

## THE RELATIONSHIP BETWEEN ANTI-PREDATOR BEHAVIOUR AND AGGRESSION AMONG CONSPECIFICS IN THE THREE-SPINED STICKLEBACK, *GASTEROSTEUS ACULEATUS*

By FELICITY ANN HUNTINGFORD\*

Department of Experimental Psychology, Oxford

**Abstract.** The response of individual breeding three-spined sticklebacks to territorial intruders of various species was compared with their response to a predator outside the breeding season. The nature of the territorial response to the different intruders, as revealed by factor analysis, was very similar and the level of aggression shown to the different species co-varied across individuals and during each breeding cycle. The level of territorial aggression of the individual fish was positively correlated with their 'boldness' towards a predator. These results provide tentative support for the idea that anti-predator behaviour and aggression towards conspecifics are linked in this species. Supporting evidence for and possible adaptive significance of these results are discussed.

The question of whether inter- and intra-specific aggression share common motivating factors (used here to refer to any changes within an animal which influence its response to constant stimuli) remains a source of controversy and there is little satisfactory evidence on this point for any species. The experiments described below were designed to provide such evidence in the case of the three-spined stickleback, *Gasterosteus aculeatus*. The reason for this choice of species was that comparisons between the ten-spined stickleback (*Pygosteus pungitius*) and the three-spined stickleback have shown a concordance between the degree of 'boldness' towards a predator (Benzie 1965) and of territorial aggression towards conspecifics (Wilz 1971). It seemed possible that a similar situation might prevail within a single species of stickleback.

The rationale of the experiments is simple and takes advantage of the marked differences in behaviour which exist naturally between individual sticklebacks. The same fish were observed in situations designed to elicit a range of anti-predator and social behaviour. These were, during the breeding season, in the presence of territorial intruders of a variety of species (reproductive tests) and, outside the breeding season, in the presence of a hunting pike (pike tests).

The levels of aggression and related behaviour shown by each fish in these situations were compared. Positive correlations between them would support the idea that something inside the fish

is affecting the levels of anti-predator boldness and social aggression in the same way; in other words, that they have motivating or inhibiting factors in common.

The sticklebacks used in these experiments were caught in various rivers and ponds in the Oxford area. They were housed in groups for most of the year in tanks of 100 × 35 × 40 cm and fed on *Daphnia* and *Tubifex*.

### Reproductive Tests: Aggression Within the Breeding Season

The breeding behaviour of *Gasterosteus* is well documented. The fish migrate in the spring to shallow water (Baggerman 1958) where the males establish territories (Van den Assem 1967), build nests and court females (Tinbergen 1951, 1953a) and care for the developing young (Van Iersel 1953). Several nests may be built and several broods reared in this way in the course of a single breeding season.

The experiments described below were designed to answer three questions about the behaviour of breeding sticklebacks, the first two being preliminary to the third:

(i) Do the individual fish differ in the level of aggression they show under standard conditions?

(ii) If so, are these differences consistent across the whole breeding cycle? The overall level of aggressive behaviour has been shown to vary at different stages in the breeding cycle (Symons 1965; Wootton 1971; Black 1971) but this might not affect all individuals in an equivalent way.

\*Present address: Department of Zoology, University of Glasgow.

Table I. Mean Levels of Eleven Scores over One Breeding Cycle

Score	Condition	Intruder				
		P5	G5	P30	G30	N
Lunges: no. per 5 min	PE	89	121	67	91	35
	E	159	149	122	122	72
	Y	193	195	147	161	121
	PE2	51	104	42	87	23
Bites: no. per 5 min	PE	42	51	23	27	12
	E	105	107	73	62	21
	Y	146	150	123	109	66
	PE2	21	47	22	41	12
Zig-zags: no. per 5 min	PE	10	8	10	18	11
	E	2	2	3	3	3
	Y	0	0	0	0	1
	PE2	9	9	11	10	10
Spines: no. per 5 min	PE	14	14	14	14	11
	E	11	12	7	11	10
	Y	3	6	5	7	8
	PE2	15	13	13	12	10
Spines: duration (s)	PE	254	121	198	221	256
	E	178	171	211	184	269
	Y	157	203	358	237	227
	PE2	212	155	204	232	231
Nest activity duration (s)	PE	6.0	5.5	8.7	4.6	13.0
	E	14.3	10.8	15.5	13.7	26.9
	Y	8.1	9.1	34.1	24.2	19.8
	PE2	5.2	4.1	9.3	5.2	8.0
Nest activity: no. per 5 min	PE	2	2	2	1	2
	E	2	2	3	2	3
	Y	2	3	5	3	4
	PE2	1	2	3	2	2
Proportion facing < 10	PE	0.956	0.965	0.988	0.792	0.864
	E	0.987	1.000	0.985	0.919	0.971
	Y	1.000	1.000	0.996	0.996	0.999
	PE2	0.968	0.970	0.979	0.820	0.813
Proportion facing > 20	PE	0.015	0.019	0.007	0.005	0.014
	E	0.000	0.000	0.001	0.002	0.002
	Y	0.000	0.000	0.000	0.000	0.000
	PE2	0.009	0.021	0.008	0.006	0.010
Bout length facing < 10	PE	12	123	16	16	12
	E	19	137	10	91	24
	Y	16	36	10	47	10
	PE2	13	82	13	20	10
Bout length facing > 20	PE	2	1	1	2	1
	E	0	0	0	4	0
	Y	0	0	0	0	0
	PE2	0	0	1	3	2
Proportion facing > 20	PE	0.015	0.019	0.007	0.005	0.014
	E	0.000	0.000	0.001	0.002	0.002
	Y	0.000	0.000	0.000	0.000	0.000
	PE2	0.009	0.021	0.008	0.006	0.010

Table I. Continued

Bout length facing	PE	12	123	16	16	12
< 10	E	19	137	10	91	24
	Y	16	36	10	47	10
	PE2	13	82	13	20	10
Bout length facing	PE	2	1	1	2	1
> 20	E	0	0	0	4	0
	Y	0	0	0	0	0
	PE2	0	0	1	3	2

$N = 27$  for pre-egg (PE) tests;  $N = 23$  for egg tests (E);  $N = 13$  for young tests (Y);  $N = 7$  for second pre-egg tests (PE2). The behaviour patterns are defined as follows: lunge: a rapid dart from a facing position at 5 cm or less towards the intruder with the mouth closed; bite: contact made with glass with the mouth open and then closed; zig-zag: one towards and away movement of the courtship dance; spine raising: any detectable movement of the spines from the lowered position; nest activity: activity of any kind centred on the nest; facing: still, with the head pointed straight towards the intruder, from less than 10 cm, from 10 to 20 cm and from more than 20 cm.

(iii) It is known that sticklebacks will defend their territories against a range of intruders. How similar are the responses of territorial male sticklebacks to intruders of a variety of species, and are individual differences reflected consistently in the reaction to all intruders?

The analysis of the results will use both direct, simple methods and multi-variate analysis.

