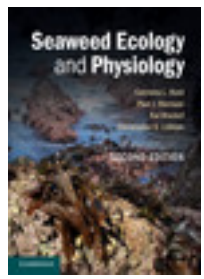

Seaweed Ecology and Physiology

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Chapter

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2

Life histories, reproduction, and morphogenesis

2.1 Introduction

The basic patterns of alternation of sporophyte and gametophyte must be regarded as a theme on which many variations are played (Fig. 2.1). Each generation may reproduce itself asexually, and sexual reproduction should be taken to include meiosporogenesis as well as gametogenesis and mating (Clayton 1988). Asexual reproduction allows an economical population increase but no genetic mixing, whereas sexual reproduction allows genetic mixing but is more costly because of the waste of gametes that fail to mate (Clayton 1981; Russell 1986; Santelices 1990). Most seaweeds use both means of reproduction, and, as Russell (1986) has noted, where there are isogametes, these can function equally as asexual swarmers. Vegetative reproduction by "multicellular propagules", defined by Cecere *et al.* (2011) as "a vegetative, multicellular structure which detaches from the parent thallus and gives rise to a new individual", is also common, for example *Halimeda* (Walters *et al.* 2002). However, their roles in species' dispersal, and forming overwintering and resting "organs" that allow the survival of unfavorable environmental conditions, is unknown (Russell 1986; Cecere *et al.* 2011). Clonal seaweeds may spread by stolons and/or rhizomes, giving a significant competitive edge in the space race (sec. 1.2.3, 4.2.3). Some floating algal populations depend entirely on vegetative reproduction by fragmentation (sec. 3.3.7).

Culture studies are critical in establishing the range of possible life histories that can occur. Sufficient variations have been discovered in the basic pattern – between and within species – that today's generalizations must be viewed only as working hypotheses. New variations in what are considered to be well-known life cycles are regularly uncovered. For example, male gametophytes of *Laminaria digitata* can reproduce themselves via fragmentation (Destombe *et al.* 2011). Although a basic alternation of a sporophyte (typically diploid) and a gametophyte (typically haploid) is common among seaweeds various extras and shortcuts are known (Fig. 2.1).[1] Indeed, a better generalization may be that almost any alternation is possible, and even no alternation at all. Moreover, the term "alternation" is a misnomer, in that it implies only two phases and a regular progression from one to the other; clearly that is not always the case (e.g. *Scytosiphon*, Fig. 2.2). Maggs (1988, p. 488) concluded that "life-history patterns seem to be more labile than morphological features, and the role of life-history variability in speciation, and in ecological success, should not be underestimated".

2.2 Theme and variations

Three basic types of algal life histories are recognized (e.g. Dring 1982; Bold and Wynne 1985; Graham *et al.* 2009). An alternation of two phases is called haplodiplontic (Fig. 2.1). Genera such as *Ulva* and *Chondrus* are examples of an isomorphic alternation of generations, having sporophytes and gametophytes that are vegetatively indistinguishable (not counting the

[1] Life-history diagrams herein are as follows: *Acetabularia* (Fig. 1.12), *Scytosiphon* (Fig. 2.2), *Halimeda* (Fig. 2.3), *Ectocarpus* (Fig. 2.4), *Laminaria* (Figs. 2.5 and 2.8), *Nereocystis* and *Pyropia* (Fig. 2.10). See also Fig. 2.7.

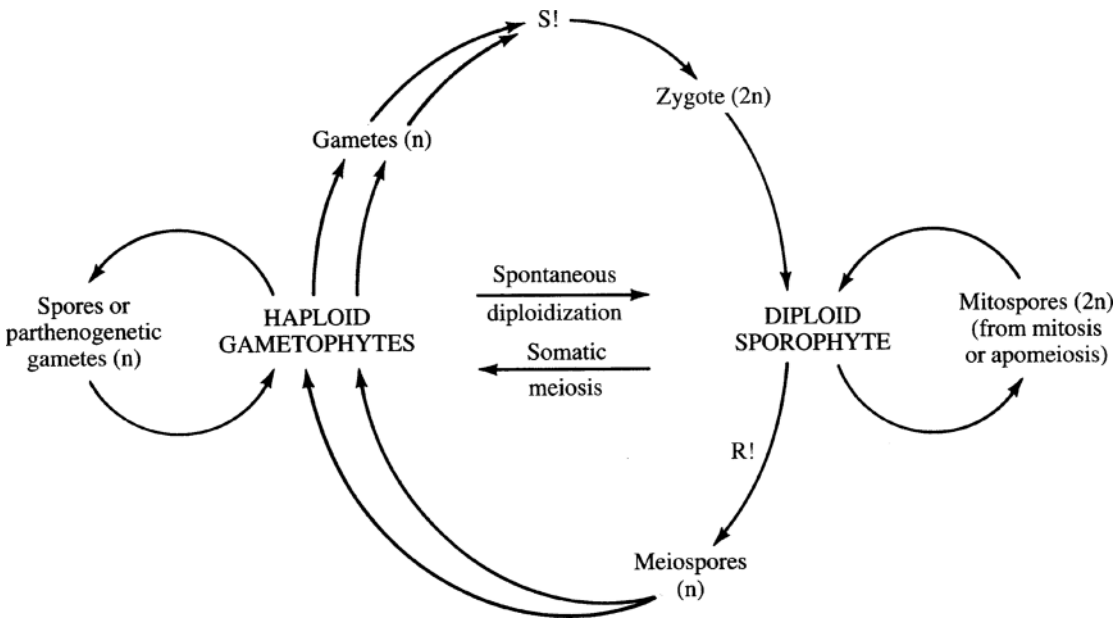


Figure 2.1 The basic pattern of an alternation between haploid (n) gametophyte and diploid ($2n$) sporophyte generations (the central cycle in the figure) is a theme upon which there are many variations. Some possible seaweed life-history progressions are indicated by the smaller cycles to the right and left of the figure. Most species use only a small part of this range. R!, meiosis (reduction division); S!, syngamy.

carposporophytes of red algae, which are not free living). Sometimes chemical differences occur between isomorphic phases, as in *Chondrus* in which different forms of carrageenan are found in the walls, even of the carpospores and tetraspores (Bellgrove *et al.* 2009; sec. 5.5.2). At reproduction, the two phases may become distinguishable by the reproductive structures. Heteromorphic generations often fall into two different functional-form groups, such as erect fronds versus creeping filaments or crusts (Figs. 2.2, 2.7, 2.8, and 2.10). A classic example is the well-known story of Drew's (1949) linking of *Conchocelis* (filamentous) and *Porphyra unbilicalis* (a blade) and its impact on the Japanese nori industry (sec. 10.2.1). Similarly, the crustose red *Erythrodermis allenii* is part of the *Phyllophora traillii* life history (Maggs 1989), and the unicellular green seaweed "*Codiolum*" is a life-history phase of various green seaweeds (see Graham *et al.* 2009). Some seaweeds exist in only one phase (Figs. 1.12 and 2.3). Most common are diplontic life cycles, for example the Fucales and *Codium* in which the vegetative phase is diploid ($2n$). Here, the gametes are formed by meiosis and the gametes are the only haploid phase of the life cycle. In haplontic life cycles, the vegetative phase is haploid with the zygote being the only diploid phase. Here, meiosis occurs in the zygote, thereby restoring the haploid phase. *Chlamydomonas* and dinoflagellates are examples of algae with a haplontic life cycle, but in seaweeds this condition is quite rare.

Florideophycidae were included in the foregoing life-history generalizations in spite of the interpolation of a "carposporophyte", a diploid tissue that forms on the female gametophyte following fertilization. This structure is often regarded as an additional diminutive diploid phase, that is hemi-parasitic because it obtains some nutritive support from the female gametophyte (Maggs *et al.* 2011). (The term "triphasic" is often used to describe such life cycles, which consist of free-living gametophytes and tetrasporophytes, plus the carposporophyte). The carposporophyte is responsible for zygote amplification; from one fertilization event

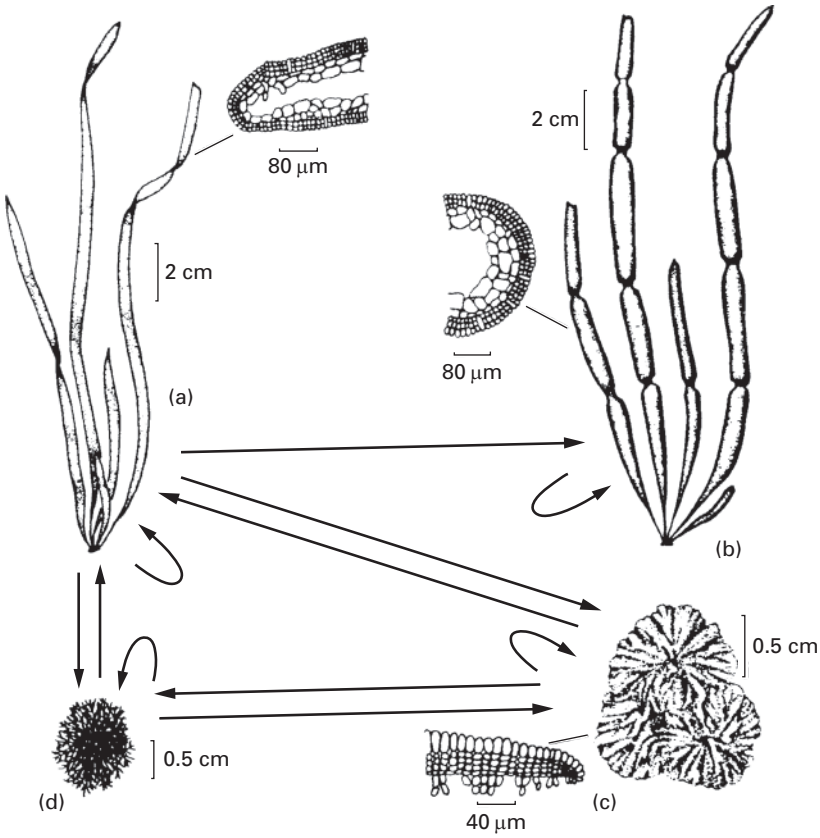


Figure 2.2 Life history and anatomical features of the *Scytosiphon lomentaria* (previously *Scytosiphon simplicissimus*) complex: (a) complanate form; (b) cylindrical form; (c) crustose form; (d) filamentous plethysmothalli. (From Littler and Littler 1983 (partly after Clayton), with permission of *Journal of Phycology*.)

thousands of carpospores (produced by mitosis) may be released and germinate into the free-living tetrasporophyte generation (see Graham *et al*. 2009). Some Bangiophycidae, including *Porphyra*, also multiply the zygote by forming "zygotosporangia", packets of diploid cells which are released upon breakdown of the cell wall, and germinate, giving rise to the conchocelis phase (Guiry 1990; Nelson *et al*. 1999). The zygote in *Palmaria palmata* develops into a large diploid phase, morphologically like the male gametophyte, that overgrows the tiny female gametophyte and produces spores by meiosis (van der Meer and Todd 1980). Replication of the zygote is one of several ways to amplify the results of sexual reproduction (see sec. 2.4; Hawkes 1990; Graham *et al*. 2009; Maggs *et al*. 2011).

