh materials. In one experiment he stabbed seven frogs with fresh spines from *T. draco*; three died within several hours, one developed swelling at the site of the injury, became weaker, developed paralysis in both hind limbs but subsequently became asymptomatic. The remaining animals showed little more than lethargy and increased salivation.

Gressin's excellent thesis (1884) contains many interesting observations on the weeverfishes and their venom. He obtained a rather pure venom by merely pressing on the base of the opercular spine, working his fingers up the spine and drawing off the " drop of liquid " from the tip with a syringe. The product was clear and light blue in color, contained several different types of cells, was coagulated by strong acids, bases and by heat. In frogs the venom produced inactivity, increased respiration, prostration, convulsions and sometimes death. It also depressed cardiac rate. In rats it produced a similar sequence of events. Autopsy revealed ecchymosis and necrosis at the site of inoculation, and congestion of the heart, kidneys, liver and brain. He also noted that potassium permanganate had no effect on the action of the venom.

Pohl (1893) found that the toxin was inactivated by 25% and 95% alcohol, and by ether and chloroform. In frogs he observed paralysis, prostration, decreased sensory perception and cardiovascular changes, particularly slowing of the heart rate and defective filling of the ventricle. Phisalix (1899) found that glycerine extracts of tissues from the opercular and dorsal spines produced paralysis, local swelling and necrosis, and death when injected into guinea pigs.

Briot (1902) demonstrated *in vitro* hemolytic activity for the venom. He found it had no effect on the blood coagulation time. Glycerine extracts were found to be lethal to rabbits. Briot attributed the lethal effect of the toxin to respiratory paralysis, and suggested that the action was peripheral rather than central. He also demonstrated the necrotic effect of the toxin. He succeeded in immunizing rabbits against the lethal effect of the venom and found that the sera of immunized rabbits would protect other rabbits against the venom. In subsequent studies (Briot, 1903, 1904) he prepared glycerine extracts of the opercular and dorsal spines and found the extracts of the former to be much more dangerous. He also studied the kinase activity of the toxin. Briot's various studies are well conceived and give us many valuable data.

Using venom extracted by syringe from the opercular spines of freshly caught *T. draco*, Evans (1907) demonstrated a fall in blood pressure concomitant with an increase in heart contractions and respiratory changes, when the toxin was injected into rabbits and cats. His studies on the hemolytic effects of the venom yielded results quite different from those obtained by Briot. Evans (1943) reviewed his various findings in his monograph.

De Marco (1936) observed an increase in central nervous system permeability to potassium following injection of the venom into frogs. In subsequent experiments (1938) he demonstrated a more rapid exhaustion of the frog's gastrocnemius in the presence of the toxin, but no significantly deleterious effect that might be attributed to the direct action of the venom on the muscle. Maretić (1957) found that guinea pigs stabbed with the venom apparatus became restless and noisy, and subsequently developed a paresis of the hind legs. Other than one animal which developed tachycardia, he did not observe any significant systemic effects as a result of the poisoning. Local swelling

TABLE VIII. ANALYSIS OF EXTRACTS OF INTEGUMENTARY TISSUES FROM OPERCULAR AND DORSAL SPINES OF *Trachinus vipera*. (Russell and Emery, 1960)

Elemental Analysis	*Per cent by weight*	*Proximate Analysis*	*Per cent*
Carbon	22·8	Moisture	41·5
Hydrogen	3·5	Protein	21·7
Nitrogen	6·9	Lipids (ether soluble)	3·5
Phosphorous	2·2	Inorganic matter	12·9
Sulfur	0·0	Carbohydrate (calculated)	20·4

occurred at the site of the injury. He suggested that the venom contained hyaluronidase.

Russell and Emery (1960) analyzed extracts from the venom-containing tissues of *T. vipera* and *T. draco*. The freshly prepared extracts had a fishy taste and ammoniacal odor; pH was 6·78. The results of the determinations are shown in Table VIII. Assays of the dialyzed and non-dialyzable solutions indicated that the lethal portion was non-dialyzable.

The extracts had no deleterious effect on mammalian neuromuscular transmission. When large amounts of the extract were added to the nerve-muscle bath, there was some shortening of the muscle and a very gradual depression of both the directly and indirectly elicited contractions, but at no time was there any evidence of a differential that could be attributed to changes at the neuromuscular junction.

In cats the venom produced a precipitous fall in systemic arterial pressure concomitant with changes in the pulse pressure, cardiac rate, pulmonary arterial pressure, venous and cisternal pressures, respiration, and the electrocardiogram and electroencephalogram (Fig. 19). The findings are similar to those produced by stingray venom and certain of the venoms of other fishes.

The electrocardiograms demonstrate that the venom can produce both changes in rhythm and injury to the heart muscle. The fall in pulmonary artery pressure indicates either a failure of the heart to maintain an effective stroke volume, or a decrease in pulmonary resistance. The latter seems unlikely; studies of pulmonary artery blood flow using a gated sine-wave electromagnetic flowmeter indicate that the blood flow in this vessel is reduced during the period of decreased pressure. The findings of lowered pulmonary arterial pressure and flow, a decrease in heart rate with various degrees of auriculo-ventricular block, and evidence of heart muscle injury may be interpreted to mean that some degree of cardiac failure is probably responsible for the fall in systemic arterial pressure and the rise in venous and cisternal pressures. Smaller amounts of weeverfish venom produced transient vasoconstriction or vasodilatation, depending on the quantity injected. With these doses there is little or no deleterious effect upon the heart, although there may be some changes in cardiac rate and in respiratory rate.