### Methods

The fish were kept at a temperature of about 20°C in tanks illuminated for 16 hr per day, conditions under which they readily come into breeding condition (Baggerman 1958). As soon as the male fish began to develop their reproductive colours, they were placed alone in an aquarium of 40 × 35 × 35 cm. Most of the fish built nests and were then said to be in the pre-egg phase (PE phase). The day on which the nest was completed is referred to as PE0 and subsequent days as PE1, PE2, etc.

On PE2 and PE3 the fish were subjected to a battery of five tests, in random sequence and at intervals of at least 4 hr. When these tests had been completed, a gravid female was introduced into the tank. Males which courted successfully and fertilized the eggs were said to have entered the egg phase (E phase); the successive days were labelled in the same way, with the day of fertilization E0. The same series of five tests were performed on E1 and E2, with the same interval between tests. The day on which the first eggs hatched (usually about E6) marked the change to the young phase (Y phase) and was labelled Y0. On Y0 and Y1 the five tests were performed again. Some of the fish built a second nest after their babies had been removed. They were

then said to be in the second pre-egg stage and the five tests were carried out for the last time on these fish. Numbers of sticklebacks tested fell off during the course of the experiment as not all obtained eggs, reared young or built a new nest. Twenty-seven fish were tested on the pre-egg phase, twenty-three in the egg phase, thirteen in the young phase and seven in the second pre-egg phase.

All five tests involved the introduction of an intruder, confined in the bulb of a long-necked glass flask, into the territory of the individual male being tested, whose behaviour during the next 5 min was recorded. Resident fish were allowed a period of 5 min to become accustomed to the flask alone before a test was started.

The intruders in the five tests were: a *Gasterosteus* male in breeding colours, 5 cm from the nest (G5); a *Pygosteus* male in breeding colours, 5 cm from the nest (P5); a *Gasterosteus* male in breeding colours, 30 cm from the nest (G30); a *Pygosteus* male in breeding colours, 30 cm from the nest (P30); a female smooth newt, *Triturus vulgaris*, in breeding condition, 5 cm from the nest (N).

This choice of intruders permitted a comparison of the aggressive response of nesting sticklebacks to conspecifics, to a member of a closely related species and to a fresh-water animal which might well invade its territory in the wild. In this way a range of inter- and intra-specific responses was investigated. As conspecifics are rivals in the early stages of the breeding cycle (Van den Assem 1967), a particularly strong response to these intruders might be expected, in the pre-egg phase at least.

A tape-recorded commentary of the behaviour of the resident fish towards each intruder was

**Table II. Spearman Rank Correlations Between the Scores of Individual Sticklebacks Tested with *Gasterosteus* at 5 cm at Various Stages of the Breeding Cycle ( $N = 13$ )**

	Lunges	Bites	Spines (No.)	Spines (Duration)	Nest activity (No.)	Nest activity (Duration)
Pre-egg tests v. egg tests	0.58*	0.59*	0.23	0.23	0.29	0.79**
Pre-egg tests v. young tests	0.62*	0.66**	0.41	0.31	0.11	0.08

\* $P < 0.05$ , \*\* $P < 0.01$ .

**Table III. Correlations Between the Scores of the Individual Sticklebacks in Some Different Pre-egg Tests ( $N = 27$ )**

	Lunges	Bites	Zig-zags	Spines (No.)	Spines	Nest activity (No.)	Nest activity (Duration)
G5 v. G30	0.70**	0.73**	0.37*	0.50**	0.47**	0.95**	0.53**
P5 v. P30	0.56**	0.66**	0.34*	0.56**	0.50**	0.07	0.15
P5 v. G5	0.66**	0.74**	0.36*	0.36*	0.01	0.73**	0.19
P30 v. G30	0.71**	0.74**	0.38*	0.71**	0.13	0.05	0.21
G5 v. N	0.62**	0.48**	0.06	0.36*	0.03	0.46**	0.08

\* $P < 0.05$ , \*\* $P < 0.01$ .

Key to test: G5 *Gasterosteus* at 5 cm; P5 *Pygosteus* at 5 cm; G30 *Gasterosteus* at 30 cm; P30 *Pygosteus* at 30 cm; N newt at 5 cm.

made and later transcribed for analysis. The following aspects of their behaviour were particularly noted: attendance at the nest, the position of the spines and finally whether they attacked, courted or merely observed the intruder. The head-down threat posture described by Tinbergen (1951) occurred very rarely in these tests and was therefore not recorded.

### Results

While it is quite possible that individual variation between animals is reflected in the sequences in which particular behaviour patterns are performed, as well as in their frequencies, a sequence analysis produced no obvious, easily quantified differences between the subjects of these experiments. The subsequent analysis therefore concentrated on the frequencies and durations of the behaviour patterns recorded. Eleven behavioural measures were selected for further analysis on the grounds that they fulfilled one or more of three criteria; that they gave a reasonably large amount of data, varied across tests or across conditions or had proved useful in previous studies of stickleback behaviour. These are defined in the caption to

Table I, which shows the mean values of these scores in each test and for each stage of the breeding cycle.

The possibility that the result of a given test was influenced by the time of day or the position in the whole sequence of tests at which it performed was tested and dismissed.

(i) **Individual differences.** A significant proportion of the variance of many of the behaviour patterns studied was due to differences between individual sticklebacks ( $P < 0.05$  on an analysis of variance). The persistence of these differences was studied by calculating Spearman rank order correlation coefficients between the scores of individual fish on a single test (G5) in the pre-egg and young phases (Table II). For several of these scores, including lunges and bites, the correlation was significant at the 5 per cent level at least. These results, which agree with those of Black (1971), indicate that with respect to several of the behaviour patterns studied here the individual differences found in the pre-egg phase were also reflected in the tests performed at a later stage of the breeding season.

Spearman rank order correlations between the scores of the individual fish in the various