Ploidy levels within a particular life-cycle phase are often assumed, but studies have sometimes demonstrated the unexpected. For example, most *Codium* species studied in the western Atlantic are diploid and reproduce via haploid gametes. However, *C. fragile* ssp. *fragile* (formerly known as ssp. *tomentosoides*) is haploid, reproducing parthenogenetically (Kapraun and Martin 1987; Prince and Trowbridge 2004). Furthermore, various morphological forms may be expressed at a given ploidy level, and the same morphology may be formed at different ploidy levels: There is no necessary connection between ploidy and

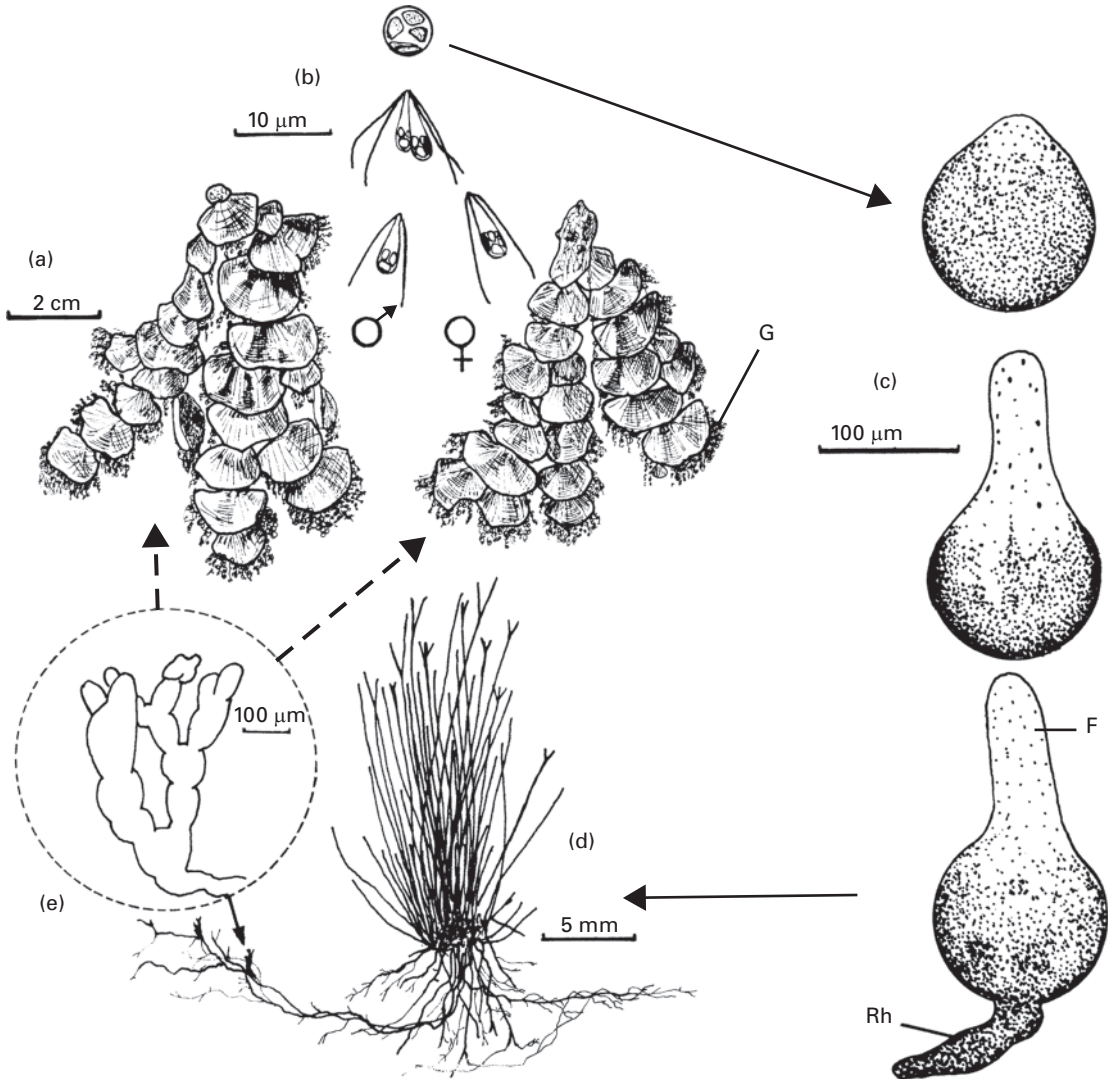


Figure 2.3 Diplontic life history and siphonous development in *Halimeda tuna*. Fertile male and female thalli (a), shown hanging downward, release biflagellate gametes from external gametangia (G) to form a zytoge (b). (c, d) Bipolar germination of the zygote leads to rhizoids (Rh) and erect free filaments (F). Subsequently (e), buds form on horizontal filaments and grow into the calcified, segmented fronts. (From Meinesz 1980; *Phycologia*, with permission of Blackwell Scientific Publications.)

form, even though, in general, gametophytes are haploid and sporophytes are diploid. Several seaweeds are known in which an apparent alternation of generations occurs with no ploidy change (e.g. *Petalonia* and *Scytosiphon*, Kapraun and Boone 1987). *Desmotrichum* can form filamentous microthalli or parenchymatous macrothalli, depending on temperature and day length. Also in response to temperature and day length, *Elachista stellaris* alternates between a diploid macrothallus, which produces meiotic spores, and a

microthallus that can reproduce the macrothallus by spontaneous diploidization. Diploidization has also been reported in *Boergesenia forbesii*, which has an isomorphic life history (Beutlich *et al.* 1990); in this case it results in a preponderance of diploids in the population. Polyploidy can be developed in *Gracilaria tikvahiae* mutants; polyploid tetrasporophytes (e.g. 3*n*, 4*n*) were found to be robust, but polyploid gametophytes (again, 3*n*, 4*n*) were stunted (Zhang and van der Meer 1988; sec. 1.4.2). Endopolyploidy (chromosome division without nuclear division) is apparent for *Saccharina latissima* (formerly *Laminaria saccharina*) and *Alaria esculenta*, and for vegetative sporophytes of both species the nuclear DNA content ranged from 2C to 16C (Garbary and Clarke 2002).

Variations in these basic patterns include several reproductive shortcuts (Fig. 2.1). In sporangia that are expected to be meiotic, such as red algal tetrasporangia and brown algal unilocular sporangia, spores may be formed by mitosis instead, giving rise to more seaweeds of the same ploidy level; this is called apomeiosis (e.g. *Ectocarpus*, Fig. 2.4). For instance, *Dasya*

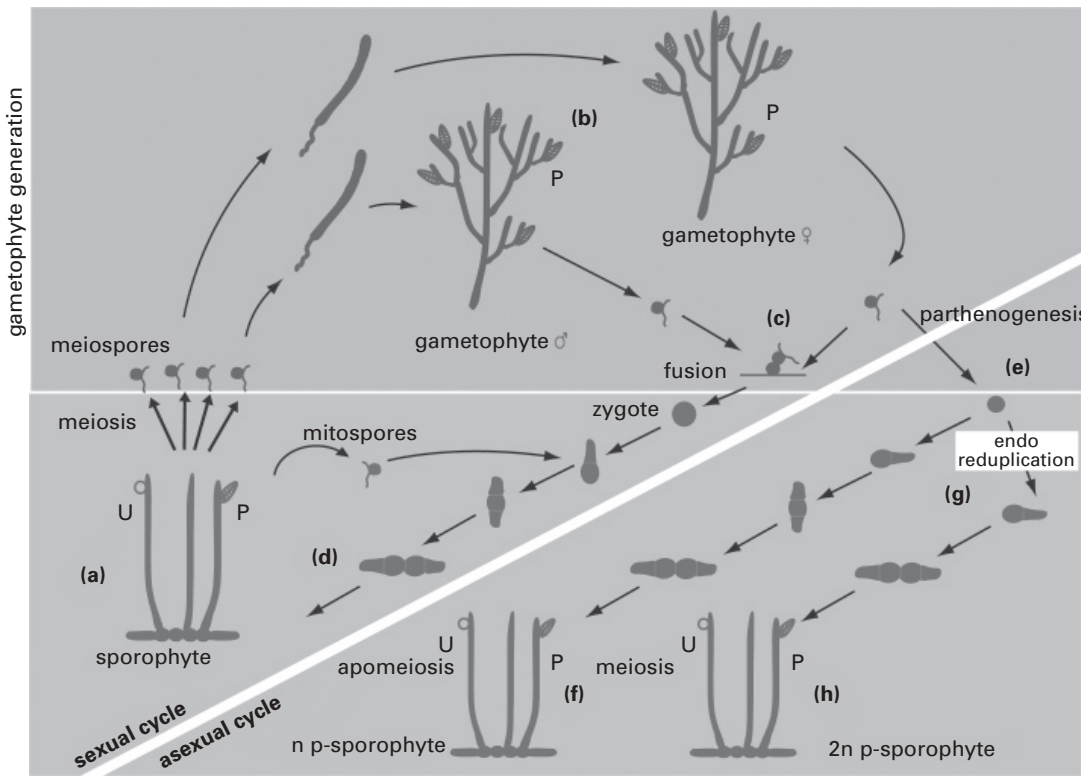


Figure 2.4 The life cycle of *Ectocarpus*. (a) Mature sporophytes produce upright filaments, which develop unilocular (U, single-compartment) sporangia. The first cell division in unilocular sporangia is meiotic and followed by a number of mitoses to give around 100 male and female meiospores. No septa are laid down between these daughter cells. (b) Meiospores grow into the dioecious gametophyte generation which has only one reproductive structure, the plurilocular (P, many compartments) gametangium, in which male or female gametes are produced through mitosis. (c) Gametes can fuse to form a zygote that grows into (d) a heterozygous sporophyte or, alternatively, (e) can develop parthenogenetically to give rise to haploid parthenosporophytes. (g) Endoreduplication may occur in a proportion of parthenosporophytes, giving rise to (h) diploid parthenosporophytes. Sporophytes and parthenosporophytes may also produce plurilocular sporangia, in which spores are formed by mitosis (= mito-spores) and grow as clones of the parent. (From Bothwell *et al.* 2010, reproduced with permission.)

ocellata has a sexual life history in Southern Portugal with tetrasporophyte and gametophyte generations, whereas in Northern Ireland the populations are apomeiotic tetrasporophytes (Maggs 1998). Five types of complications can arise in red algal life histories (Maggs 1988; see also Hawkes 1990): (1) formation of monosporangia, bisporangia, polysporangia, parasporangia, or vegetative propagules in a species that also forms tetraspores; (2) simultaneous occurrence of gametangia and tetrasporangia (mixed-phase reproduction); (3) bisexuality in a normally unisexual species; (4) direct development of tetrasporophytes from tetraspores (exclusively or mixed with gametophytes); (5) direct development of gametophytes from carposporophytes (exclusively or mixed with tetrasporophytes).

Parthenogenesis is the development of a gamete without fertilization. For isogamous species, parthenogenesis can occur in both male and female gametes, in anisogamous reproduction it is typically the female gamete, and in oogamous reproduction it is the female egg that develops parthenogenetically (Oppliger *et al*. 2007). Parthenogenesis is rare in red algae (Kamiya and West 2010) but widely reported in laboratory studies of brown and green seaweeds: within the orders Laminariales (Gall *et al*. 1996; Oppliger *et al*. 2007), Fucales (Maier 1997; Clayton *et al*. 1998), and Ectocarpales (Bothwell *et al*. 2010), and for green seaweeds, *Ulva* (Stratmann *et al*. 1996) and *C. fragile* ssp. *tomentosoides* (Kapraun and Martin 1987; Prince and Trowbridge 2004). However, its occurrence in natural populations and ecological significance have yet to be determined (Oppliger *et al*. 2007). Apospory is a process whereby diploid gametophytes are produced directly by sporophyte cells (i.e. without spores). Thus apospory differs cytologically from somatic meiosis in that no ploidy change occurs, but the morphological effect is the same. Apogamy is the production of haploid sporophytes directly from gametophyte cells, and it differs from spontaneous diploidization in having no ploidy change. Apospory and apogamy are detectable only in heteromorphic life histories, such as those of *Alaria crassifolia* (Nakahara and Nakamura 1973), *Desmarestia* species (Ramirez *et al*. 1986) and a number of red seaweeds (Murray and Dixon 1992).

Ectocarpus siliculosus, including mutant strains, is being used as a model alga to elucidate life-cycle events and gain a genetic understanding of the variable relation between ploidy level and morphology, a line of inquiry that will lead ultimately to the roles and origins of alternating generations. The basic life cycle of *E. siliculosus* is that of an alternation between sporophyte and gametophyte, but there are many developmental alternatives (see Charrier *et al*. 2008 who review D. Müller's extensive contributions; Peters *et al*. 2008; Bothwell *et al*. 2010). Sporophytes can form either plurilocular sporangia that mitotically produce spores (mitospores) that give rise to genetically identical sporophytes, or they can produce unilocular sporangia within which meiosis gives rise to 1*n* meiospores which germinate to give the male and female gametophyte generation (Fig. 2.4). The gametophytes produce plurilocular gametangia that release gametes, which typically fertilize to become a heterozygous diploid sporophyte. However, for any un-fused gametes there are two possible developmental pathways: (1) they can form haploid sporophytes parthenogenetically (*n*-parthenosporophytes) which themselves can form either plurilocular sporangia and mitospores, or unilocular sporangia by apomeiosis; or (2) they undergo endoreduplication (i.e. nucleus divides without cell division) which results in 2*n*-parthenosporophytes (Fig. 2.4). Diploid sporophytes can also be produced from tetraploid sporophytes (Coelho *et al*. 2007). This "extreme developmental plasticity" of *Ectocarpus* is controlled genetically and not by the ploidy level of a particular stage in the life cycle (Bothwell *et al*. 2010). Using mutants, a regulatory locus "Immediate upright" (*IMM*) that controls aspects of the sporophyte developmental program was identified (Peters *et al*. 2008), and Coelho *et al*. (2011) found the "master regulator" *OUROBOROS* which controls the transition from gametophyte to sporophyte, probably by repressing the gametophyte developmental program.