The depression in central nervous system activity seen following the intravenous injection of a lethal dose of the toxin (Fig. 19), can be attributed to ischemic anemia produced by the lowered systemic arterial pressure. The wave pattern is typical of that which occurs during cardiovascular failure from any of a number of causes. However,

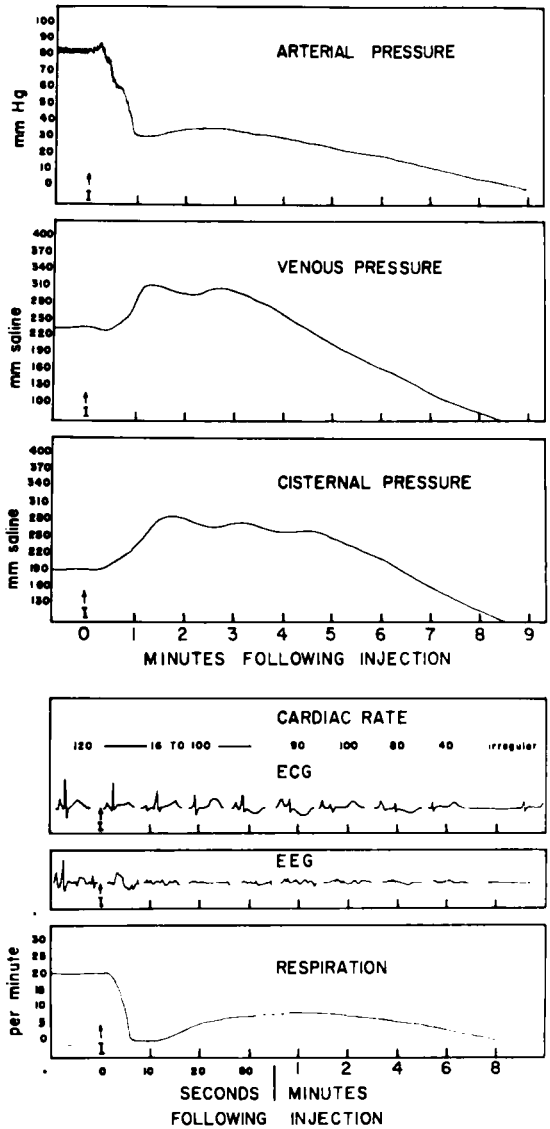


FIG. 19. Effects of a lethal dose of an extract from the tissues of the dorsal spines of *T. draco.*

this finding by itself does not exclude the possiblity that the venom might have a direct effect on central nervous system activity.

The rate and depth of respirations following injection of weever toxin are subject to considerable variation. In small doses the venom

produces a slight increase in rate. With slightly larger doses there may be either an increase or decrease in rate, and with lethal doses there is usually a decrease in rate leading to complete cessation of respirations. It is always difficult to interpret such respiratory changes in the presence of profound alterations in the parameters of the cardiovascular system. It would not be feasible in this review to discuss the possible relationships between the changes in the two systems. We have attempted to do this in our several publications. Here, it is sufficient to observe that the present evidence indicates that a part of the respiratory crisis can be attributed to the changes in the cardiovascular system. It should be noted that neither continuous artificial respiration or direct stimulation of the diaphragm significantly alters the cardiovascular crisis, nor do these measures save the life of the animal (Russell and Emery, 1960).

Carlisle (1962) found that the dialyzable fraction of the venom produced the stabbing pain characteristic of the whole venom, when he injected it into himself. The non-dialyzable fraction failed to provoke the pain but did produce a rise in pulse rate and some respiratory distress. He concluded that the systemic effects of the toxin were due to the non-dialyzable fraction, while the pain was a consequence of some constituent of the dialyzable part of the venom. He also discovered that the dialyzable fraction contained a large amount of 5-hydroxytryptamine, and that it was associated with a substance of low molecular weight which acted as a histamine releaser. The non-dialyzable fraction contained a neutral amino polysaccharide lacking in sulfur, and two separate albumins. Carlisle suggested that these three substances may not exist separately, but may " represent a complex muco-substance of combined polysaccharide-protein nature."

Skeie has recently published several papers on the weeverfishes and their venoms. Although he describes all other studies as " rather haphazard " (1962a) and criticizes certain investigations and methods, as well as the compendium of knowledge, his own studies generally confirm the findings of previous investigations. He describes (1962b) a technique for extracting the venom from opercular spines. The method is similar to that which has been employed for extracting venom from certain other fishes and should be of particular value where large numbers of weevers are available. As Skeie notes, even with this method " a rather large amount of solid sediment consisting of cell remnants and a few unavoidable scales " are obtained. Using this technique he extracted the toxin from 600 weevers into 60 ml of solution. He found the DML_{100} (LD_{100}) in two mice to be 0·0004 ml. On the basis of this formula he calculated that each weever contained

sufficient venom to kill 250, 17 g mice. In studies upon mice with nineteen batches of weever toxin he found the average venom content to vary from 6–1066 LD_{100} per weever, or from 640–2560 LD_{100} per ml venom. He also found that it is possible to quantitate the venom on tissue cultures, and by studies on skin reactions in guinea pigs.

Haavaldsen and Fonnum (1963) separated three protein fractions by electrophoresis from the venom of *T. draco*. On a paper chromatogram they obtained two spots, one of which was identified as histamine and the other as a catecholamine. Photofluorimetric studies revealed the presence of adrenaline and noradrenaline in high concentrations. The toxin also showed considerable cholinesterase activity; it did not contain 5-hydroxytryptamine, lecithinase or phosphodiesterase.