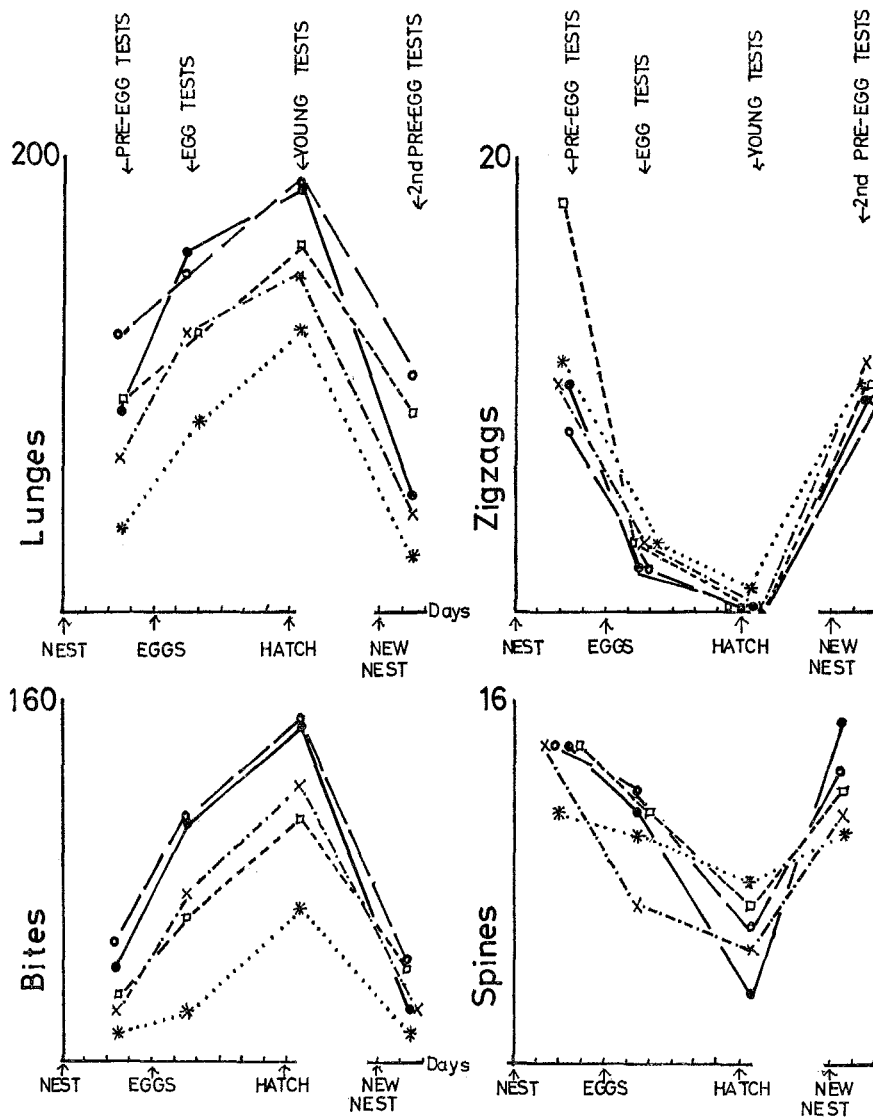


Fig. 1. Changes in mean level of four behaviour patterns over successive days of the breeding cycle. ( $N = 27$  for pre-egg tests;  $N = 23$  for egg tests;  $N = 13$  for young tests;  $N = 7$  for second pre-egg tests.) Key to tests: ●—● *Pygosteus* at 5 cm; ○—○ *Gasterosteus* at 5 cm; × ····× *Pygosteus* at 30 cm; □ — — □ *Gasterosteus* at 30 cm; \* . . . \* newt at 5 cm.

pre-egg tests (i.e. with different intruders) are shown in Table III. Again these were significant for a number of scores, including lunges and bites. It appeared that individual differences in aggression towards conspecifics showed also in response to heterospecific intruders.

(ii) **Stage in the breeding cycle.** The level of lunges and bites, increased during a breeding cycle (Fig. 1 and Table I). The reverse was true for zig-zags and the number of spine-raising bouts, while for both these groups the scores returned to their original level at the start of

the next breeding cycle. These results are in broad agreement with those of Wootton (1971) and Black (1971) although these authors studied the changes in behaviour over the breeding cycle on a day-to-day basis and only looked at the response to conspecifics. In the present study, the changing levels of all these scores in tests with heterospecifics closely paralleled those where the intruder was of the same species as the resident fish.

(iii) **Species of intruder.** At all four stages of the breeding cycle, higher levels of bites and lunges were obtained when the intruder was a member of either fish species than when it was a newt (Table I, Fig. 1). For the duration of spine raising, this situation was reversed. In addition, intruders near the nest produced higher levels of the first two responses and lower levels of spine raising than those at a distance from the nest. This distance effect is in agreement with results of Tinbergen (1953b), Van den Assem (1967) and Black (1971).

In the pre-egg tests, conspecifics elicited higher levels of lunges and bites and fewer bouts of spine raising than did *Pygosteus* intruders at the same distance from the nest. Thus in the early stages of the breeding cycle conspecifics were attacked more vigorously than intruders of other species. In the egg phase and in the young phase this difference was no longer apparent, but it reappeared in the second pre-egg phase. All these results were tested for significance with an analysis of variance followed by the Student Newman Keuls test and are reported here if  $P < 0.05$ .

In order to get a clearer picture of the relationships between the various elements of the reaction of these fish to territorial intruders and to find a composite measure of this response, the results of the pre-egg tests were subjected to Principal Components Analysis followed by Varimax rotation. Simple accounts of these techniques are provided by Hope (1968) and Child (1970). Briefly, a Principal Components Analysis determines and expresses the relationships between the original variables, replacing these by new orthogonal axes, or components, which reflect their correlations. The first component is chosen to account for as much as possible of the total variance; the second is then set up to account for the greatest possible proportion of the remaining variance, and so on, always subject to the condition of orthogonality. The relative importance of each of the original variables in specifying the structure of a given

component is expressed in a set of 'loadings' for that component. The positions of the subjects with respect to a component can be calculated from these loadings and the scores on the original variables. Since the first few components often account for a large part of the total variance, these may be used to describe the behaviour of the fish with considerable economy. A study of the largest loadings on a given component may allow it provisionally to be identified in biological terms, although this is by no means essential for the method to be valuable. Varimax rotation is an extension of Principal Components Analysis in which the position of the components is moved, to produce maximum distinction between their structures, while keeping them orthogonal (see Child 1970). The new axes which result from this rotation are called 'factors' rather than 'components' since the analysis has moved away from a straightforward Principal Components Analysis.

All the eleven scores for each test were included in this analysis. Fig. 2 displays the loadings for the first four factors resulting from the separate analyses of the five tests as well as the average loadings from all tests. On the basis of the relative sizes of their loadings, these factors are tentatively labelled as indices of 'aggression' (factor 1 with high loadings for lunges, bites and a measure of facing from close up), of 'curiosity' (factor 2 with high loadings for facing from close up opposed to facing from a distance), of 'nest activity' (factor 3 with high loadings for the two measures of nest activity) and finally 'sex' (factor 4 with high loadings for zig-zags). These labels are as much a shorthand statement of the loadings on these four factors as a claim that they really do represent real dimensions in the behavioural organization of the fish, although this does seem a likely explanation of the results. However, regardless of whether or not the factors are given names, their structures in the tests with the different types of intruder are readily comparable. This, together with the fact that the individual scores on the first two factors in the different tests are significantly positively correlated (Table IV), supports the conclusion reached above that in these sticklebacks the territorial response to conspecifics and to other species have much in common.