Haplodiplontic life cycles are thought to have evolved repeatedly within each seaweed lineage and therefore the life cycles should represent adaptations to particular environments (Bessho and Iwasa 2010). A question that has received much attention is why both generations persist, and various genetic and

ecological theories have been forwarded (e.g. Bell 1997; Hughes and Otto 1999; Bessho and Isawa 2009, 2010). Genetic models include: (1) DNA damage can be repaired only in diploids favoring their retention in the life cycle; (2) Deleterious mutations accumulate in the diploid phase but because they are often recessive their effect is masked; in the haploid generation such deleterious alleles are selectively eliminated; (3) Diploids will favor the accumulation of advantageous mutations; (4) Parasites may prefer diploid hosts. Some ecological models that have been proposed are: (1) Haploids are often smaller and therefore have lower nutrient and energetic requirements, including lower energetic costs of DNA replication; (2) For heteromorphic alternations, having both haplontic and diplontic generations means two niches can be inhabited at once, thereby exploiting differences in the environment (e.g. light, temperature), or escaping herbivory (sec. 4.3.2). Bessho and Iwasa (2009) suggest that a heteromorphic alternation of generations is better adapted to environments with marked seasonality than isomorphic life cycles. Results from "The *Porphyra* Genome" project indicate that cytoplasmic ribosomal proteins (RPs) are differently expressed in the haploid conchocelis phase compared to the diploid bladed phase; it is hoped that this line of inquiry will provide further insights into both the developmental regulation of life-history phases, and the biochemical mechanisms underpinning niche adaptation of different life-history phases (Chan *et al.* 2012a).

Another interesting question is how in some isomorphic seaweeds one phase is numerically dominant over the other (e.g. Van der Strate *et al.* 2002b; Scrosati and Mudge 2004). Fierst *et al.* (2005) reported 34 species of red algae for which one phase was dominant within the population compared to the alternate phase (see their Table 2). There are also differences between orders, with gametophytes dominating the Gigartinales while tetrasporophytes prevail in the Gracilariales and Ceramiales (Thornber 2006). Reasons for these disparities require testing but candidate theories include differential reproductive output of the different phases and differential susceptibility to various biotic and abiotic factors (Thornber and Gaines 2004; Fierst *et al.* 2005). Two studies support the latter hypothesis. Thornber *et al.* (2006) found that the snail *Chlorostoma funebralis* (previously *Tegula funebralis*) much preferred gametophytes of *Mazzaella flaccida* (previously *Iridaea flaccida*) compared to sporophytes, because the carposporphytes on the gametophytes are large and protrude further out from the blade surface, making them easier to graze. Verges *et al.* (2008) found that the sea hare *Aplysia parvula* preferentially grazes male gametophytes of *Asparagopsis armata*, whereas the female cystocarps are strongly chemically defended and consumed much less, explaining why the sex ratio of *Asparagopsis* gametophytes is 1:1 early in its growth season but biased towards females (70%) later in the year.

2.3 Environmental factors in life histories

The life history of a species is a continuous interaction between the organism and its biotic and abiotic environments (Fig. 2.5). A seaweed begins life as a single undifferentiated cell, with the potential to produce the whole organism through the expression of its genetic information. The genotype interacts with the environment to produce the phenotype. The environment of a cell consists of the physical and chemical influences of the other cells in the seaweed, plus the environment of the seaweed itself. The environmental history of a seaweed, because it affects growth and form, in a sense becomes recorded in its body (e.g. Waaland and Cleland 1972; Niklas 2009). Thus individual seaweeds of the same genotype that are grown under different environmental conditions will grow into phenotypically distinct individuals.

The switch from vegetative growth to reproduction (which in most seaweeds involves very little growth) often depends on environmental factors such as temperature and light (Lüning and tom Dieck 1989; Lüning 1990; Santelices 1990). Kelp gametophytes, for example, may reproduce when they are only a few cells in size, or they may grow vegetatively almost indefinitely, depending on light quality and quantity (reviewed by Bartsch *et al.* 2008). They have been used for studies of minimum irradiance requirements for growth and reproduction because of their extreme

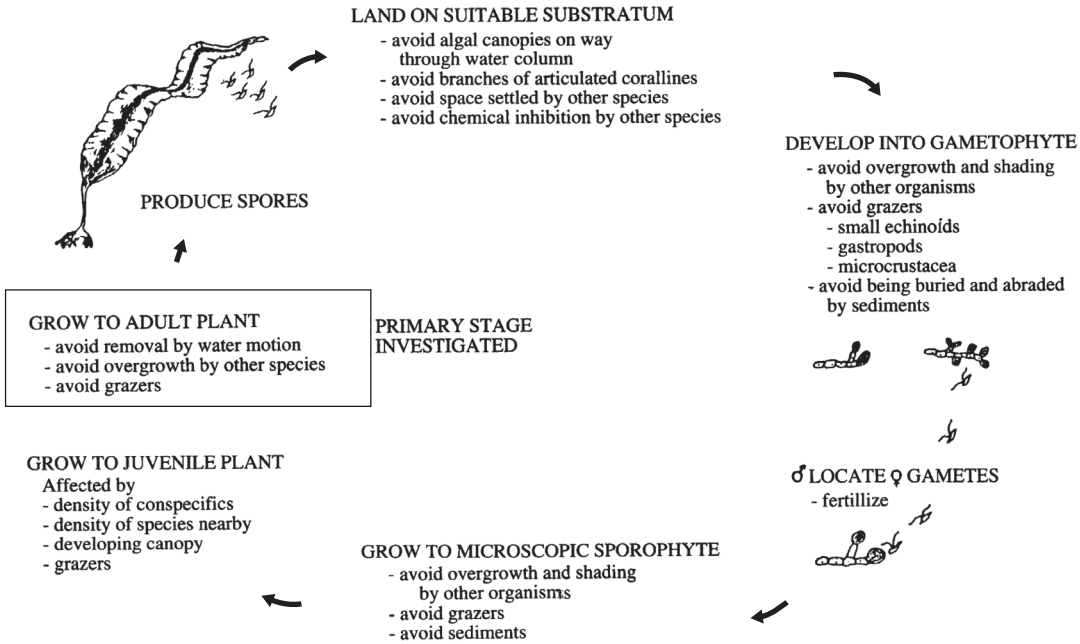


Figure 2.5 Life history of a laminarian alga, showing some of the major (chiefly biotic) environmental hazards that must be overcome at each stage. In addition, success will be affected by abiotic factors such as light, temperature, water motion. (From Schiel and Foster 1986, *Oceanogr. Mar. Biol. Rev.*, with permission of Aberdeen University Press, Farmers Hall, Aberdeen, AB9 2XT, UK.)

shade environment and ease of culture. A pre-requisite for growth, of course, is that the energy trapped and carbon fixed must exceed the totals used in respiration. Chapman and Burrows (1970) showed that development of *Desmarestia aculeata* gametophytes depends on the mean daily irradiance (i.e. (irradiance × photoperiod)/24). At the lowest irradiances tested, gametophytes did not mature, though they survived and were able to develop later when irradiance was increased. More detailed studies by Lüning and Neushul (1978) showed that various kelp gametophytes were saturated for vegetative growth at 4 Wm^{-2} (about 20 μmoles photons m^{-2} s^{-1}; see sec. 5.2.1 for explanation of units) but required two to three times that irradiance for reproduction. Blue light, alone or as part of white light, is required for kelp gametogenesis; in red light, gametophytes grow only vegetatively. The ability of these algae to grow vegetatively in extremely dim light and reproduce only when irradiance increases provides a mechanism for populations to retain space after the canopy of parent sporophytes is lost. The effects of light wavelength on photosynthesis is discussed further in Chapter 5, and the importance of environmental factors in seaweed aquaculture are discussed in Chapter 10.

2.3.1 Seasonal anticipators and responders

One of the most important ways in which algae (and all organisms) respond to their environment is in the timing of reproduction, because in reproduction lies the key to the survival of the species (Santelices 1990; Pearson and Serrão 2006). Reproductive responses to the environment are particularly evident in algae with strongly heteromorphic generations, such as kelps and *Pyropia*, where different growth forms are adapted to

different environments. When conditions are suitable for the growth of one form, vegetative growth or asexual propagation is likely to occur, whereas conditions poor for that form are likely to prompt a reproductive switch to the alternative morphology. However, because of the lead time sometimes needed for reproduction, some seaweeds may need to anticipate the changes in seasons, using an appropriate cue. A useful framework for categorizing the ability of a seaweed to modify its reproductive status in response to an environmental cue is that of "anticipators" and "responders", terms coined by Kain (1989); Lüning and co-workers (e.g. Lüning 1991; tom Dieck 1991) had the same idea and termed them Types II and I, respectively (Kain 1989).

For anticipators, seasonal patterns of growth and reproduction are controlled by a free-running endogenous clock. In the absence of environmental cues, the free-running rhythm is slightly different from that observed in nature (e.g. 9–10 months for *Laminaria hypoborea*), but is entrained by environmental cues, termed "Zeitgebers". In seaweeds, light is an important Zeitgeber (Lüning 1994; reviewed by Bartsch *et al*. 2008), and for animals and other algae e.g. *Euglena* and the dinoflagellate *Gonyaulax*, temperature or nutrients are also Zeitgebers (e.g. Roenneberg and Mittag 1996; Rensing and Ruoff 2002). The maximum growth rate of anticipators may occur when environmental conditions do not seem optimal. For the red seaweed *Delesseria sanguinea*, this is in late winter when light levels are low, and the growth rate declines in summer (Kain 1989). Another example is that of the perennial brown seaweeds of Antarctica, *Desmarestia anceps*, *D. menziesii*, and *Himantothallus grandifolius*, for which reproduction and sporophyte development occurs in winter (Wiencke 1990a; Wiencke *et al*. 2009). Seasonal "responders" on the other hand sense and respond directly to the prevailing environment, and seasonal patterns of growth and reproduction are not governed by a circannual endogenous clock. Examples of responders include growth of the giant kelp *Macrocystis pyrifera* and the red seaweed *Plocamium cartilagineum* (Kain 1989; Reed *et al*. 1997).

A major criterion used to demonstrate the presence of an endogenous rhythm is "self-sustainment for more than one cycle in conditions giving no seasonal information" (Schaffelke and Lüning 1994), and such patterns have been observed in various kelp including *Pterygophora californica*, *Laminaria setchellii*, and *Laminaria hyperborea* (Lüning 1991, tom Dieck 1991; Schaffelke and Lüning 1994). Sporophytes of *L. hyperborea* were grown for 2 years under constant conditions of light, photoperiod (12:12), nutrients and temperature and yet they still continued the seasonal patterns of blade growth observed in the field, with periods of fast growth followed by periods of zero growth. The Zeitgeber that entrained the endogenous clock was photoperiod, and this was clearly shown when the experimental year was shortened from 12 months to 6 or 3 months: the same seasonal pattern occurred, but instead of one growth period per year there were 2 or 4, respectively. An endogenous pattern of reproduction has been shown for *Dictyota dichotoma* (Müller 1963) and *Ulva pseudocurvata* (Lüning *et al*. 2008). The biochemical and molecular mechanisms underlying circannual endogenous clocks are not currently known for seaweeds (see Bartsch *et al*. 2008, p. 36).

For both seasonal responders and anticipators, environmental factors modulate growth and the onset of reproduction, and can trigger changes from one life-history phase to another. Modulating factors include temperature, light (photon dose, photoperiod, wavelength), nutrients, lunar and tidal cycles, desiccation, salinity, water motion, and biological factors including grazing and bacteria (Santelices 1990; Pearson and Serrão 2006). These factors do not act in isolation and Bartsch *et al*. (2008) sum up the effects of environmental factors on reproduction in *Laminaria*: "tissue location, temperature, irradiance as well as competition and life strategy modify reproductive output, for no simple parameter is the sole decisive trigger".

2.3.2 Temperature

Although temperature seems an obvious seasonal cue in middle to high latitudes, it should be remembered that changes in seawater temperature are directly related to the amount of light reaching the sea (which causes warming), and there is often an inverse relationship between inorganic nitrate and temperature:

thus culture studies are required to confirm if correlations with temperature are directly triggering a response, or acting indirectly. Kain's (1989) analysis of seasonal patterns of reproduction in subtidal seaweeds revealed few direct responses of temperature on seasonality of growth and reproduction, but she recognized that temperature is a key factor controlling biogeography, and the ability of seaweeds at their geographic limit to reproduce (sec. 7.3). Temperature can affect reproduction through its effects on metabolic rates. For example, in *Fucus* it may influence the timing of reproduction by speeding up gamete development; Ladah *et al.* (2008) suggest that at lower temperatures the oogonial sheath remains for longer and this results in a reduced dispersal of eggs. Similarly the time taken for sorus induction of *Laminaria* is temperature dependent, and it has been suggested temperature and photoperiod together could allow kelps to distinguish between spring and fall (Lüning 1988, see Bartsch *et al.* 2008). A further example is that of the Antarctic red seaweed, *Iridaea cordata*, for which the gametophytes take 21 months to form reproductive structures at typical Antarctic seawater temperatures of 0°C, whereas in sub-Antarctic waters of 5°C it takes just 12 months (Wiencke 1990b).