3. *Clinical problem*

Injuries inflicted by weeverfishes are not uncommon (see references, Russell and Emery, 1960). Stings by the lesser weever usually occur when the unfortunate bather treads upon the dorsal spines of one of these fish while wading on sandy grounds, or when a fisherman mishandles the fish while dislodging it from a net. Occasionally these fish become entangled in seaweed during trawling operations and are taken aboard unknowingly. The lesser weever often buries itself in the sandy or muddy ocean bottom and may remain there motionless with only its erect dorsal spines projecting above its camouflage. When the fish is stepped upon, the integumentary sheath surrounding the venom-containing tissues of the spine is torn and the venom escapes into the victim's tissues. In an interesting case history reported by Halstead (1957), a lesser weever was said to have aggressively attacked a skin diver, driving its dorsal fins into the victim's right jaw. Most stingings by these fish, however, are inflicted on the hands or forearms of the shrimp fishermen of the North Sea.

The greater weever may on occasion bury itself in the sand. However, as it does not customarily invade very shallow waters it is not usually implicated in stingings to bathers. Most injuries inflicted by *T. draco* are incurred by fishermen removing it from their nets or set lines. According to Evans (1943) the greater weever may employ its venomous spines as offensive weapons, " attacking any object approaching it with the precision of a fighting cock."

A weeverfish may inflict either a single or multiple puncture-type wound. Persons stung by these fishes report having received a sharp, immediately painful stab. The pain is described as intense or excruciating; it increases in severity during the first 20 to 50 min following the injury and may persist for 16 to 24 h if treatment is not undertaken.

The pain is out of proportion to that which might be produced by a similar wound from a non-venomous fish. During the initial 5 to 10 min following the stinging the pain is usually confined to the area about the stab, but subsequently it spreads to involve the entire affected extremity. Axillary pain is common in patients stung on the hand by weeverfishes. The pain is more severe than that observed following stingings by stingrays. As noted by Halstead (1957) the pain can be so severe that a victim stung by one of these fishes while in the water may experience difficulty in reaching shore. I have received similar reports from bathers along the Devon and Cornwall coasts of England. It seems likely that it is the excruciating pain rather than true muscular paralysis that is responsible for the victim's motor incapacity.

The amount of bleeding from the puncture wound appears to be about what one might expect from a similar non-venomous injury. The degree of swelling about the wound varies, although some swelling appears to be a constant finding. The tissues adjacent to the wound often appear discolored; the surrounding area may be somewhat blanched. Localized necrosis at the wound site is not uncommon, and sloughing of these tissues may occur. In 1958 and again in 1962 I examined the hands of a number of retired fishermen at Lowestoft and Great Yarmouth in England, who had been stung on the hands on numerous occasions by weevers during their younger years. Many of these fishermen exhibited arthritic changes in both hands, and while such changes are not uncommon in fishermen who handle nets, these cases seemed more severe than I have seen in fishermen elsewhere. It is possible that repeated stings, and the effects of the venom and low grade infection, might well have been a contributing factor in these cases of arthritis.

In severe cases of envenomation by weevers there may be weakness, dizziness, nausea, primary shock and respiratory distress. Fishermen at Ijmuiden, Holland, told me that there was often an urgency to urinate, and that in severe stings there was considerable axillary and chest pain as well as changes in pulse rate and respiration. The case histories of the three deaths I have been able to secure indicate that in each case the fatality could be attributed to a secondary infection.

A few thoughts on the treatment of weeverfish sting seem indicated, and perhaps this is an appropriate place to present an additional reflection or so on the therapeutics of the injuries produced by venomous fishes in general. After having seen and treated a good many such injuries during the past several decades I have been impressed with the differences between the advice found in medical texts and that suggested and used by fishermen or life guards or persons familiar

with envenomations by fishes. I am distressed to note that in most cases the non-professional advice has not only proved to be more effective, but often more rational. Much of the advice given in texts devoted to tropical medicine, where the problem of venomous animal injury is most often discussed, stems from the false and antiquated idea that all venoms are related chemically, and thus all respond to similar therapeutic measures. From the early studies on snake venoms a number of remedies found their way into the therapeutics for venomous marine animal injuries. Among these were alcohol, formaldehyde, urine, potassium permanganate, ammonia, gold salts and cassava bread. While all these measures have been found to be ineffective, some are still advised in an occasional medical text. The more recent fads for antihistamines, corticosteroids and ice water as " shotgun " therapeutic methods are slowly waning fortunately for the patient. One cannot help from reaching the conclusion, after studying the many hundreds of experiments that have been done with various drugs for the treatment of venomous animal injuries, that the test tube is a rather naïve human being.

It is refreshing to find that a thorough review of the literature for the past several centuries often reveals a highly effective method of treatment, based on trial and error. When I suggested (Russell and Emery, 1960) that the use of hot water in weeverfish stings might be effective I did so on the basis of its very effective use in stingray and scorpionfish injuries, and on the basis of a limited number of case histories and observations on weeverfish stings that I studied in England, France and Holland during 1958. Subsequently, I reviewed the quite extensive earlier literature on this problem and found a great number of statements concerning the effectiveness of heat, in one form or another, in the treatment of weeverfish poisonings.

Gressin (1884), for one, notes that " one could, following the custom of the fishermen of the Channel, put the stricken member in a bath of hot milk, which, according to their saying, calms its admirably." Evans (1921) notes " that the fishermen have acted on the line of heat. The most frequent remedy is to plunge the part into boiling vinegar; another plan is to hold the affected limb over the funnel of the boiler which, in a smack, drives the donkey engine." Patrick Russell (1758) states that fishermen in the German Sea often apply a very hot poultice to the wound, which is a " most efficacious cure ". Similar reports on the use of heat for this type of injury have been noted in the papers of more recent workers.