The scores of each stickleback on the 'curiosity', and the 'aggression' factor are used to quantify their territorial response. Both these aspects of the behaviour of breeding sticklebacks might be

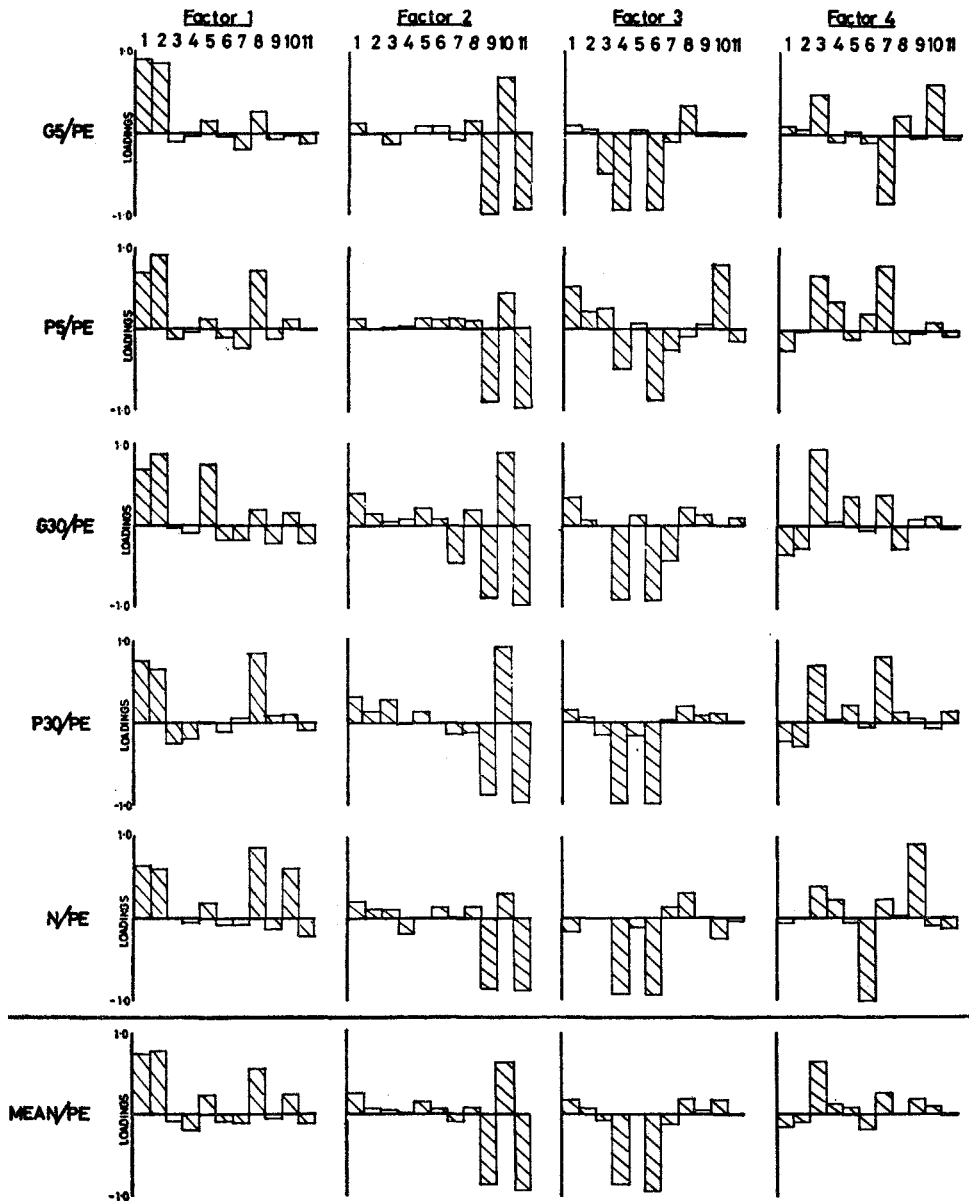


Fig. 2. The structure of the first four factors from the five pre-egg tests ( $N = 27$ ). Key to behaviour patterns: (1) number of lunges; (2) number of bites; (3) number of zig-zags; (4) number of bouts of nest activity; (5) number of spine-raising incidents; (6) duration of nest activity; (7) duration of spine raising; (8) mean bout length of facing from  $< 10$  cm; (9) mean bout length of facing from  $> 20$  cm; (10) proportion of time facing from  $< 10$  cm; (11) proportion of time facing from  $> 20$  cm. Key to tests: G5 *Gasterosteus* at 5 cm; P5 *Pygosteus* at 5 cm; G30 *Gasterosteus* at 30 cm; P30 *Pygosteus* at 30 cm; N newt at 5 cm.

Table IV. Correlations Across Tests of Individual Scores on the 'Aggression' Factor ( $N = 27$ )

P5	G30	P30	N	
0.51**	0.64**	0.51**	0.31	G5
	0.67**	0.59**	0.59**	P5
		0.78**	0.63**	G30
			0.64**	P30
				N

\* $P < 0.05$ , \*\* $P < 0.01$ .

Correlations Across Tests of Individual Scores on the 'Curiosity' Factor

P5	G30	P30	N	
0.41*	0.58**	0.59**	0.33*	G5
	0.81**	0.65**	0.62**	P5
		0.71**	0.55**	G30
			0.55**	P30
				N

\* $P < 0.05$ , \*\* $P < 0.01$ .

Key to tests: G5 *Gasterosteus* at 5 cm; P5 *Pygosteus* at 5 cm; G30 *Gasterosteus* at 30 cm; P30 *Pygosteus* at 30 cm; N newt at 5 cm.

expected to have counterparts in the behaviour they show outside the breeding season.

### Discussion of Tests Within the Breeding Season

Taken together, the results considered so far provide answers to the three questions posed above. There are significant differences between individual sticklebacks in a number of behaviour patterns, including some which seem to be aggressive in nature. These individual differences remain consistent over the different phases of the breeding cycle. The positive correlation between the scores of individual fish in response to the different species of intruder, their parallel changes over the breeding cycle and the similarity of the results of multivariate analysis on the different tests indicate that the aggressive response to con- and hetero-specific territorial intruders have much in common in breeding sticklebacks.

### Some Experiments Outside the Breeding Season

Non-breeding sticklebacks rarely show behaviour of such an obviously aggressive nature

as the bites and lunges described above. However, there do appear to be differences in the degree of boldness that the individual fish show in a number of disturbing situations. The experiments performed outside the breeding season were designed (i) to quantify such differences in boldness in sticklebacks when confronted with a predator and (ii) to relate these differences to the behaviour shown by the sticklebacks when they come into breeding condition.

### Pike Tests

In spite of the protection afforded by their spines, sticklebacks are preyed upon by a number of aquatic predators. Of these, young pike were chosen for the present experiments since, although they are difficult to keep, their behaviour in response to prey is relatively easy to observe and has already been described by Hoogland, Morris & Tinbergen (1956) and by Benzie (1965). That small pike do, in fact, prey on sticklebacks in the wild has been demonstrated by analysis of stomach contents of these predators (Frost 1954; Healey 1956). Therefore in attempting to quantify interactions between sticklebacks and young pike, one is studying a situation which might well occur in natural conditions.

### Methods

The pike used in these experiments measured between 15 and 20 cm. They lived singly in glass tanks of  $100 \times 35 \times 35$  cm with gravel bottoms planted with a single, sparse clump of weed in one corner. Each pike was screened off from the rest of the room and all observations were made from behind a screen as these fish are easily disturbed. Before each test they were fed freely with minnows until they stalked two fish in succession without striking at them. In this way the pike were discouraged from eating the test fish and yet still showed some hunting behaviour. In the relatively rare cases that a stickleback was captured, even if it was subsequently released, it was not used in later tests.