Nevertheless, some differences in the kinds of reproduction at different temperatures have been noted in seaweeds. Müller (1963) found that *Ectocarpus siliculosus* produced unilocular sporangia at 13°C and plurilocular sporangia at 20°C, although for other strains this is not temperature dependent (Charrier *et al.* 2008). Changing the temperature in which *Myriotrichia clavaeformis* sporophytes are grown determines whether sexual or asexual organs are produced, whereas for gametophytes the effect is triggered by photoperiod (Peters *et al.* 2004b). The formation of erect thalli in some isolates of *Scytosiphon lomentaria* var. *complanatus* (currently *Scytosiphon complanatus*) studied by Correa *et al.* (1986) was dependent on temperature and independent of photoperiod (Table 2.1), in contrast to the better known photoperiodism of the typical variety, as discussed later. (In other isolates of *S. lomentaria*, this morphogenetic switch apparently is not responsive to either temperature or photoperiod.) In some species, different steps in reproduction have different temperature optima. In the conchocelis stage of *Pyropia tenera* (previously *Porphya tenera*) in Japan, the temperature optimum for monosporangium formation is 21–27°C, whereas for monospore release it is 18–21°C (Kurogi and Hirano 1956; see also Dring 1974). Chen *et al.* (1970) found that conchosporangia of *Wildemania miniata* (previously *Porphyra miniata*) from Nova Scotia were formed at higher temperatures (13–15°C in this case), but conchospores were released only with low temperatures (3–7°C) and short days. Such interactive effects of light and temperature on seaweed reproduction appear common, for example: *Desmarestia firma* (Anderson and Bolton 1989), *Helminthocladia stackhousei* (previously *Helminthora stackhousei*; Cunningham *et al.* 1993), *Liagora californica* (Hall and Murray 1998), *Hydropuntia cornea* (previously *Gracilaria cornea*; Orduña-Orjas and Robledo 1999), *Lessonia variegata* (Nelson 2005). In some cases the responses are quantitative (e.g. higher fertility at lower temperatures), in others qualitative (i.e. fertile vs. nonfertile) (Maggs and Guiry 1987). For instance, the initiation of growth of the macrothalli of *Dumontia contorta* is strictly controlled by day length, but the initials do not grow out unless the temperature is less than 16°C (Rietema 1982).

Table 2.1 Influences of temperature and day length on formation of upright fronds by *Scytosiphon complanatus* (previously *Scytosiphon lomentaria* var. *complanatus*) in Nova Scotia.

Temperature (°C)	Day length (h)	Crusts with uprights (%)
0	14	100
5	14	100
10	8	100
10	12	100
10	16	100
15	12	3.8
15	16	0.3
20	12	0
20	16	0

Source: Correa *et al.* (1986), with permission of Blackwell Scientific Publications.

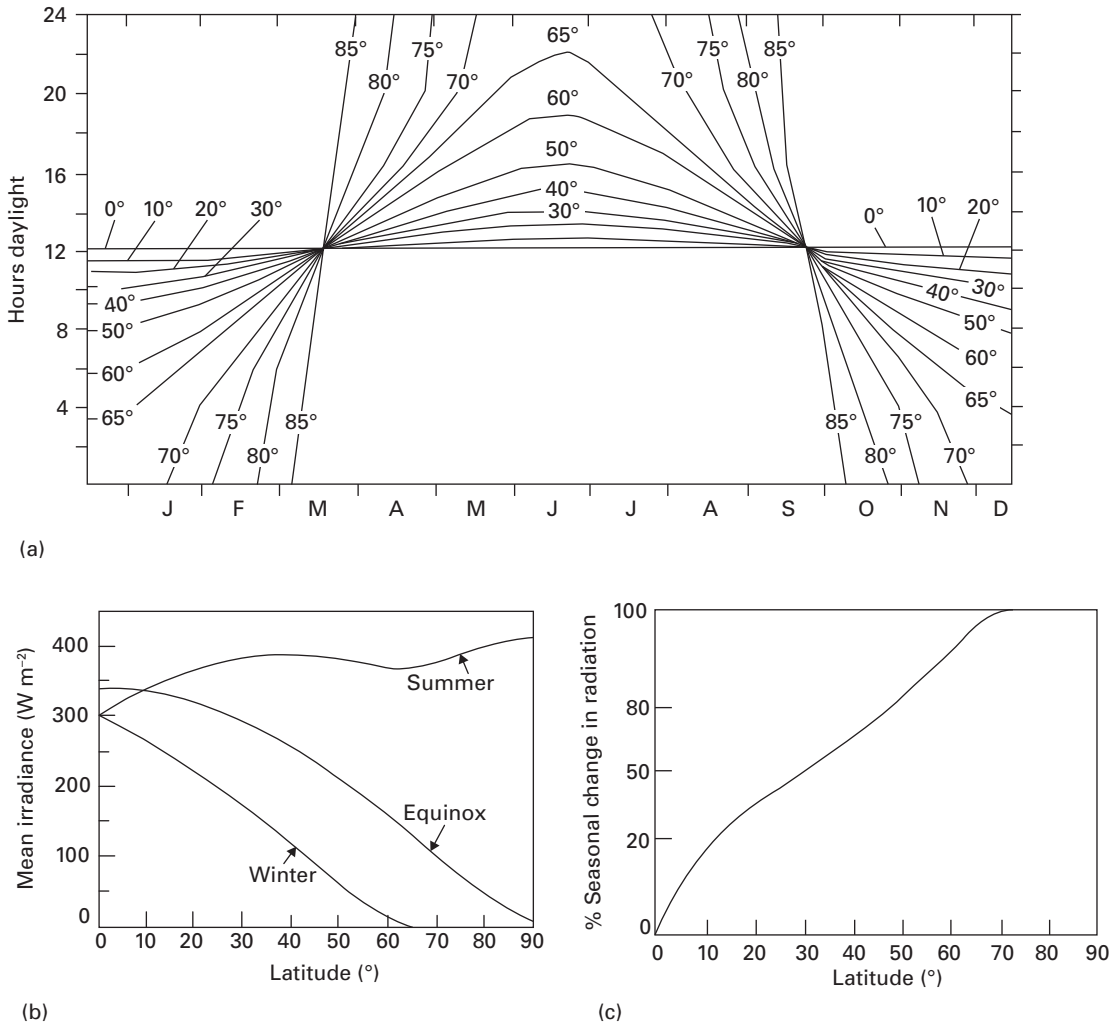


Figure 2.6 Variations in day length and irradiance at different latitudes. (a) Daylight hours in the northern hemisphere (southern hemisphere values may be obtained by 6-month transposition of the abscissa scale). (b) Mean energy flux with a cloudless sky for the months containing the equinoxes and solstices (mean values for both hemispheres). (c) Percentage seasonal change in energy flux with a cloudless sky (recalculated from b). (Part a from Drew 1983, with permission of Clarendon (Oxford University) Press; b and c from Kain 1989, with permission from British Phycological Society.)

2.3.3 Light: photoperiod and wavelength

Although temperatures are involved in reproductive cues, temperatures may undergo seasonal cycles, and such cues are erratic. A more dependable seasonal cue is day length (photoperiod) (Fig. 2.6). As one progresses to farther northern and southern latitudes, day length changes becomes increasingly pronounced, and it has been known since the 1930s that flowering plants respond to photoperiod. Some plants flower when days are long (LD plants) or short (SD plants),

others require a specific sequence of long days followed by short days (long-short-day plant), or short days followed by long days (short-long-day plant), while others are insensitive to day length i.e. day-neutral (e.g. Taiz and Zeiger 2010). Notwithstanding these names, plants actually measure the length of uninterrupted night, not day length (Buchanan *et al.* 2000). Photoperiodic responses were expected in temperate seaweeds, but not until 1967 was a true photoperiodic response demonstrated, for the conchocelis phase of *Porphyra* (Dring 1967). For terrestrial plants, the biochemistry and molecular biology of photoperiodic responses are well understood (Buchanan *et al.* 2000) but we know little of these processes in seaweed.

Most seaweeds that show photoperiodism are SD. Dring (1988) suggested that this bias could be due to inadequate controls and that there was no reason to suppose that algae would respond more often to SD than LD. Nevertheless, only a few studies demonstrate LD responses (see Pang and Lüning 2004). Higher plants, green algae, cyanobacteria, and fungi, have a family of photoreceptors, the phytochromes, which detect light in the red region of the spectrum and are involved in their systems for measuring and responding to light/dark cycles (Mathews 2006; Sharrock 2008). The presence of phytochromes has not been confirmed for red and brown seaweeds, but phytochrome-like proteins have been detected in *Corallina* and *Gelidium* and responses to red/far-red light suggest its presence in *Porphyra* (López -Figueroa *et al.* 1989; Figueroa *et al.* 1994; see Kain 2006). Red-light effects could occur through red light absorbed by phycobiliproteins (in red algae), which have structures very similar to that of phytochrome (reviewed by Rüdiger and López–Figueroa 1992).

Photoperiodic effects have to be distinguished from the effects of the total irradiance received, which also changes seasonally (Fig. 2.6b,c) and can have strong effects on seaweed growth and development. For example, some kelp and *Desmarestia* gametophytes, have minimum requirements for accumulated total daily irradiance or a certain irradiance intensity in order to reproduce (Chapman and Burrows 1970; Lüning and Neushul 1978; Wiencke 1990a). The classic means of demonstrating a true photoperiodic effect in terrestrial plants is the nightbreak experiment, in which a long night (e.g. 16 h) is broken by a short exposure to weak light; night breaks show that the alga is counting time rather than an accumulation of photosynthate over 24 h (Lüning *et al.* 2008). For flowering in terrestrial plants, the length of night break and the timing during the dark period both influence the effectiveness of the break on initiating flowering. The effect on SD plants is to spoil the inductive effect; the plant measures two short nights and a LD response is triggered. In LD plants, a night break is usually inductive. Converse experiments, either (1) with a long day (16 h) broken by a short dark period and a regular night, or (2) with the cycle extended (e.g. to 32 h) to give a long night and a long day, are inductive in SD plants, just as is a regular light:dark 16:8-h day. Phytochrome has two alternate forms which absorb red light (Pr) and far-red light (Pfr), respectively, and when these wavelengths are given in sequence in a night break, the last one determines the effect: red light spoils the long night, but far-red light does not (and even counters the effect of red light). The night-break technique remains a widely used tool for screening plants and seaweeds for photoperiodism (Hwang and Dring 2002; Taiz and Zeiger 2010). However, some seaweeds that have SD photoperiodic responses are insensitive to a night break and other pigment systems may be involved (Rüdiger and López-Figueroa 1992). Therefore, rigorous application of the night break as a diagnostic tool for photoperiodism might obscure photoperiodic responses that are mediated by physiological mechanisms other than phytochrome (Dring 1988; Hwang and Dring 2002).