In controlled experiments in humans we have recently found that the methods suggested for the treatment of stingray injuries are

equally effective in alleviating the severe pain and other symptoms provoked by the venom of the weeverfishes. The reader is referred to page 346 of this contribution for a more thorough review on this treatment. Maretić (1957) has used intravenous calcium gluconate with good success for relieving the pain of the injury. Local injections of procaine may be of some value in less severe poisonings, and intramuscular or intravenous meperidine is of definite value in those cases in which there is severe pain after the first hour following the injury.

C. *Scorpionfish*

At least 80 members of the family Scorpaenidae, the scorpionfishes or rockfishes, have been implicated in poisonings to man, or have been studied by venomologists. Included in this group are the zebrafishes, sculpins, stonefishes, bullrout and waspfish (Fig. 12, 13). They are widely distributed throughout all tropical and most temperate seas. A few scorpionfishes are found in Arctic waters. Many species are strikingly colored, and several are of unusual shape. They are most often found in or around rocks, coral reefs or kelp beds, with which they often blend very favorably. Some species bury themselves in the sand, and almost all species have the habit of lying motionless and partially concealed for long periods of time. Thus, they are often handled or trod upon unknowingly, that is, until envenomation occurs.

Halstead (1959) has divided the scorpionfishes into three types on the basis of the morphology of their venom organs. While this classification has certain shortcomings, it does provide a simple method for reviewing this family of venomous fish.

Structure	*Pterois*	*Scorpaena*	*Synanceja*
Fin spines	Elongated and slender	Shorter and heavier than in *Pterois*	Short and stout
Integumentary sheath	Thin	Moderately thick	Very thick
Venom glands	Small and well-developed	Larger and more highly developed than in *Pterois*	Large and highly developed
Venom ducts	Not evident	Not evident	Well-developed

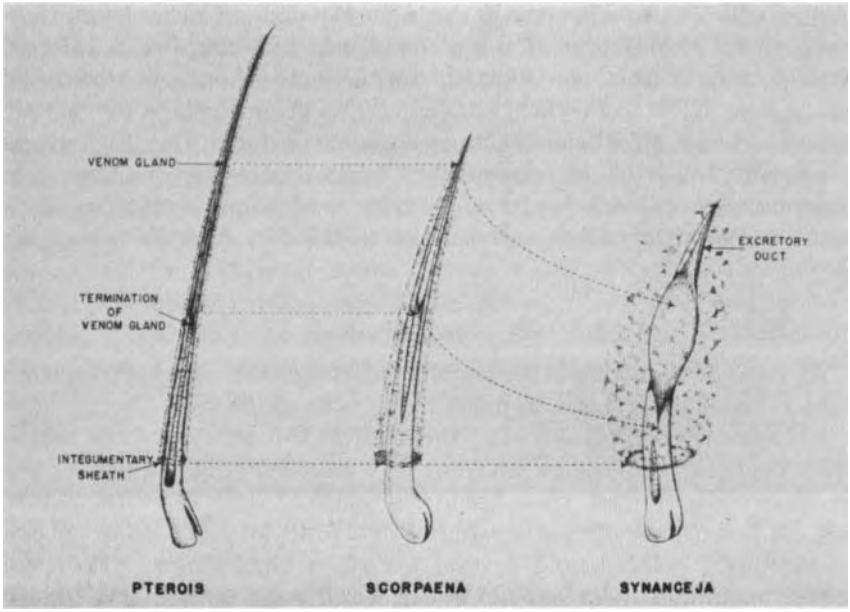


FIG. 20. Dorsal stings from three types of scorpionfish. (From Halstead, 1959.)

1. *Zebrafishes* (*Pterois*)

The zebrafishes, lionfishes, turkeyfishes or butterfly cods (genera *Pterois* and *Dendrochirus*) are spectacularly colored scorpionfish, usually found in shallow waters around coral heads, in underwater caves and about underwater debris. Because of their brilliant colors and long delicate fins they are easily seen and rarely contacted accidentally (Fig. 12). Little is known about the habits of these fishes, or the manner in which they use their venom apparatus. However, some interesting observations have been made by Steinitz (1959) that indicate the manner in which the fish may make use of its venom apparatus in defense.

(*a*) *Venom apparatus.* The venom apparatus has been described in considerable detail by Bottard (1889), Pawlowsky (1927), Tange (1953) and Halstead *et al.* (1955a). It consists of 13 *dorsal spines*, 3 *anal spines*, 2 *pelvic spines*, the enveloping *integumentary sheaths*, and the *glandular complex* lying within the *anterolateral grooves* of the spines.

The dorsal spines are long, slender and almost straight, except at the base and the tip where they incline slightly caudally (Fig. 20). The anterolateral grooves originate just above the base of the spine

and extend the entire length of the sting. They appear as two deep channels separated by the anteromedian ridge. The spine is invested in a thin layer of gaily colored, fibrous connective tissue, the integumentary sheath. Within the anterolateral grooves are fusiform shaped " *venom glands* " which may occupy up to three-quarters of the total length of the spine. The glandular structures within the grooves are composed of several types of tissues, including the venom-producing cells, large polygonal shaped cells with pinkish-gray, finely granular cytoplasm. According to Halstead *et al.* (1955a) these cells may measure 270 $\mu \times 75\ \mu$ or more, and are collected together in connective tissue compartments within the anterolateral grooves; they are covered in turn by the integumentary sheath. There does not appear to be a glandular duct through which the venom is secreted or discharged. Envenomation probably occurs through mechanical pressure on the spine.