Sticklebacks were tested singly and on one occasion only. The method of introduction which proved least disturbing to all participants was to remove each fish to be tested from its home tank and hold it for 5 min in an opaque container before tipping it gently into the clump of weeds when the pike was at the far end of the tank and facing in the opposite direction. In this way the initial experience of the sticklebacks on first being introduced to the pike's tank was made as similar as possible.



The fish were observed for 15 min, during which time a continuous record of the stickleback's behaviour was tape recorded. In particular, its distance from the pike, the position of its spines, the details of its response to the pike (approach or retreat, etc.), and the type of swimming it showed, were recorded. These are explained in the caption to Fig. 3, but the last category needs some additional discussion. Undisturbed sticklebacks swim in a highly characteristic way, using only their pectoral fins to produce an irregular forward movement, called here 'jerky swimming': at any disturbance they either stop moving ('still') or start to swim in a more typical fish-like manner using the tail to propel themselves forward ('continuous swimming'). The type of swimming that sticklebacks use in an alarming situation may therefore serve as an indicator of 'boldness'. This possibility was tested in a separate experiment in which the swimming method of sticklebacks was observed before and after exposure to a stimulus known to alarm them (an overhead moving object, Phillips 1962). A marked and significant decrease in the proportion of time spent in jerky swimming was observed following this treatment (74 per cent before, 24.5 per cent after,  $P < 0.01$  on an analysis of variance). This aspect of the fishes' behaviour, therefore, seemed a likely indicator of their degree of boldness.

At the end of the 15-min period in the pike tank, the stickleback was returned to its home. Three pike were used to test eighty-one sticklebacks.

The behaviour of the pike was rather variable; not only do they show individual differences in behaviour, but the behaviour of a single pike may change from one test to the next. As attempts to use model predators failed, live pike had to be used. As far as possible the problem was avoided by using the pike only when they were fairly inactive. However, the variability of the pike remains a source of inaccuracy in these experiments.

## Results

An initial examination of the results of these experiments showed considerable differences between sticklebacks as far as most of their behaviour patterns were concerned. The fifteen variables used in the following analysis are listed in the caption to Fig. 3.

In order to interpret this large body of data the results of the pike tests were subjected to a

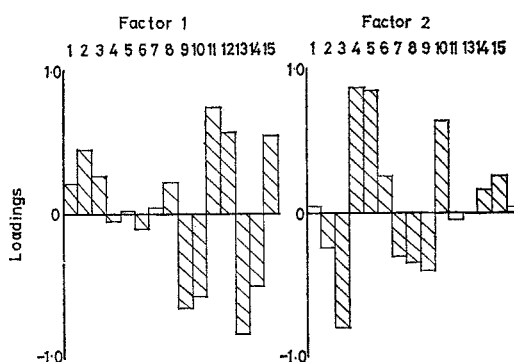


Fig. 3. Structure of the first two factors from the pike tests. ( $N = 81$ .) Key to behaviour patterns: (1, 2) number and duration of 'still'; (3, 4, 5) latency, number and duration of jerky swimming; (6, 7) number and duration of spine raising; (8) latency of spine lowering (9) number of retreats (movements from a facing position away from the pike through a distance of at least 2 cm); (10) number of approaches (movements from a facing position towards the pike through a distance of at least 2 cm); (11) least distance from pike; (12) average distance from pike; (13, 14, 15) number, duration and mean distance of facing (motionless in a position pointing towards the pike).

Principal Components Analysis followed by Varimax rotation of the new axes. The loadings of the first two factors, the only ones which will be discussed here, are shown in Fig. 3. The first factor accounts for about 22 per cent of the total variance and has high loadings for: duration of still, least distance, average distance and average distance of facing; with high loading in the opposite direction for: number and duration of facing bouts, approaches and retreats. It thus opposes behaviour which serves to maintain a distance between the stickleback and the pike to that which takes the stickleback close to the pike, giving it information about the larger fish while keeping it ready to flee. The convention used for labelling such bimodal factors was to refer first to those variables whose loadings are positive and then to those with negative loadings. The nature of the loadings on this first factor is therefore summarized in the label 'precaution-investigation'.

The second factor, accounting for 21 per cent of the total variance, has high loadings for number and duration of jerky swimming and number of approaches, opposed to latency of first jerky swimming and first spine lowering, duration of spine raising and number of retreats. Jerky swimming appears to be characteristic of an undisturbed stickleback, while

spine raising is in some situations typical of an alarmed fish (Symons 1965). In addition, an approach is the behaviour of a bold fish and retreat the reverse. On these grounds, this second factor opposes behaviour likely to be performed by a fish which is not easily disturbed with that typical of a more timid animal. Therefore it has tentatively been labelled a 'boldness-timidity' axis, on the convention described above.

The scores of the individual fish on the 'precaution-investigation' and the 'boldness-timidity' factors are used to summarize these two features of their anti-predator response. The second factor is considered especially important since 'boldness-timidity' probably comes closest to the type of reaction to a predator which might conceivably be related to aggressive behaviour of the sort shown by breeding sticklebacks.

Thus, it is possible, with the help of multivariate analysis, to quantify the anti-predator behaviour of sticklebacks for comparison with their behaviour in other situations.

#### Unfamiliar Environment Tests

However, since the pike tests proved hard to standardize, another means of assessing the boldness of sticklebacks outside the breeding season was thought necessary. Benzie (1965) found differences in behaviour between *Gasterosteus* and *Pygosteus* in a novel environment. For most prey species a strange environment must be potentially dangerous at least until it has been explored. It seemed possible that a similar situation might also show up differences in behaviour between individuals of the same species and to this end a modified version of Benzie's apparatus was designed.

#### Methods

A large tank (210 × 35 × 35 cm) was divided equally into three by opaque Perspex partitions with vertically sliding metal doors. The front and back walls of each compartment were divided into 5-cm squares. The central compartment in which the fish lived was planted with artificial water weed. This was called the home tank. Of the other two compartments one was planted with a different type of artificial weed at different parts of the tank and called the weeded tank while the other, the bare tank, was left unplanted. Each compartment was illuminated from above with a 40-watt bulb.

Fish were placed in the home tank in groups of eight and allowed four days to settle down. They were then watched for 5-s periods in a regular sequence until twenty records had been

made for each fish. During each 5-s observation records were made of the position of the fish in the tank, its type of swimming and whether or not its spines were raised.

At the end of this initial observation period one of the doors was opened, the fish were edged gently into one of the experimental tanks with a small net and the door was then closed. Twenty more observations were made on the fish in this new situation and they were then returned to their home tank.