There are also many records of blue-light effects, such as the formation of uprights in *Scytosiphon* (Fig. 2.7) (Dring and Lüning 1975; see reviews by Dring 1984a, 1988; Rüdiger and López-Figueroa 1992). Tetrasporangium formation in *Rhodochorton purpureum* takes place during short days and is inhibited by a night break of red light but not far-red light, and yet the red-light inhibition is not reversed by subsequent exposure to far-red light (in contrast to the case with flowering plants). Moreover, a night break by blue light is also inhibitory (Dring and West 1983). Eggs are released from oogonia of *Dictyota* when blue light is

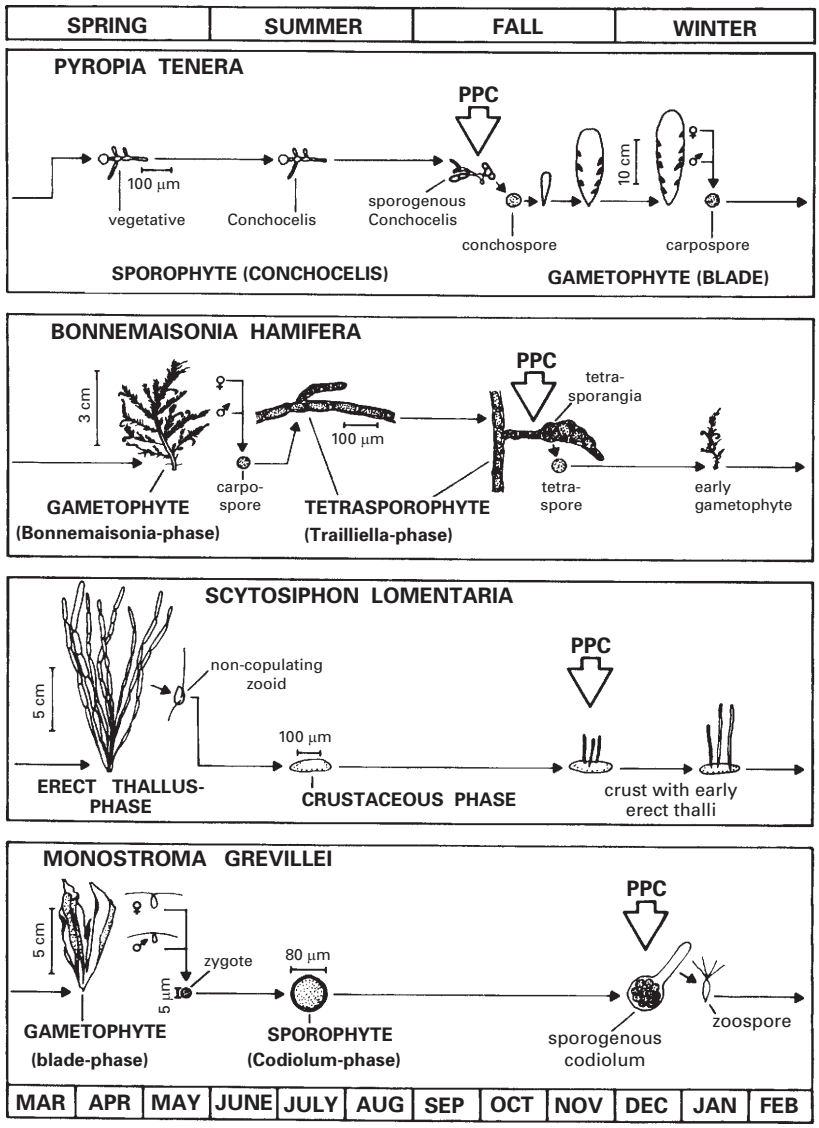


Figure 2.7 Annual cycles of four short-day algae. PPC, short-day signal. The responses are as follows: *Pyropia tenera* (previously *Porphyra tenera*) forms conchospores; *Bonnemaisonia hamifera* forms tetrasporangia; *Scytosiphon lomentaria* (in Europe) forms new erect thalli from the crust; *Monostroma grevilleri* (codiolum phase) forms zoospores. (From Dring and Lüning 1983, *Encyclopaedia of Plant Physiology*, new series, vol 16B, with permission of Springer-Verlag, Berlin.)

switched on (*Dictyota*), whereas egg release by *Laminaria* is inhibited by blue light (Dring 1984a; Fig. 2.8). Blue-light effects (see Table 2.3) are typically attributed to "cryptochromes", which are a sub-group of the large cryptochrome/photolyase (CPF) family of blue-light sensitive flavoproteins, and found in animals, cyanobacteria, the unicellular red algae *Cyanidioschyzon merolae*, unicellular green algae, terrestrial plants,

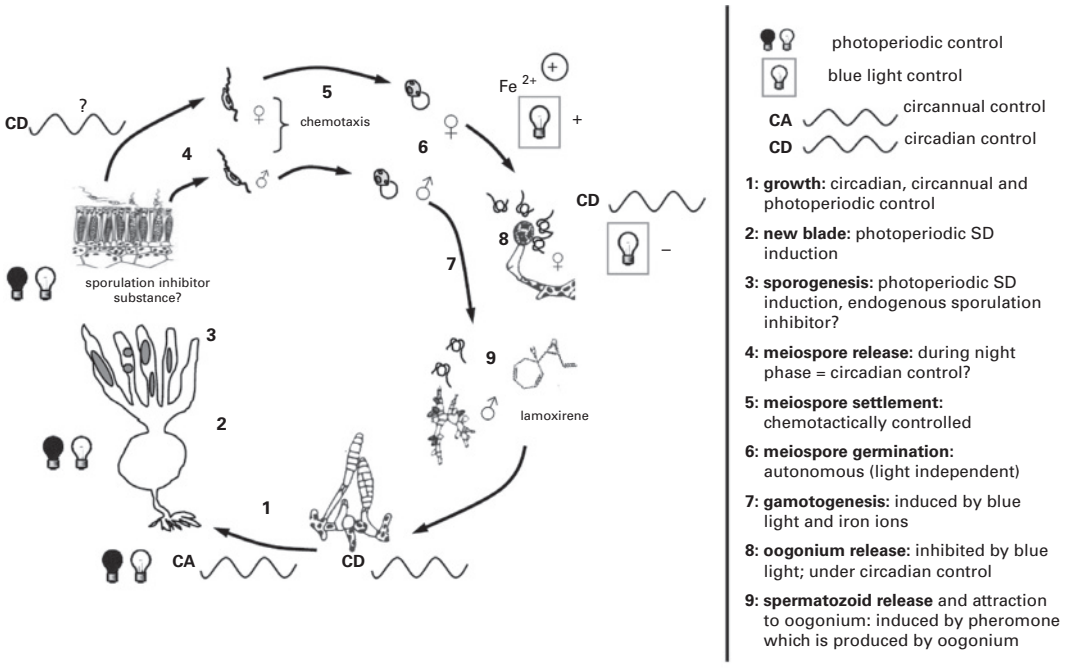


Figure 2.8 Schematic representation of life-cycle control in *Laminaria sensu lato* by abiotic and endogenous factors assuming that regulation processes are similar within the genus. (From Bartsch *et al.* 2008, reproduced with permission.)

and diatoms (e.g. Cashmore 2005; Asimgil and Kavakli 2012). Plant cryptochromes (plant CRY) regulate circadian rhythms and growth. The question of how CPFs evolved to perform a very wide range of physiological roles in prokaryotic and eukaryotic lineages is receiving much attention. However, although CPFs have been found in the red and brown lineages, and cryptochromes have been implicated in blue (and green) light detection in red seaweeds (Kain 2006), experimental validation of their existence in these seaweeds is required.

Other red- and blue-light photoreceptors have been found in seaweeds (Hegemann 2008; see sec. 1.3.3). The filamentous green seaweed *Mougoutia* uses neochrome to detect the red/far-red and UV-A/blue regions of the spectra (e.g. Suetsugu *et al.* 2005). The blue-light receptor aureochrome has been found in *Fucus*, *Vaucheria*, and the diatom *Thalassiosira* suggesting that it is common to the photosynthetic stramenopiles (Takahashi *et al.* 2007; Ishikawa *et al.* 2009). Dring (1988, p. 169) concluded that "photoperiodic responses in algae may be controlled by a variety of pigment systems analogous to the variety of pigments involved in algal photosynthesis". The application of molecular tools to identify candidate genes involved in photoreception, and of methodologies developed for photoperception and photocontrol in higher plants, would help advance this field for seaweeds.

Many seaweeds exhibiting photoperiodic control over reproduction have heteromorphic life histories,

in which the algae use the cue to switch to a different phase, assumed a priori to be better adapted to the conditions in the next season. Best studied are species of Laminariales, which are strongly heteromorphic (Bartsch *et al.* 2008; Figs 2.5 and 2.8). *Laminaria* sporophytes grow vegetatively under long days under the control of circadian and circannual rhythms and photoperiod; new blade production is triggered by SD. The onset of sporogenesis is cued by SD and sori form on the older, distal tissue; this process may also involve a "sporulation-inhibiting substance" released by meristematic tissue but not distal tissue (also see *Ulva* later). The microstages of the life cycle are not under photoperiodic control. Meiospore germination is dependent on light dose, whereas gametophytogenesis is triggered by a specific dose of blue light, which means that when the sporophyte canopy is removed, increased blue light triggers gametogenesis. Iron (Fe^{2+}) also induces gametogenesis, while other nutrients (nitrogen, phosphorus) modulate the life cycle but do not act as a cue (Fig. 2.8).

Sporophyte development by *Undaria pinnatifida* is a rare example of a long-day photoperiodic response (Pang and Lüning 2004). This is a "facultative" LD response whereby LD triggers sporophylls to develop, but in the absence of a LD signal, sporophylls will eventually develop under a SD regime. *Undaria* are winter annuals with sporophyll production occurring in spring (i.e. longer days), and so the LD response clearly matches this seasonal cycle. The formation of hairs on the blade surface are also triggered by LD and these probably enhance nutrient acquisition during spring (see secs. 2.6.2 and 6.4.2).

Early field studies on *Ulva* on the Pacific coast of the United States showed a periodicity of propagule release, with gametes being released at the beginning of the spring tide series and spores 2–5 days later (Smith 1947). Over 30 years later, the endogenous, environmental and biochemical control of these cycles are being elucidated (Lüning *et al.* 2008). In a field experiment that lasted 2 years, *Ulva pseudocurvata* was shown to release gametes for a 1–5-day period every 14 days in fall and winter, and every 7 days in summer. In the laboratory, *U. pseudocurvata* has a free-running "sloppy" cycle of gamete release about every 7 days, that can be synchronized to a 1-month cycle by providing artificial moonlight. The difference between the summer (7-day) and winter (14-day) cycles may be due to insufficient light in winter to supply the energy required for reproduction. Gamete release occurs only after a minimum of 1 h darkness (quantified as < 0.001 μmole photons m^{-2} s^{-1}), followed by 5–9 minutes of light, explaining the sunrise release of gametes observed in the field. Red and blue wavelengths were equal in triggering gamete release, and gametophytes were more sensitive to these wavelengths (PDF of 0.01 μmole photons m^{-2} s^{-1}) than green light (0.1 μmole photons m^{-2} s^{-1}); the ability to detect such low PFDs suggests highly sensitive photoreceptors (phytochromes, cryptochromes, and rhodopsins). Gametes are only released from the marginal tissue, whereas tissue close to the holdfast is purely vegetative: this is explained by a "swarming inhibitor" (see below, Stratmann *et al.* 1996). Another finding of this study is that *Ulva* gametophytes become progressively smaller as the seasons progress from fall to winter, as they "sporulate away their thallus toward winter in a controlled way governed by the annual course of day length" (Lüning *et al.* 2008, p. 871). This is a photoperiodic SD response, because a night break spoils the effect. In winter, a perennial holdfast remains and therefore *Ulva pseudocurvata* is not an annual, it is a pseudo-perennial, with an overwintering holdfast that grows a new blade again in spring.

In *Ulva mutabilis* three regulatory factors have been identified that control the life-cycle progression from vegetative growth to gametogenesis (Stratmann *et al.* 1996), confirming the suggestion of Nilsen and Nordby (1975) that vegetative thalli release substances that inhibit sporulation, and Jónsson *et al.* (1985), who found that complex glycoproteins inhibit gametogenesis. Sporulation Inhibitor-1 (SI-1) is a very high molecular mass glycoprotein associated with the extracellular matrix (ECM) proteins that is released into the surrounding medium, and acts to maintain a vegetative thallus. Concentrations of SI-1 decrease as the thallus ages and when concentrations are below a critical level, gametogenesis occurs. A second compound, SI-2, with a low molecular mass, was isolated