(*b*) *Chemistry and toxicology.* Saunders and Taylor (1959) found that water extracts of the integumentary sheaths and underlying glandular tissues from the dorsal spines of *P. volitans* were somewhat turbid, reddish-orange in color, and had a pH of approximately 7. The toxin was unstable at room temperatures; lyophilized and glycerol-treated extracts were found to retain 40 to 90% of their original lethal activity following 1 year's storage at $-20°C$. The toxin was non-dialyzable, and in mice had an intravenous LD_{50} of 1·1 mg protein/kg body weight.

In rabbits, small doses produced a slight decrease in arterial blood pressure without particular effect on the electrocardiogram, and an increase in respiratory rate. Larger doses caused a more profound fall in blood pressure, an increase in respiratory rate, and evidence of myocardial ischemia or injury, or conduction defects. Lethal doses produced a precipitous fall in arterial blood pressure and extensive electrocardiographic changes. Respirations were markedly depressed and finally ceased; the blood pressure continued to fall. On opening the chest following cessation of respirations the auricles, and occasionally the ventricles, were usually found to be beating; some irregular electrical activity persisted for several more minutes. In several of the animals, initiation of artificial respiration within one half minute following respiratory arrest was ineffective in prolonging life.

Intravenous injection of the venom into mice produced ataxia, circling movements, and partial or complete paralysis of the legs. This syndrome was followed by a period of inactivity, during which

there was evidence of muscular weakness. According to the authors, the apparent skeletal muscle weakness was more pronounced with the venom of this fish than that observed following injection of the venom of the stonefish. Cessation of respirations preceded death. It was found that approximately 2500 LD_{50} for mice were present in each extract prepared from the combined tissues of the dorsal spines of one fish.

(*c*) *Clinical problem.* Poisonings by zebrafishes have been reported by Bottard (1889), Halstead *et al.* (1955a), Ray and Coates (1958), Steinitz (1959), Saunders and Lifton (1960), and others. Envenomation gives rise to immediate intense, sometimes burning pain, which radiates from the injured area more rapidly than in the case of weeverfish or stingray poisoning. The pain often becomes unbearable within a few minutes following the injury, and may cause the victim to thrash about in considerable agony. The area about the wound may be blanched. The victim may complain of numbness or parasthesia about the injury or even over the entire injured part. Weakness, dizziness and shock may quickly ensue. In cases of shock there is often bradycardia, hypothermia and respiratory distress. Cyanosis has been reported. Edema develops rapidly and may be quite severe within an hour. The wound site is often discolored and markedly tender. Necrosis and sloughing of the tissues about the wound may occur. The pain often persists for 8 to 12 h, and the injured part may be sore and swollen for several weeks.

On 16 May, 1961 I received a telephone call from a tropical fish dealer in Los Angeles who had just been stung by a 17 cm *P. volitans.* At the time of the phone call, which was approximately 3 min following the injury, the patient complained of intense burning pain at the site of the injury (distal third of the right index finger), pain through the palm of the hand and into the forearm, and a feeling of nausea, anxiety and weakness. I advised the patient to put the entire right hand in a bucket of hot water and to be driven to the nearest receiving hospital. The patient was seen 20 min later, at which time he stated that while the pain was less severe than it had been 10 min previously, it was still intense, and that he had a dull aching sensation through the forearm and up into his right axilla. He complained of some weakness but no nausea. His color was dusky and his skin cool and moist. The injured finger was swollen, red and tender to deep pressure, and superficial sensory stimuli over the area of injury were not felt. The patient's blood pressure was 100/60, pulse 64, respirations 12 and somewhat shortened during inspiration. White blood cell count, urinalysis and the electrocardiogram were normal.

During the next hour the patient received an intravenous injection of 250 ml of 5% glucose in saline, containing epinephrine, 10 ml of calcium gluconate and 5 ml of methocarbamol. Three hours following the injury the patient was asymptomatic, except for the parasthesia over the right index finger. The finger was still markedly swollen and the edema extended into the palm of the hand. The hand was immobilized. There were no subsequent complaints or findings of significance. The edema persisted for several days, and the distal portion of the injured finger was tender for several weeks. An area of a few millimetres in diameter around the two stab wounds became necrotic and sloughed out.

Stings by *P. volitans* and related species provoke more severe pain, and appear to be considerably more dangerous, than stings by the stingrays, sculpins or weeverfishes. The severity of the signs, as noted in the various cases presented in the literature, indicates that this fish is capable of producing a fatal poisoning in man. If the initial pain does not respond to hot water, meperidine hydrochloride may need to be given. Cardiovascular tone can be maintained with intravenous fluids and epinephrine; it is advisable to give oxygen.

2. *Scorpaena* (*Scorpaena*)

This group has a far greater distribution and range than any of the other venomous fishes, with the possible exception of the stingrays. Its various members are known in different parts of the world as scorpionfishes, sculpins, rockfishes, sea pigs, bullrouts, waspfishes, bullheads or blobs. According to Halstead and Mitchell (1963), members of the genera *Apistus*, *Centropogon*, *Gymnapistes*, *Hypodytes*, *Notesthes*, *Scorpaena*, *Scorpaenodes*, *Scorpaenopsis*, *Sebastapistes*, *Sebastodes*, *Sebastolobus*, and *Snyderina* all belong to this group (Fig. 12, 13). These fishes vary considerably in their size, shape and color, as well as in the kind of habitat in which they are found and the kind of life upon which they feed. In spite of their differences they have very similar venom apparatuses.