Initially, three different experimental procedures were used; the fish were transferred from the home tank to the weeded tank, from the home tank to the bare tank or from the home tank to a small holding net before being returned to the home tank. These were carried out in a random sequence. However, transfer from the home tank and back produced somewhat anomalous results; in addition the sequence in which the different procedures were performed was found to have no effect on their results. Therefore in later experiments where a larger number of fish were to be tested (104 altogether) the only transfers used were from the home tank to the weeded tank and to the bare tank, in that order.

This experimental set-up is reminiscent of the open-field test, which has often been used to investigate emotionality in rodents and which has been criticized by Archer (1973). Different measures of emotionality in a single test do not always correlate well, nor do the results of open-field tests always agree with other measures of emotionality. In addition, exploratory behaviour as well as emotional behaviour may affect an animal's activity in the open field (Candland & Nagy 1969; Denenberg 1969) and these confounded effects are hard to unravel. In the experiments described above, the fish are introduced into an unfamiliar environment where the exploratory behaviour that such conditions might be expected to produce is probably initially inhibited by fear and only begins to appear after some time in the strange environment (Russell 1967). Individual differences in the behaviour shown by sticklebacks in these experiments may therefore result from differences in boldness or in the tendency to explore, or both. The introduction of an independent measure of boldness, the proportion of jerky swimming, should help to clarify the situation. However, it is as well to remember that the results of these experiments are likely to be complex.

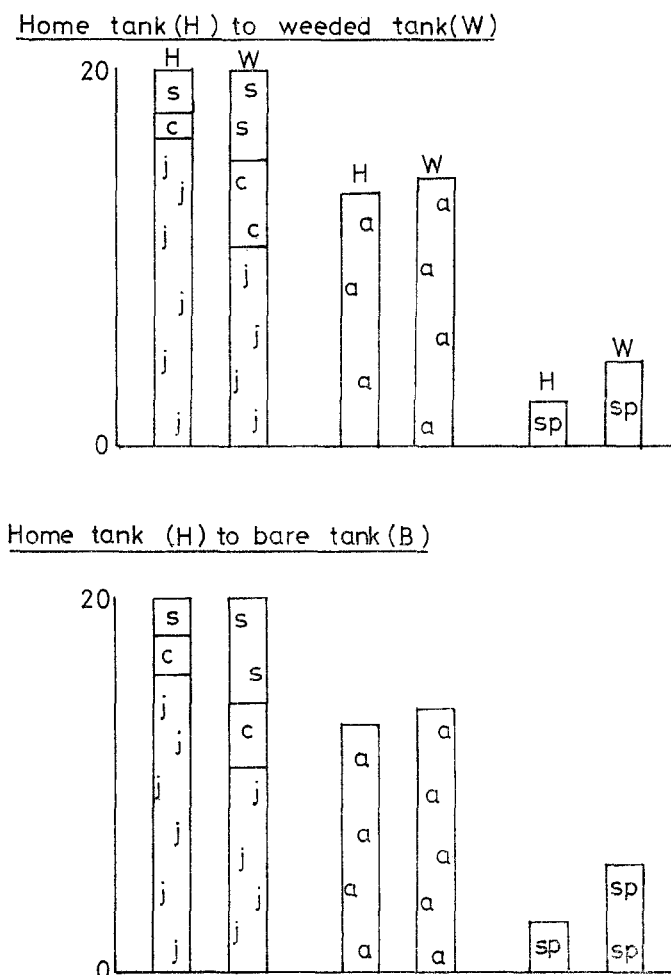


Fig. 4. Change in behaviour on transfer from a familiar to an unfamiliar environment. ( $N = 104$ .) Key to behaviour patterns: (j) proportion of records showing jerky swimming; (c) proportion of records showing continuous swimming; (s) proportion of records showing still; (sp) proportion of records showing any degree of spine raising; (a) activity (the number of squares entered at least once during a test).

## Results

The means of five scores taken for each fish in each observation period are shown in Fig. 4.

The experimental treatment depressed jerky swimming and elevated continuous swimming, still and spine raising, while having only a slight effect on activity. These changes are more marked on transfer to the weeded tank than to the bare tank. In addition, there were significant differences between the individual fish in the extent to which their behaviour was changed by transfer to the unfamiliar environment.

(For each of these results  $P < 0.05$  on an analysis of variance.)

The results of the experiments were analysed by the method of principal components in an attempt to find some composite score which might reflect 'boldness' in these tests. (Rotation of the axes proved unnecessary in this case.) The first two components from the analysis of each condition in which the fish were observed are shown in Fig. 5. The structure of these components in the four analyses are very similar. The first component opposes behaviour

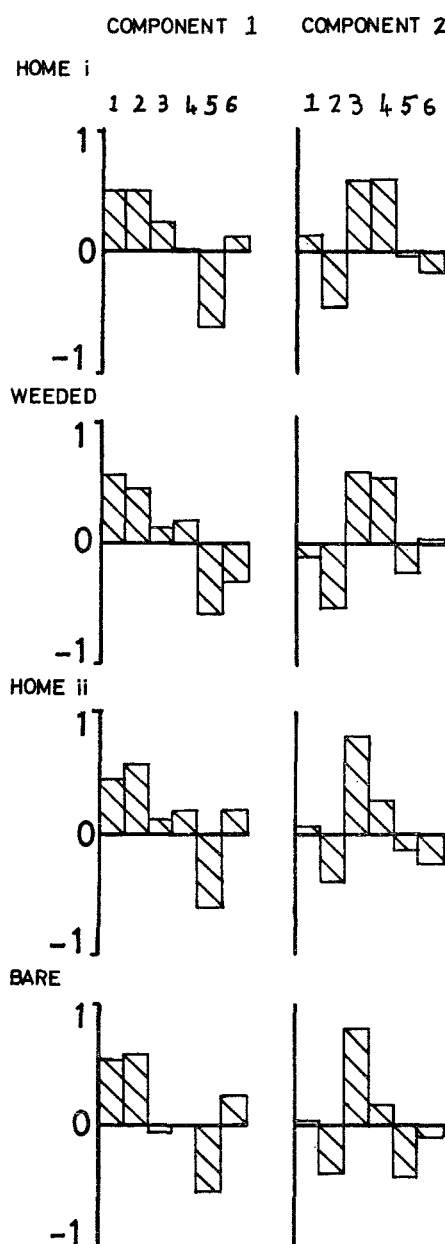


Fig. 5. Structure of the first two components from the unfamiliar environment tests. Key to behaviour patterns: (1) activity; (2) proportion of records showing jerky swimming; (3) proportion of records showing continuous swimming; (4) proportion of records showing creepy swimming—irregular creeping across the floor of the tank; (5) proportion of records showing still; (6) proportion of records with spines raised to any extent. ( $N = 104$ .)

of an active fish (activity and jerky swimming) to that of an inactive fish (still) and has therefore been labelled as an index of 'activity'. Bearing in mind the fact that jerky swimming is reduced when sticklebacks are alarmed (see above) component 2 (with high positive loadings for continuous or creepy swimming and negative loadings for jerky swimming: see Fig. 5) has tentatively been identified as a 'timidity—boldness' axis, using the convention described above.