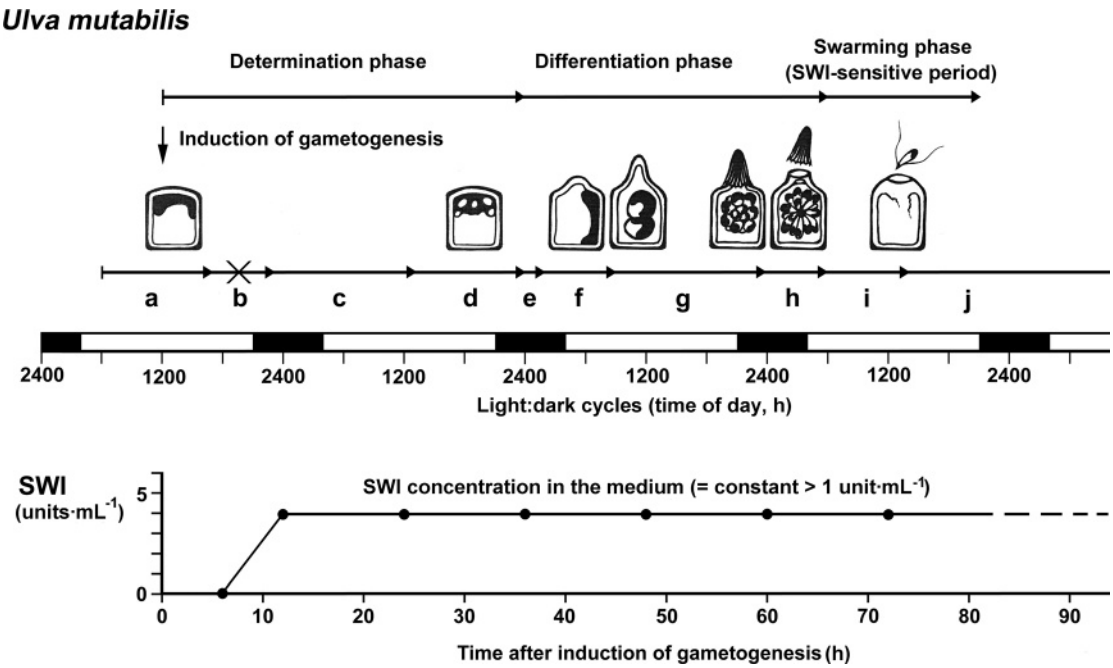


Figure 2.9 Time course (h) of induced gametogenesis and discharge of gametangia from *Ulva mutabilis* in relation to swarming inhibitor (SWI) –synthesis and action (summary of new and previous results). Top panels: Gametogenesis was induced in *U. mutabilis* [mutant slender sl-G (mt+)]. The "determination phase" and "differentiation phase" are defined as described by Stratmann *et al.* (1996). The "swarming phase" is the time period when gamete release can be induced by light or a medium change, or later may occur spontaneously. (a) Regular vegetative G1 cell-cycle phase in which gametogenesis can be induced by removal of sporulation inhibitors SI-1 and SI-2 from the medium. (b) Regular vegetative S cell-cycle phase in which the genome is replicated, normally, or, after induction of gametogenesis, the period of SWI synthesis and excretion. (c) Next G1-phase after induction of gametogenesis. (d) Next S-phase after induction of gametogenesis and accumulation of starch granules. (e) Time of irreversible commitment to gametangium differentiation. (f) Period of progamete formation, chloroplast reorientation, and papilla initiation. (g) Period of progamete multiplication to 16 cells, and papilla maturation. (h) Period of gamete and pore cap maturation. (i) Period when the exit pores are open and when gamete release can be induced by light and (or) depletion of SWI in the medium. (j) Period when the gametangia become insensitive to SWI, and gamete release may occur spontaneously and asynchronously. Bottom panel: The time course of SWI accumulation in the medium was measured in parallel to gametogenesis by the SWI assay at the times indicated in the figure in a sample containing 10 mg *Ulva* fragments · mL^{-1}. (From Wichard and Oertel 2010, reproduced with permission.)

from the space between the two cell wall layers, and is thought to act as positive regulator through interactions with SI-1 because concentrations do not change as the life cycle progresses. The conversion of vegetative cells to gametangia occurs only when SI-1 is removed: when this happens the cells enter a "determination phase" during which they can return to vegetative growth if SI-1 is added back (Fig. 2.9). The length of this phase is 23–46 h, and depends on the time of day at which induction of the cell-cycle progression was begun. Cells then enter a "differentiation phase" that is 28 h long; they are now committed to becoming gametes and are no longer susceptible to SI-1. The flexible timing of the determination phase means that irrespective of the time of day (i.e. time of cell cycle) when the life-cycle progression is induced, gamete

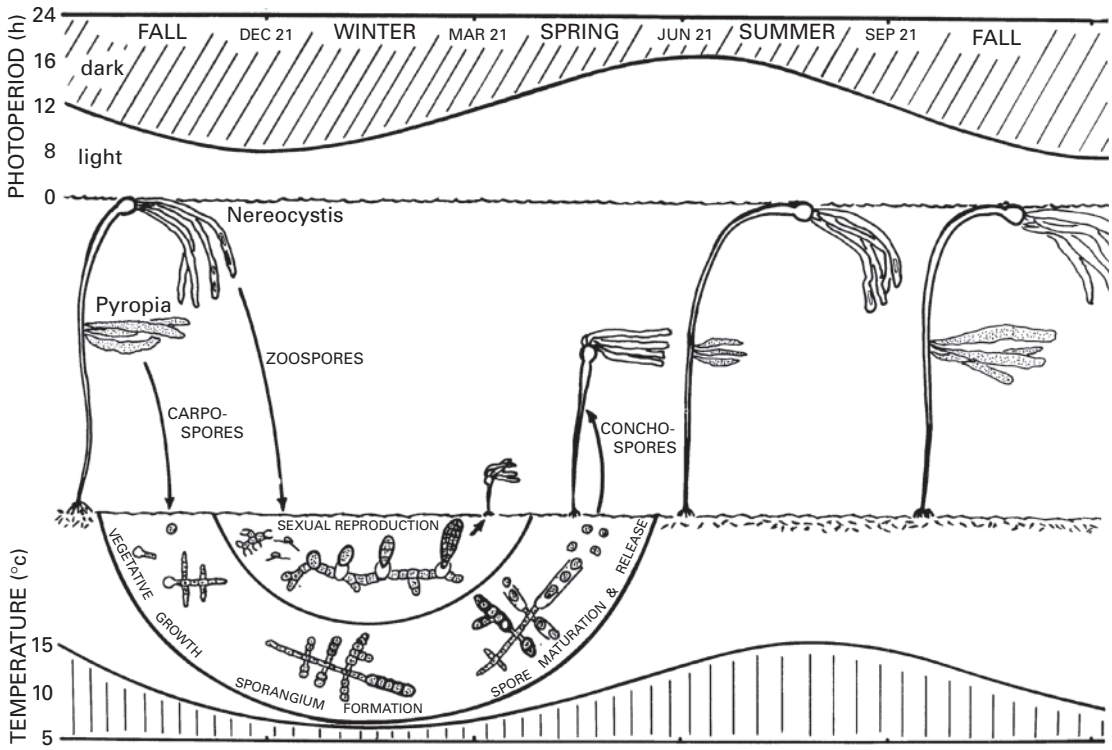


Figure 2.10 Life history and seasonal occurrence of the annual, epiphytic alga *Pyropia nereocystis* (previously *Porphyra nereocystis)* and its annual host seaweed, *Nereocystis luetkeana*. The top part of the diagram shows seasonal photoperiod variation at the Puget Sound study site; the lowest part of the diagram traces water temperatures. Carpospores from *Pyropia* blades form the shell-boring conchocelis stage, which releases conchospores in response to long days after short days, as the new annual crop of *Nereocystis* sporophytes elongates. Zoospores from *Nereocystis* form microscopic male and female gametophytes, and sexual reproduction (not photoperiodic) results in sporophytes. (From Dickson and Waaland 1985, with permission of Springer-Verlag, Berlin.)

release always occurs between 0500 and 0800 h. A third compound, a low molecular weight "Swarming Inhibitor" (SW-1) is released during the determination phase, triggering the release of motile gametes (Stratmann *et al.* 1996). SW-1 is also present in *Ulva lactuca*, although there are species-specific differences in timing of gametogenesis and its relationship to SW-1 concentrations (Wichard and Oertel 2010).

The more critical the timing of reproduction, the more complex the environmental cues need to be. Short days, for instance, occur in both fall and spring, as well as through the winter. *Pyropia nereocystis* (previously *Porphyra nereocystis*) grows on the stipes of the annual kelp *Nereocystis luetkeana*. The bladed phase of the *P. nereocystis* appears when the host stipe has completed elongation but before the stipe becomes covered in other algae. Moreover, the stipes are high in the water column, whereas the sporophyte of *P. nereocystis* is on the bottom, in old shells. To time its spore release for spring, *P. nereocystis* responds to a dual photoperiod: prolonged short days followed by prolonged long days (Fig. 2.10) (Dickson and Waaland 1985). (Tests were run at 8:16 and 16:8 light:dark photoperiods, and critical photoperiods were not

determined.) The response was also better in cooler water, typical of spring, than in warmer water, typical of fall. The conchospores are released in slime strands that may produce a "bola" effect for increasing the chances of snagging and sticking to the slippery young kelp stipes. Related bladed species have less critical photoperiodic control. For example, in *Pyropia torta* (previously *Porphyra torta)* from the same region (Puget Sound), conchospores can form in any photoperiod, but they mature and are released only when there are short days (Waaland *et al.* 1987); this species is a winter annual on rocky intertidal substrates.

"Short day" and "long day" obviously are relative terms, and for a seaweed with a wide latitudinal range, what is a short day in higher latitudes may be a long day in lower latitudes. Compare, in Figure 2.11, for instance, the effects of 11-h days on *Scytosiphon* from Tjörnes (66ºN) and from Punta Banda (32ºN) (Lüning 1980). However, intraspecific differences in critical day length do not always correlate with latitude, as Rietema and Breeman (1982) found in *Dumontia contorta*. Moreover, photoperiod responses sometimes are altered by temperature (Fig. 2.11 and Table 2.2) and may not be exhibited in the presence of high nitrogen levels (such as are created in standard culture media).

We still know very little about reproductive phenology in the tropics. There are seasonal changes in the environment, albeit more subtle than those in mid-latitudes and there are strong seasonal variations in growth and reproduction of the flora, but the cues are unknown (Price 1989). A dramatic example of reproductive synchronicity in the tropics is the mass spawning of 17 species of Bryopsidales on Caribbean coral reefs (Clifton 1997; Clifton and Clifton 1999). These algae become fertile overnight, then release their entire cellular contents as gametes (termed holocarpy) in the morning, forming a dense cloud in the water. Up to nine species release gametes on the same morning, but do so at slightly different times, which could reduce hybridization. The entire event from onset of fertility to death of the parent material lasts just 36 h. All that is left of the adults are white cell walls that mostly disappear within 24–48 h, either via water motion or grazing, most likely freeing up space on the reef for the propagules to settle. While the precise cues that trigger the onset of fertility are unknown, they may include a combination of light and temperate because on cloudy mornings, gamete release was delayed by around 20 minutes and a laboratory study indicated a trend of spawning being delayed by 8 minutes per 1ºC decline in temperature.

The more equable conditions in the tropics are perhaps reflected in the apparently low numbers of tropical algae with heteromorphic life histories. Nevertheless, since photoperiodic effects have been shown in temperate isomorphic species, and there are day length changes except very close to the equator (e.g. the range in Guam, at 13º N, is 11–13 h) (Fig. 2.6a), it would be interesting to look for latitudinal effects in widely distributed heteromorphic species such as *Asparagopsis taxiformis* or *Tricleocarpa fragilis* (previously *Tricleocarpa oblongata*). If tropical algae near their northern or southern limits (e.g. in Bermuda, Hawaii, or southern Queensland) show photoperiodic responses, what do they do near the equator? And if they are day-neutral, to what environmental cues do they respond?

2.3.4 Other factors

How do the results of laboratory experiments on the temperature and photoperiod requirements for reproduction relate to conditions in the "real world", especially of the intertidal zone where other environmental factors (secs. 3.1 and 7.1) come into play? Breeman and Guiry (1989) described how tides alter reproductive timing in *Bonnemaisonia hamifera* sporophytes. These are SD algae, requiring a narrow range of warm temperatures (Table 2.2). Lüning (1980a) had predicted reproduction only during a short time in early fall, when the days become short enough but the sea is still warm (Fig. 2.12 and Table 2.2). Whereas, in general, phenology *in situ* bore out the predictions (Breeman *et al.* 1988), two factors confounded the predictions: (1) High spring tides at the beginning or end of the day shortened the effective day length, allowing reproduction to start earlier than predicted. (2) Low water of spring tides in the middle of the day exposed seaweeds to warm air temperatures,