(*a*) *Venom apparatus*. The venom apparatus of several of the European scorpionfishes has been described by Bottard (1889), Tuma (1927), and by Pawlowsky (1927). Halstead *et al.* (1955b) has described the venom apparatus of the California scorpionfish *Scorpaena guttata*. This fish may be taken as a representative for the group. It has twelve *dorsal spines*, three *anal spines*, two *pelvic spines*, and their enveloping *integumentary sheaths*. The spines are shorter and heavier than in *Pterois* (Fig. 20). When the moderately thick integumentary sheath is

removed from a dorsal spine, a slender, elongated, fusiform strand of greyish tissue is found lying within the distal one-half or two-thirds of the *glandular grooves* on either side of the sting. The venom is contained within these tissues. The *venom glands* are similar to those found in *Pterois*, although more highly developed. It is not known how functional the anal and pelvic spines are.

(*b*) *Chemistry and toxicology.* According to Saunders (1960) the venom of *Scorpaena guttata* has similar pharmacological actions and certain similar chemical properties to those of *Pterois* and *Synanceja*. We have injected extracts from the tissues of the dorsal spines of *Scorpaena guttata* and have found that they produce a fall in systemic arterial pressure, an initial increase in systemic venous pressure followed by a decrease, changes in respiration rate and depth, and changes in the electrocardiogram and electroencephalogram. The various changes are quite similar to those provoked by the venoms of the stingray and weeverfish. From the standpoint of the physio-pharmacological effects, it would appear that the venoms of *Urolophus*, *Trachinus*, *Scorpaena*, *Pterois* and *Synanceja* have much in common.

(*c*) *Clinical problem.* Stingings by *Scorpaena* are not uncommon (Bottard, 1889; Pawlowsky, 1927; Hiyama, 1950; Halstead, 1951; Maretić, 1957, and Whitley, 1963). Elsewhere I have reported that approximately 300 persons a year in the United States are stung by *S. guttata* or related species (Russell, 1961). During the period 1953–60 a total of 247 injuries from this species was reported to our Laboratory by the Sportsfishing Association of Southern California, the California Department of Fish and Game, and by various bait stand operators on the public piers from Malibu south to Oceanside, California. From these 247 poisonings we have collected 100 case histories. The following description of " sculpin poisoning " is based on these cases.

Approximately 80% of the stings were inflicted upon fishermen while they were attempting to dislodge the fish from their hook, or while removing the fish from their fishing bag. Most of the remaining stings occurred in " bait boys ", whose task it is to clean fish aboard sportsfishing boats, or in housewives who had not been adequately forewarned of the danger of being stabbed by the dorsal spines of this fish. Envenomation on a finger is followed almost immediately by intense, sometimes pulsating pain in the area of the injury. The pain radiates so that within 3 to 10 min it may involve the entire finger or hand. The area around the wound may appear ischemic; bleeding does not seem to be affected. The finger becomes red and

swollen. The pain may extend up the forearm and into the axilla within 15 min of the sting. Nausea, vomiting, weakness, palor, syncopy, an urgency to urinate, conjunctivitis, increased perspiration, headache and diarrhea have all been reported. Parasthesia about the injured part and even up the forearm are not uncommon. Swelling and tenderness of the axillary nodes occurred in at least 30% of the untreated cases. The severe pain usually subsides in 3 to 8 h; the swelling and tenderness may persist for several days. In most cases there are no subsequent deleterious effects.

In severe stingings (and four of the victims in this series were hospitalized for treatment of the systemic effects of the venom), the pain may be excruciating, causing the victim to thrash about in agony. Primary shock may occur, and in two cases the victims were taken to the hospital under oxygen administration. Respirations may become labored and painful. Pulmonary edema has been reported and " abnormal electrocardiograms " demonstrated. In one case known to the author the patient had a pulmonary embolism and was hospitalized for 24 days.

As a " bait boy " on the Billings Barge off Ocean Park, California during the 1930's, I received numerous stings from this fish. I recall that some of them were so painful that they caused me to vomit and on several occasions precipitate episodes of migraine. My fingers were often swollen for several days following an envenomation. I do not recall that I ever became immune to the pain produced by the venom, even though I suspect I must have been stung at least twenty times over a 4-year period. On some days, two of us cleaned as many as seventy-five of these fish during a single afternoon, and at least one person was stung every day or so while handling, or mishandling, *Scorpaena guttata*. The pain following the stinging of this fish is more severe than that experienced following a stinging by *Urolophus halleri* or *Trachinus vipera*. I do not think it is as painful as that produced by *Pterois*, although on this point I cannot speak from experience. The treatment for *Scorpaena guttata* envenomations has long been hot water and the protocol as suggested under stingray injuries.

3. *Stonefishes* (*Synanceja*)

While most of our knowledge about this group comes to us through studies on *Synanceja horrida*, Halstead and Mitchell (1963) suggest that members of the genera *Choridactylus*, *Erosa*, *Inimicus*, *Leptosynanceja* and *Minous* should also be included in the group, in view of the similarities in their venom apparatuses. These fishes are perhaps the most dangerous of the venomous piscines; certainly, the stonefish

or dornorn, devilfish, goblinfish, sea toad, lumpfish, lupo or stingfish, *Synanceja horrida* (Fig. 13), is the most venomous piscine known.

There are a number of species of *Synanceja*, and they vary in color from brown or green to scarlet with gray markings. The adult of most species is 10 to 15 inches in length, although according to Whitley (1963) they may reach 2 ft in length and may weigh over 3 lb (Halstead *et al.*, 1956). They inherit their name from the fact that they so remarkably resemble stone or coral. They have the habit of lying motionless in coral or rock, or partially buried in the sand or mud, and so still can they lie that molluscs and other benthonic marine animals may often crawl over them (Whitley and Boardman, 1929). They are sluggish fish, and if handled out of the water and then returned they usually settle on the substratum rather than swim away. According to Endean (1963), if approached while they are swimming they will frequently turn themselves so that their dorsal spines point toward the intruder. They sometimes feign death by floating upside down and motionless. The venom apparatus of this fish appears to be a purely defensive weapon, as it is in *Pterois* and *Scorpaena*.