The individual scores on the second component are higher in the two experimental than in the two home tanks. In other words, if this component really does reflect the behaviour systems after which it was named, the fish are more timid in both unfamiliar environments; this agrees with what is known of the behaviour of many fish species (e.g. Russell 1967). When the magnitude of the change in level on this component for each fish in the two transfers is compared, a small but significant positive correlation (0.2) emerges. Thus whatever the second component is measuring, the extent to which it is changed in an unfamiliar environment would appear to be a fairly consistent feature of the behaviour of individual sticklebacks. The average of the two values for this change is taken as an overall measure of the disturbance caused to a stickleback by transfer to a strange environment.

If these unfamiliar environment tests do provide a satisfactory substitute for the more variable pike tests, the behaviour of the individual fish in the two situations should compare well. Figure 6 shows the scores of forty-four fish tested with the same pike on the 'boldness—timidity' factor in the pike test plotted against their average change in level of the 'timidity—boldness' component in the unfamiliar environment tests. Clearly the scores are quite closely correlated. One explanation for this correlation might be that since the pike tank itself is unfamiliar to the sticklebacks these two experiments are simply measuring the same thing. This seems unlikely to be the whole story since most of the sticklebacks in the pike tests reacted to the predator early on in the test and continued to do so throughout the 15 min. The unfamiliar environment tests are therefore considered to serve well as a substitute for the pike tests.

Thus the experiments performed outside the breeding season have quantified the boldness of the individual sticklebacks both in the

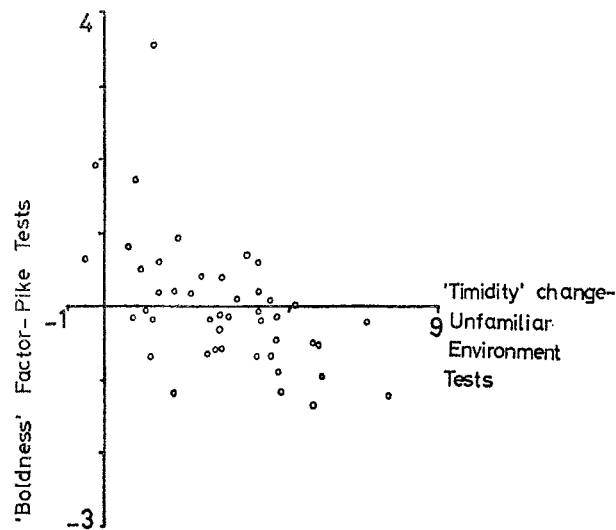


Fig. 6. Comparison of scores on the 'boldness-timidity' factor of the pike tests with the mean depression of the 'timidity-boldness' component in the unfamiliar environment tests. ( $N = 44$ .)

presence of a predator and in an unfamiliar and therefore potentially dangerous environment.

#### Comparison with the Behaviour Shown During the Breeding Season

The main aim of these experiments was to compare the behaviour of the individual sticklebacks in an anti-predator situation with that shown towards territorial intruders during the breeding season. Especially relevant are those behaviour patterns which correspond to 'boldness' or 'timidity' on the one hand and 'aggression' on the other. (These labels are written in quotes to stress their provisional nature.) Correlations between these are shown in Table V. Here, three measures of the territorial response of the fish are compared with two measures of their behaviour in the pike tests and two from the unfamiliar environment tests.

No significant correlations emerge between the unfamiliar environment 'activity' factor and any of the reproductive tests measures. Whatever is responsible for the differences in territorial response of individual sticklebacks, it does not seem to be different in activity, at least as this is measured in the unfamiliar environment tests.

As far as the 'curiosity' factor is concerned, the correlation with the change in the 'timidity-boldness' component is negative, although not significant at the 5 per cent level. This indicates a relationship between the behaviour of stickle-

Table V. Spearman Rank Correlations Between Scores in Tests Inside and Outside the Breeding Season. ( $N = 25$ ; the Scores of Two Fish which Contracted White Spot Disease are Omitted from this Comparison)

	Reproductive tests		
	Mean score in all tests on 'curiosity' factor	Mean score in all tests on 'aggression' factor	Mean score in tests with conspecifics on 'aggression' factor
<i>Unfamiliar score on 'activity' component tests</i>			
Mean score on 'activity' component	0.10	-0.07	-0.02
Mean change on 'timidity-boldness' factor	-0.25	-0.43*	-0.42*
<i>Pike tests</i>			
Score on 'investigation'-precaution' factor	0.13	0.13	0.17
Score on 'boldness-timidity' factor	0.25	0.29	0.48**

\* $P < 0.05$ , \*\* $P < 0.01$ .

backs in an unfamiliar environment and their 'curiosity' as reflected in the territorial situation. Thus it may well be that 'exploration' as well as 'emotionality' is indeed influencing the behaviour of the sticklebacks in the unfamiliar environment tests (Archer 1973).

The 'timidity-boldness' scores for the unfamiliar environment tests show a significant negative correlation with the reproductive 'aggression' scores. Thus sticklebacks which are least disturbed on transfer to a strange environment tend to be the most aggressive when they come into breeding condition.

Similarly, the correlations between the scores of the fish on the 'boldness-timidity' axis from the pike tests and on the 'aggression' axis in the reproductive tests are also positive, although only significantly so (at the 5 per cent level) when the response to conspecifics alone is used. In general, the results of the comparisons based on behaviour of sticklebacks in the pike tests agree well with those found when the more reliable unfamiliar environment tests are used.

Altogether, these results show that 'boldness' in various situations outside the breeding season and 'aggression' during the breeding season vary together when the behaviour of individual sticklebacks are compared. Hence in this species at least there may well be motivational links between anti-predator defence and aggression towards conspecifics.

Some evidence in support of this finding is available. In a rather inconclusive study of differences between sticklebacks with different numbers of lateral scutes, Moodie, McPhail & Hagen (1973) found fish with seven lateral scutes to be less bold in the face of a predator than were other types, in the winter at least. Moodie (1972) also noticed that, in a different population of sticklebacks, fish with seven scutes were less aggressive in defence of their territories and more easily frightened away from their nests. Anthour (1968) looked at individual differences in behaviour of sticklebacks in a strange environment. He found positive correlations between the tendency of sticklebacks to explore in these conditions and their later social dominance. His time-scale was different to that of the present experiments, the exploration tests being carried out shortly before the fish started breeding. However, the results of both these studies are at the least compatible with those of the present experiments.

### Discussion

The experiments reported above have shown that the aggressive response of breeding male sticklebacks to the different species of territorial intruder have much in common and co-vary on several time-scales and that the degree of boldness shown towards a predator by non-reproductive fish and their aggressiveness during the breeding season are positively correlated. There are various possible explanations for these results, in terms of both causes and functions.

The fact that changing levels of territorial aggression across the breeding cycle are reflected in the response to intruders of various species implies a motivational link between inter- and intraspecific aggression in this context. This may be a common response of both patterns to some varying internal factor such as hormone level; however, it may also be that the sticklebacks are merely reacting to the common stimulus situation provided by the various intruders. That quite subtle visual cues are important in eliciting attack in sticklebacks has been shown by Muckensturm (1967) and in the present tests the fish did react differentially to conspecifics in the pre-egg phase. These facts militate against the latter explanation.