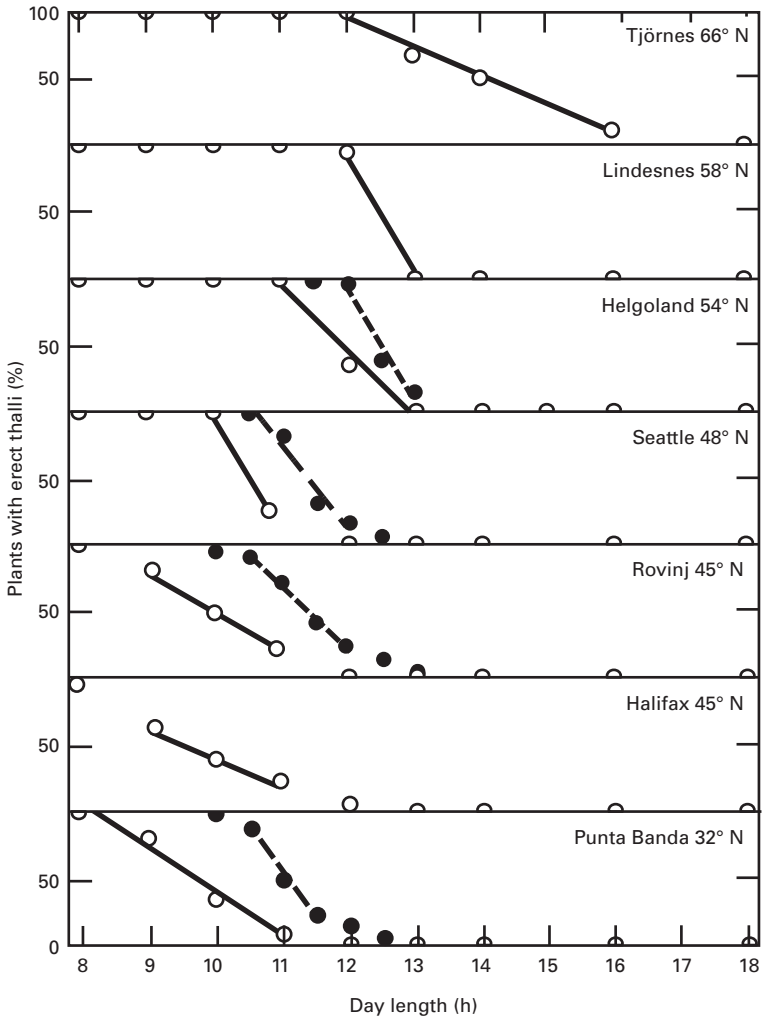


Figure 2.11 Effects of day length on erect thallus formation by different geographic isolates of *Scytosiphon lomentaria* at 10°C (open circles) and 15°C (filled circles). Each value is based on a count of 250 individuals. (From Lüning 1980a, with permission of The Systematics Association.)

when water temperatures were below the threshold, and allowed reproduction to resume. Brief exposures to a suitable combination of conditions sufficed for induction, and the reproductive period stretched from September into December. [At their study sites in Ireland, the times of high and low spring tides are always the same; that is not the case everywhere (sec. 3.1.1)]. In another example (Breeman *et al.* 1984), light was so reduced at high tide, because of turbidity and the fucoid canopy, that SD conditions prevailed all year for mid-intertidal populations of *Rhodochorton purpureum*.

Lunar and tidal cycles have also been found to trigger reproduction. Gametogenesis in *Dictyota diemensis*

Table 2.2 Effects of photoperiod and temperature on tetrasporangium formation in the trailliella phase of *Bonnemaisonia hamifera*[a]

Parameter	Response to day length (at 15°C)										
Hours light per day	8	9	10	10.5	11	12	12.5	13	14	15	16
Percentage fertile	93	92	48	16	6	0	0	0	0	0	0
Parameter	Response to water temperature (at 8 h light per day)										
Temperature (°C)	10		12		15		17		20		23
Percent fertile	0		0		97		73		0		0

[a] 150 plants in each experiment were grown in enriched seawater (containing less than 20 μM NO_3^-).
Source: Lüning (1981b), with permission of Gustav Fischer Verlag.

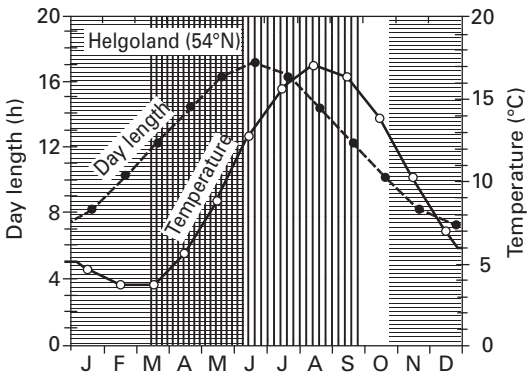


Figure 2.12 Predicted tetrasporogenesis in the trailliella phase of *Bonnemaisonia hamifera* in Helgoland, approximately the same latitude as the site in Ireland where Breeman and Guiry tested the prediction. The "window" for reproduction in September–October occurs between too warm seas (vertical hatching) and too short photoperiod (horizontal hatching). (From Lüning 1981b, *Ber. Deutsch. Bot. Ges.*, vol. 94, with permission.)

begins the day after a full moon and is completed with gamete release 10 days later (Phillips *et al.* 1990). For *Monostroma*, tides, rather than endogenous clocks, were considered responsible for synchronizing gamete release (Togashi and Cox 2001; compare to *Ulva* above). Some fucoid algae have fortnightly periodicity of gamete release, and the timing differs for the same species growing at different geographic locations, suggesting that the timing is entrained by tidal and diurnal cues, rather than lunar cycles (reviewed by Pearson and Serrão 2006). Mid-intertidal *Fucus vesiculosus* tends to release eggs late in the day and at a low tide compared to high-intertidal *F. spiralis* which releases eggs throughout the day, at both low and high tides. The suggestion is that as a selfing-hermaphrodite, *F. spiralis* requires less synchrony of egg release compared to dioecious *F. vesiculosus* (Ladah *et al.* 2008).

Seaweeds that do not experience strong seasons of temperature and photoperiod may still require environmental cues for reproduction. The deep-water brown alga *Syringoderma floridana* has macroscopic sporophytes and microscopic gametophytes. Most of the two-celled gametophytes develop right on the sporophyte, because the zoospores have very limited motility (Henry 1988). In culture, sporogenesis was induced by low-temperature shock or by transfer to a nutrient-rich medium. [Sudden changes in the surrounding medium can induce reproduction in some seaweeds (Chapman 1973; DeBoer 1981).] Gametophytes matured and released gametes predictably 2 days after settlement of zoospores, at 20°C. Henry (1988) suggested that the arrival of a water mass high in nutrients (probably also relatively cool) or a low-temperature water mass followed by a warm one would induce simultaneous sporogenesis throughout a local population. Synchrony evidently is vital to seaweeds with such small and short-lived gametophytes, and here temperature acts as a non-seasonal cue. The effects of UV radiation and water motion on reproduction are discussed in secs. 5.2.2 and 8.2.2, respectively.

2.4 Fertilization biology

After reproduction has been initiated in response to environmental cues, sporogenesis or gametogenesis takes place, and finally spores or gametes are released. Three types of sexual reproduction are traditionally recognized: isogamy, anisogamy, and oogamy (e.g. Graham *et al.* 2009). Oogamy involves a non-motile female gamete or egg, for example the brown orders Fucales and Laminariales, and all red seaweeds, that is fertilized by a smaller male gamete. Among brown algae, many so-called isogamous and anisogamous species actually behave oogamously, with the female gamete settling before fertilization, and Motomura and Sakai (1988) show that *Saccharina angustata* (previously *Laminaria angustata*) eggs have vestigial f1agella that are shed when the egg is released from the oogonium.

Santelices (2002) provides a framework for the fertilization ecology of seaweeds, based on the "brooders" versus "broadcasters" scheme used for marine invertebrates. "Broadcasters" (external fertilization) include species that liberate gametes into the water column, and for these to ensure fertilization success, there must be tightly controlled synchronicity, for example the mass spawning events on coral reefs (Clifton 1997) or synchronous release of gametes in the Fucales (Pearson and Serrão 2006). Most green and brown seaweeds are placed in the broadcaster category, and this mechanism is also common in marine invertebrates (e.g. sea urchins). Brooders (internal fertilization), in contrast, require efficient sperm collection mechanisms, a mechanism analogous to pollen collection in terrestrial plants. Many red seaweeds fall into this category, with their trichogyne acting as the "sperm-collector" (see Figs. 2.16 and 2.17). The trichogyne, consisting of a tube along which sperm travel to mate with the large egg at the end, resembles that of a flowering plant's pollen tube. Kelp gametophytes in which the egg remains on the female could also be classified as brooders. Santelices (2002) acknowledges that his framework may be an oversimplification given the diversities of algal life cycles, but nevertheless it provides a useful structure for testing hypotheses relating to the fertilization ecology of seaweeds.

In species with free-living male and female gametophytes, triggering of gamete release and attraction of one gamete to the other increase the chances of successful syngamy. In the Laminariales and Desmarestiales male antheridia do not release their sperm until they detect the pheromone from mature female gametophytes; the same compound (lamoxirene, Fig. 2.13) acts as antheridium releaser and sperm attractant (Müller *et al.* 1985; Müller 1989). This can result in a "mass release" of sperm, and the time from chemical signal to response is just 8–12 seconds for *Laminaria digitata* (Pohnert and Boland 2002). In the field, male and female kelp gametophytes need to be sufficiently close to one another that the male can detect the pheromone released by the female, and for *Macrocystis pyrifera* and *Pterygophora californica*, spore density must be greater than 1 spore mm^{-2} for successful recruitment (Reed 1990a).

The role of pheromones in sexual reproduction is best studied in brown algae for which 12 have been identified, and these each have structural isomers, leading to a high diversity of these signaling molecules (Fig. 2.13; Pohnert and Boland 2002). However, not all brown algae use pheromones. *Sargassum muticum* is monoecious, and fertilization takes place under a blanket of mucilage, while the oogonia are still strapped to the conceptacle by their mesochiton; presumably, self-fertilization can occur. *Himanthalia* eggs also apparently do not chemically attract sperm. Moreover, *Dictyopteris* and *Feldmannia mitchelliae* (previously *Hincksia* [*Giffordia*] *mitchelliae*) secrete compounds similar to those in Figure 2.13, from both gametophytic and sporophytic tissues, but these substances do not act as pheromones in these genera (Kajiwara *et al.* 1989; Müller 1989).

Although pheromones have been detected in a large number of brown algae, there is surprisingly little variation in their structure and they are not distributed along taxonomic lines (Müller 1989; Pohnert and Boland 2002). Female eggs typically release a blend of several pheromones, although usually one of these compounds is the most biologically active. All the

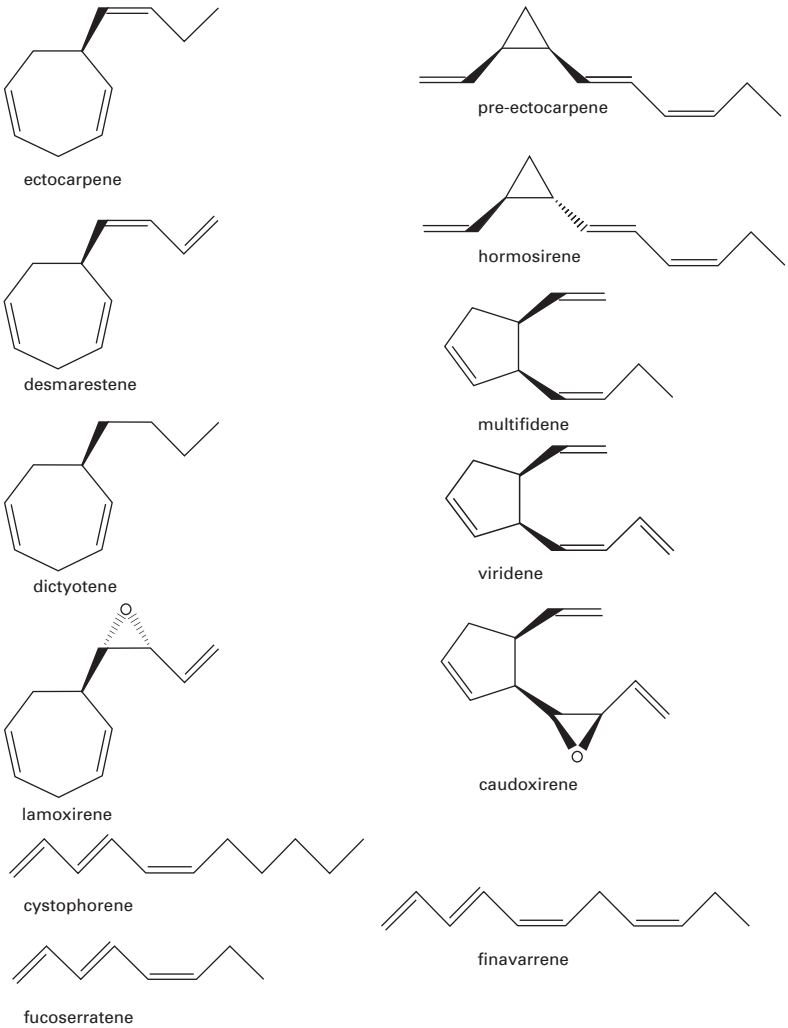


Figure 2.13 Identified brown algal pheromones. (From Pohnert and Boland 2002, © The Royal Society of Chemistry 2002.)

compounds are simple, non-polar, volatile hydrocarbons, either open-chain or cyclic olefinic hydrocarbons. The structural similarity between the 12 brown algal pheromones indicates a common biosynthetic pathway from fatty acids, and a few tailoring enzymes are required for their activation (Pohnert and Boland 2002; Rui and Boland 2010). Their insolubility and volatility prevent their concentrations building up in the water and enable the female gametes to maintain steep concentration gradients at their surface. The range of attraction probably is no more than 0.5 mm (Müller 1981). The pheromones are highly active, and so only minute quantities of attractant are released: 5 million *Macrocystis* eggs yielded 2.9 μg of lamoxirene (Müller *et al*. 1985).