(*a*) *Venom apparatus*. The venom apparatus of *Synanceja* has been described by a number of workers. The reader is referred to the papers by Duhig and Jones (1928a), Gail and Rageau (1956), Halstead *et al.* (1956), and Endean (1963) for a more thorough review on this subject. The venom apparatus of *Synanceja horrida* consists of 13 *dorsal spines*, 3 *anal spines*, 2 *pelvic spines*, the thick warty *integumentary sheaths* enveloping these spines, the 2 *glandular grooves* in each spine, and the *venom glands* and their *ducts* within these grooves. The dorsal spines are short, stout and straight. The two anterolateral-glandular grooves extend almost the entire length of the spine. The glands appear as two large fusiform masses at the middle and toward the tip of the spine (Fig. 20). The distal end of each gland terminates in a duct-like structure lying within the grooves. These ducts extend from the glands to the tip of the spine. The microscopic anatomy of the venom gland of *Synanceja* is quite different from that of *Pterois* and *Scorpaena*, and the reader is again referred to the papers by Halstead *et al.*, and Endean for a further consideration of these structures. Only the dorsal spines appear to be functional as a venom apparatus in the stonefish.

Some years ago Dr. H. Flecker of Australia sent us the dorsal spines of a stonefish, and we attempted to determine the LD_{50} of saline extracts from the venom glands. We were unable to obtain a lethal product from the last four dorsal spines. It is now clear (Wiener, 1959) that some spines may not possess venom glands, and as Wiener notes,

this finding may be responsible for Dr. Flecker's belief that stonefish stings are not always dangerous.

(*b*) *Chemistry and toxicology.* Bottard (1889) described the fresh venom of *Synanceja* as clear and bluish in color, but noted that it becomes opalescent and cloudy after the fish dies. It could be coagulated by nitric acid, alcohol, ammonium and heat. It was weakly acid. On microscopic examination he found the venom to appear like an albuminous liquid, containing large refractile round cells with a single, small centrally-placed nucleus. Bottard also conducted a number of pharmacological studies on the venom. He aspirated the venom from the dorsal fin glands and injected it into frogs, dogs and himself. In frogs the toxin produced paralysis in the hind legs, and death within several hours. In a dog the venom caused vocalization, incontinence, tremors, and evidence of anorexia and thirst. Necrosis developed at the injection site. On injecting a drop of venom into his own leg Bottard experienced intense pain and localized parasthesia. Necrosis developed at the site of injection, and 10 years later he still had a scar at the site.

Duhig and Jones (1928a,b), and Duhig (1929) found that subcutaneous injections of *S. horrida* venom into guinea pigs produced irritability, tremors, respiratory distress, convulsions, paralysis and loss of corneal reflexes. These investigators also demonstrated that the venom had a strong hemolytic effect *in vitro*, although Wiener's work indicates that this activity is probably not very significant, and was not observed *in vivo* (Wiener, 1963).

Gail and Rageau (1956) injected the contents of one dorsal spine gland from *S. verrucosa* into a frog, which died in extensor paralysis at about 4 h. A rat envenomated with the dorsal spines of the same species, defecated, exhibited respiratory difficulties, became hypothermic, developed spasmatic contractions over the abdomen and marked weakness in the hind legs. Death occurred at approximately 7 h; at autopsy blood was found in the thorax. A second rat experienced similar signs when the contents of two venom glands were injected subcutaneously. A hematoma developed at the injection site. The animal died at approximately 16 h. A dog receiving the contents from five glands developed agonal signs within 45 sec: vocalization, trismus, convulsions, relaxation of the sphincters, respiratory distress and loss of consciousness. Death occurred within 1 min of the injection, and was attributed to cardiac collapse and cessation of respirations.

An excellent series of studies on the venom of *S. horrida* has recently been conducted by Saunders and his colleagues (Saunders, 1959a,b, 1960; Saunders and Tökés, 1961; Saunders *et al.*, 1962).

These workers found the venom to be a clear, colorless fluid with a pH of 6·8, and having a nitrogen content of 2% and a protein content (biuret) of approximately 13%. The intravenous LD_{50} in mice was approximately 200 μg of protein/kg body weight. On the basis of this determination they suggested that extracts of the spines from one fish would contain 10,000 to 25,000 LD_{50} for mice. The lethal fraction was non-dialyzable. Lyophilized or glycerol-treated extracts were found to have retained 50 to 100% of their original lethal activity following storage for 1 year at —20°C. Intravenous injection of small amounts of the venom into rabbits produced a slight fall in arterial blood pressure accompanied by an increase in respiratory rate, with no electrocardiographic changes. These effects were transient. Larger doses caused more marked changes in arterial pressure and respiratory rate, and alterations in the electrocardiogram. With lethal doses (10 μg protein/kg body weight), these changes were markedly accentuated; first degree atrioventricular block and ventricular fibrillation were sometimes seen. In a typical experiment the rabbit died in 90 sec.

In subsequent experiments, Saunders and his colleagues suggested that extracts prepared from the dorsal spines of a single fish contained approximately 3000 LD_{50} for mice. In rabbits, the mean intravenous lethal dose/kg was approximately one-tenth the LD_{50} for mice. This amount produced ataxia, paralysis of the limbs and neck, convulsions, respiratory arrest and death. While respiratory arrest was a terminal event, artificial respiration was ineffective in prolonging the life of the animal. On starch gel electrophoresis these investigators found that the venom contained 7 to 10 bands. Material lethal to mice could be recovered from only one of these bands. This fraction had an approximate LD_{50}, in mice, of 15 μg of nitrogen/kg body weight. Re-electrophoresis of this lethal fraction gave a single band.