A possible causal explanation for the longer term correlation between anti-predator boldness and social aggression is that what is really varying between the individual fish is their 'fearfulness' rather than their 'aggression' and 'boldness'. This 'fearfulness' might be suppressing the response to a predator and to a conspecific to a similar extent and may thus be responsible for the correlations described above. Although it has been shown that aggression and fear may vary independently in several fish species (Heiligenberg 1965; Rasa 1969), there is insufficient information on this point in sticklebacks for this explanation to be accepted or dismissed at present.

Thus while it can be said that the aspects of a stickleback's anti-predator and social aggression measured in these experiments do probably depend, in part at least, on common internal factors, the precise form of these needs further investigation.

At the functional level, there would seem to be good reasons why social and anti-predator aggression should co-vary, both within the breeding season and on a longer time scale.

During the breeding season when sticklebacks are caring for their babies, conspecifics, not being averse to cannibalism, present much the

same threat to the developing young as do members of other small, carnivorous species. In such a situation, the consequences of failing to act aggressively are equally serious whether a territorial intruder is a con- or a heterospecific. It would therefore be advantageous if both these aspects of territorial behaviour were to reach a peak at the time when the young are most vulnerable.

On a longer time scale, predation on adult fish could act as a convergent selection pressure on social aggression and anti-predator boldness. For example, one possible strategy for dealing with high levels of predation in a small animal with no effective means of wounding a predator might be to become increasingly timid in the presence of any potential predator and to reduce the frequency of any behaviour which makes an animal conspicuous. Social aggression falls into the latter category, especially in sticklebacks where high levels of aggression prolong courtship. Such a solution would favour low levels of social and anti-predator aggression where predation is high but would allow higher levels of both when this pressure is relaxed. Studies by Seghers (1970, 1974) and Ballin (1974) on population of guppies subjected to different levels of predation indicate that such a condition may prevail in this species. This sort of covariance might be attained more easily if these two aspects of behaviour were controlled by the same internal factors.

### Acknowledgments

I would like to thank Dr J. M. Cullen for supervising this work and Dr R. Anderson for help in its analysis.

### REFERENCES

- Anthour, M. (1968). Activité et dominance chez l'épinoche (*Gasterosteus aculeatus* L.). *C. r. hebdomadaire. Acad. Sci., Paris D*, **256**, 1265-1239.
- Archer, J. E. (1973). Tests for emotionality in rats and mice: a review. *Anim. Behav.*, **21**, 205-235.
- Baggermen, B. (1958). An experimental study on the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). *Arch. Néerl. Zool.*, **12**, 105-317.
- Ballin, P. J. (1974). Geographic variation in courtship behaviour of the guppy, *Poecilia reticulata*. M.Sc. thesis, University of British Columbia.
- Benzie, V. L. (1965). Some aspects of the anti-predator responses of two species of stickleback. D.Phil. thesis, Oxford University.
- Black, R. (1971). Hatching success in the three-spined stickleback (*Gasterosteus aculeatus*) in relation to changes in behaviour during the parental phase. *Anim. Behav.*, **19**, 532-541.
- Candland, D. K. & Nagy, Z. M. (1969). The open field test: some comparative data. *Ann. N.Y. Acad. Sci.*, **159**, 831-851.
- Child, D. (1970). *The Essentials of Factor Analysis*. London: Holt, Rinehart & Winston.
- Denenberg, V. H. (1969). Open field behaviour in the rat: what does it mean? *Ann. N.Y. Acad. Sci.*, **159**, 354-359.
- Frost, W. E. (1954). The food of the pike, *Esox lucius*, in Windermere. *J. Anim. Ecol.*, **23**, 339-360.
- Healey, A. (1956). Pike, *Esox lucius*, in three Irish lakes. *Sci. Proc. R. Dublin Soc.*, **27**, 51-68.
- Heiligenberg, W. (1965). The suppression of behavioural activities by frightening stimuli. *Z. vergl. Physiol.*, **50**, 660-672.
- Hoogland, R., Morris, D. & Tinbergen, N. (1956). The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as a means of defence against predators. *Behaviour*, **10**, 205-236.
- Hope, K. (1968). *Methods of Multivariate Analysis*. London: University of London Press.
- Moodie, G. E. E. (1972). Morphology, life history and ecology of an unusual stickleback (*Gasterosteus aculeatus*) in the Queen Charlotte Islands, Canada. *Can. J. Zool.*, **50**, 721-732.
- Moodie, G. E. E., McPhail, J. D. & Hagen, D. W. (1973). Experimental demonstration of selective predation on *Gasterosteus aculeatus*. *Behaviour*, **47**, 95-105.
- Muckensturm, B. (1967). La signification de la livrée nuptiale de l'épinoche. *Revue de Comp. Animal*, **3**, 39-64.
- Phillips, G. C. (1962). Survival value of the white colouration of gulls and other sea birds. D.Phil. thesis, Oxford University.
- Rasa, O. A. E. (1969). Territoriality and the establishment of dominance by means of visual cues in *Pomacentrus jenkinsi* (Pisces: Pomacentridae). *Z. Tierpsychol.*, **26**, 825-845.
- Russell, E. M. (1967). The effect of experience of surroundings on the response of *Lebistes reticulans* to a strange object. *Anim. Behav.*, **15**, 586-594.
- Seghers, B. H. (1970). Behavioural adaptations of natural populations of the guppy, *Poecilia reticulata*, to predation. *Am. Zool.*, **10**, 489-490.
- Seghers, B. H. (1974). An analysis of geographic variation in the anti-predator behaviour of the guppy, *Poecilia reticulata*. Ph.D. thesis, University of British Columbia.
- Symons, P. E. K. (1965). Analysis of spine raising in the male three-spined stickleback. *Behaviour*, **25**, 1-74.
- Tinbergen, N. (1951). *The Study of Instinct*. Oxford: Clarendon.
- Tinbergen, N. (1953a). *Social Behaviour in Animals*. London: Methuen.
- Tinbergen, N. (1953b). Fighting and threat in animals. *New Biology*, **14**, 9-24.
- Van den Assem, J. (1967). Territory in the three-spined stickleback, *Gasterosteus aculeatus* L. *Behaviour Suppl.*, **16**, 1-164.

- Van Iersel, J. J. A. (1953). An analysis of the parental behaviour of the male three-spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour Suppl.*, **3**.
- Wilz, K. J. (1971). Comparative aspects of courtship behaviour in the ten-spined stickleback, *Pygosteus pungitius* L. *Z. Tierpsychol.*, **29**, 1-10.
- Wootton, R. J. (1971). Measures of the aggression of parental male three-spined sticklebacks. *Behaviour*, **40**, 228-226.
- (Received 1 October 1974; first revision 29 January 1975; second revision 5 March 1975; third revision 19 May 1975; MS. number: 1370)