Wiener (1959a) found that the average yield of venom from a functional dorsal spine of *S. trachynis* was 0·03 ml, or 5·1 to 9·8 mg of the dried venom. When the toxin was injected intravenously into mice the LD_{50} was found to be 0·005 to 0·01 mg/15 g mouse; the subcutaneous LD_{50} was 0·04 to 0·06 mg, and the intraperitoneal LD_{50} was 0·02 to 0·03 mg. Lethal amounts of the venom caused muscular incoordination, paralysis of the hind limbs, irregular respirations, coma and convulsions. These signs were followed by prostration, cessation of respiratory movements and cardiac arrest. On opening the thorax, Wiener found the lungs to be hemorrhagic, " gelatinous in consistency and filled with frothy fluid." In guinea pigs the venom caused necrosis at the site of injection, muscular weakness, respiratory depression, coma and death. Examination of the thorax revealed that the lungs

were emphysematous, and showed the presence of blood stained fluid. The intravenous injection of 3·5 mg of venom into an 8 kg dog produced cardiac and respiratory arrest in less than 2 min. With smaller amounts of the toxin there was a rise in the blood pressure followed by a fall. During this fall, pulse pressure tripled and respirations became deep and irregular.

Wiener (1959a) also found that lyophilized venom stored in a desiccator for several months did not lose its lethal activity. The venom gave all the reactions of a protein; it was destroyed on heating and inactive at a pH below 4, and at a high pH. Toxicity was lost in 48 h when the venom was stored in solution at 4°C.

In an interesting report, Austin *et al.* (1961) described the cardiovascular changes elicited in rabbits following injection of the venom. These findings were similar to those reported by Saunders and by Wiener. Like other investigators, they found that the death of the animal could not be prevented by the initiation of artificial respiration. Their most important finding was that records of the action potential from the phrenic nerve indicated that following injection of the venom, respiratory movements were affected before efferent respiratory center activity, and also, before conduction in the phrenic nerve was impaired. This would indicate that stonefish venom either blocked neuromuscular conduction across the phrenic nerve-diaphragm junction or paralyzed the diaphragmatic musculature. They concluded that the toxin was highly myotoxic, and suggested that the venom may depolarize cardiac and involuntary muscle as well as skeletal muscle.

(*c*) *Clinical problem.* Stingings by the stonefishes, particularly *S. horrida* and *S. trachynis*, appear to be more common than generally appreciated (Le Juge, 1871; Bottard, 1889; Coutière, 1899; Duhig and Jones, 1928a; Whitley and Boardman, 1929; Ralph, 1943; Smith, 1951; Halstead *et al.*, 1956; Gail and Rageau, 1956; Smith, 1957; Wiener, 1958; Wiener, 1959b; Phleps, 1960; Jüptner, 1960; Whitley, 1963). These works indicate that the sting of *Synanceja* is usually more serious than that inflicted by any other of the known venomous fishes.

The clinical course following poisoning by a stonefish is similar, although considerably more severe, than that previously described for the stingrays, weevers, sculpins and lionfishes. Deaths from stingings by these fishes are not uncommon, and may occur within several hours of envenomation (Smith, 1957). Necrosis of tissues at the site of the injury, and the subsequent sloughing of these tissues, is more common following stings by *Synanceja* than following injuries by the other venomous fishes. The clinical evidence to date indicates that lethality, pain and

necrosis are correlated in certain of the venomous piscines, and that they follow in this order: *Synanceja* > *Pterois* > *Scorpaena* > *Trachinus* > *Urolophus*. This contention is supported by some experimental findings, although it does not necessarily follow that these three activities can be associated with one component of the venom.

Treatment of wounds inflicted by *Synanceja* must be instituted immediately following envenomation. The use of hot water as described for stingray wounds on page 346 should be tried. Injection of emetine hydrochloride directly into the wound(s) is of value, if it can be accomplished within 30 min of the stinging. Wiener (1959b) has prepared an antivenin against *S. trachynis* venom. It appears to have a high degree of potency and has been found to be very effective. It is recommended for all cases of *S. trachynis* poisoning in which systemic symptoms develop, providing the patient is not sensitive to horse serum. Its value in poisonings by species other than *trachynis* has not been determined. I have used one ampule of Dr. Wiener's antivenin in a case of *Scorpaena guttata* poisoning, and I would like to think that my patient fared better because of it.

D. *Summary, physiopharmacology*

It appears from the several physiopharmacological studies on the venoms of *Urolophus*, *Trachinus*, *Pterois*, *Scorpaena* and *Synanceja* that these toxins produce certain identical reaction patterns, which differ for the most part only in their degree of reactivity. The cardiovascular reaction-patterns that our group has demonstrated for *Urolophus* venom have now been demonstrated for the four other genera noted. Some of the physical and several of the chemical properties identified in *Urolophus* venom have also been found in these other venoms. Most of the differences in the toxicological data presented by the various workers appears to be more directly related to technical differences (differences in the techniques employed, the preparation of the venom, the choice of the experimental animal, the route of administration of the toxin, the pH of the extract, etc.) than to actual differences in the venoms themselves. The similarity of the clinical syndromes and the effectiveness of certain therapeutic measures also indicates a relationship between these toxins.

At the Tenth Pacific Science Congress in 1961, I pointed out that the evidence to date suggested that a close relationship existed between the chemical and toxicological properties of many of the venomous fishes. This contention appears even more supportable today, and one might expect that within the next few years the marine biochemist may develop the basic structural formula(s) for these toxins. It is

sufficient to say at this time that awaiting such a development the physiopharmacologist has a good deal of work yet to keep him occupied.

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