The behavior of male gametes in the presence of an attractant varies from one species to another (Fig. 2.14). *Laminaria* sperm head straight for the

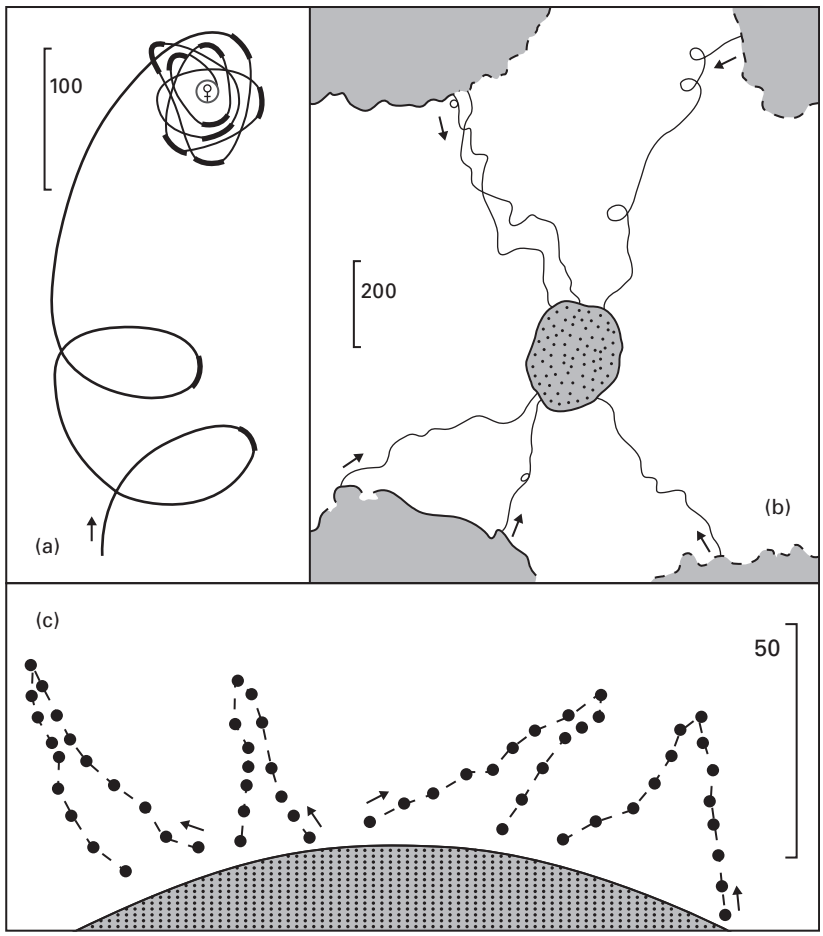
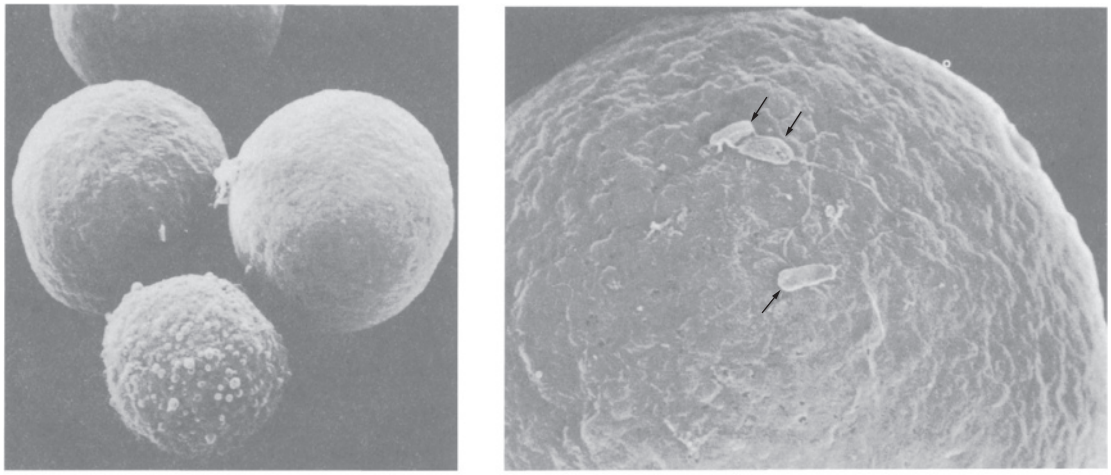


Figure 2.14 Different types of gamete approach in brown algae. (a) Chemo-thigmo-klinokinesis in *Ectocarpus siliculosus;* emphasized parts of male track indicate periods of hind-flagellum beat. (b) *Laminaria digitata:* impregnated silica particle as pheromone source in center, with tracks of individual sperm. (c) *Fucus spiralis:* return responses of individual sperm near a fluorocarbon droplet containing fucoserratene. Scales in micrometers. (From Müller 1989, with permission of John Wiley/Alan R. Liss Inc.)

egg. *Ectocarpus* males have a more complex pattern. In the water column they swim in straight lines, periodically changing direction abruptly. When they encounter a surface, they change to a wide, looping path along the surface. In the presence of attractant from the female gamete, the male changes to a circular path, the diameter of which decreases as the hormone concentration increases.

Gamete recognition is a critical stage in sexual reproduction. Because female gametes release a blend of pheromones, they may attract the sperm of different species. In *Fucus*, the same attractant works for at least three species. Similarly for the kelps, (1'R, 2S, 3R)-lamoxirene was the bioactive component of pheromones released by *Laminaria*, *Alaria*, *Undaria*, and *Macrocystis* and no species specificity of the pheromone was detected (Maier *et al.* 2001). Rather than gamete recognition by a species-specific pheromone, gamete selectivity arises from the recognition of complementary surface carbohydrates, and syngamy is



(a) (b)

Figure 2.15 Scanning-electron-microscope views of eggs, sperm, and zygotes of *Fucus serratus*. (a) Group of cells 10 min after mixing eggs and sperm. Smooth cells have been fertilized and have formed a fertilization membrane; the rough cell in the foreground is an unfertilized egg (×450). (b) Detail of fertilized egg with three sperm (arrows); the tip of the anterior f1agellum of the middle sperm is embedded in secreted cell wall material (×1600). (From Callow *et al.* 1978, with permission of The Company of Biologists.)

thus prevented (Schmid 1993; Schmid *et al.* 1994). Glycoconjugates are carbohydrates that are covalently linked to another chemical (e.g. glycoprotein = carbohydrate + protein), and are involved in a wide range of cell–cell and cell–substrate interactions (Lodish *et al.* 2008). The processes of recognition and fusion have been studied in some Fucales and Ectocarpales, taking advantage of the fact that surface receptors are not obscured by cell walls (Evans *et al.* 1982). The egg membrane initially appears lumpy because of protrusion of cytoplasmic vesicles (Fig. 2.15a; Callow *et al.* 1978). The spermatozoid probes the surface of the egg with the tip of its anterior f1agellum, apparently seeking specific binding sites (Friedmann 1961; Callow *et al.* 1978). Attachment takes place first by the f1agellum tip, and later also by the body of the cell (Fig. 2.15b). Egg membrane surfaces carry special glycoproteins with fucose and mannose units in particular patterns that fit into carbohydrate-binding sites (ligands) on sperm membrane proteins, analogous to a lock-and-key mechanism (Bolwell *et al.* 1979, 1980; Wright *et al.* 1995a, b). Some of the *Fucus* sperm surface domains that have been distinguished by monoclonal antibodies probably are specific for egg recognition. The sperm protein has been characterized; it partially activates the egg when it locks to its surface (Jones *et al.* 1988; Wright *et al.* 1995a, b).

For Florideophyte red seaweeds, fertilization is initiated by the cell–cell recognition between the liberated sperm and the trichogyne. For *Aglaothamnion sparsum*, recognition was mediated by fucose and mannose units, similar to the fucoid example above (Kim *et al.* 1996). For *Aglaothamnion oosumiense*, however, male gametes use a "double-docking" process for recognizing the appropriate female trichogyne, utilizing at least two carbohydrate moieties and the complementary receptors which are lectin-like molecules (Kim and Kim 1999). They suggest that the carbohydrate-based system observed in some red algae may not be species specific, but rather a generic mechanism, and that other currently unidentified mechanisms will exist for specific species.

When one sperm has entered the egg, no more are needed. Indeed, polyspermy is lethal; fucoid germlings develop abnormally and die after a few days (Brawley

1987, 1991, 1992a; Brawley and Johnson 1992). Mechanisms have evolved to minimize the percentage of eggs that are fertilized by more than one sperm, even though in monoecious species fertilization often takes place when oogonia and antheridia are newly released and the sperm concentration is likely to be high. A fast block to polyspermy has been shown in *Pelvetia* and *Fucus* (Brawley 1987); this block is Na^+ mediated and is replaced within about 5 min by a "slow block" corresponding to the formation of the cell wall, and an intermediate block in which the sperm receptors on the surface of the egg are degraded (Brawley 1991; reviewed by Pohnert and Boland 2002). Polyspermy blocks do not always work perfectly and for *Fucus ceranoides* 1–9% of eggs shed were polyspermic (Brawley 1992a). Polygamy was also evident in *Bryopsis* but it is not known if it is lethal in this genus (Speransky *et al.* 2000). In red algae, there is indirect evidence of mechanisms to prevent polyspermy, but this has not been confirmed (Santelices 2002).

Red algal sperm do not have flagella and therefore cannot swim towards the egg located on the female gametophyte. Correspondingly, sexual attractant pheromones have not been detected in red seaweeds. The lack of sperm motility has led to the traditional view that red seaweeds are ill-equipped for fertilization and dispersal in the marine environment, and that they compensate for this through zygote amplification (sec. 2.2; Searles 1980). However, this theory has not been born out in quantitative field studies, which show that fertilization rates of red seaweeds are similar to those of brown and green seaweeds (Kaczmarska and Dowe 1997; Engel *et al.* 1999). This led Santelices (2002) to call for a new explanation for the "triphasic" life cycle of the red seaweeds. Brawley and Johnson (1992) suggest that immobilization of the female gametes (i.e. brooders) might be an adaptive advantage to enhance fertilization: with only one gamete moving in the water column the chances of missing the other might be reduced. A range of mechanisms can enhance fertilization success despite non-motile flagella. The ability of spermatia to reach a trichogyne is improved when they are released in slime strands, as in *Tiffaniella snyderae* (Fetter and Neushul 1981). The fibrillar mucilage is elastic; it stretches out in water flow, and when it attaches to the female alga it tends to sweep the surface and deposit spermatia on extended trichogynes. There are cone-shaped appendages on spermatia from *Aglaothamnion neglectum* that are not sticky and bind only with trichogynes and hairs, though the binding is not species specific (Magruder 1984).

A popular theory in fertilization biology is that of "sperm limitation". The idea is that for broadcast-spawning marine organisms, fertilization success would be limited due to dilution of sperm in a turbulent water column. However, for fucoid seaweeds many species have fertilization levels of close to 100% indicating mechanisms that ensure fertilization success, such as timing gamete release to coincide with periods of calm water (Serrão *et al.* 1996; Berndt *et al.* 2002; sec. 8.2.2). For red seaweeds too, there is little evidence of sperm limitation (Maggs *et al.* 2011). Engel *et al.* (1999) conducted an elegant field study in which they mapped and sampled each of the 64 tetrasporophytes, 37 male and 26 female gametophytes of *Gracilaria gracilis* in a rock pool, at low tide. Using two microsatellite loci as genetic markers, they identified the exact males within the population that were responsible for fertilizing 72% of the 350 cystocarps sampled. Only 11% of males were from populations outside the rock pool. While they found no evidence of sperm limitation, there were strong differences in the male's ability to fertilize the females, indicating either male–male competition or female choice.

Sexual reproduction in red algae has been extensively studied because of its importance in systematics (reviewed by Hommersand and Fredriq 1990). Post-fertilization events in red seaweeds are complex. O'Kelly and Baca (1984) were able to observe the timing of reproductive stages in *Aglaothamnion cordatum*, which in culture produced one new axial cell per day. Like all Ceramiales, this species has a four-celled carpogonial branch (Fig. 2.16), and auxiliary cells are produced only after fertilization, in this case from the support cell and an additional auxiliary mother cell. Gamete fusion (including spermatium attachment, plasmogamy, transfer of the male nucleus down the trichogyne, and karyogamy) took 5–10 h. Carpogonia divided to form two daughter cells. Auxiliary cells

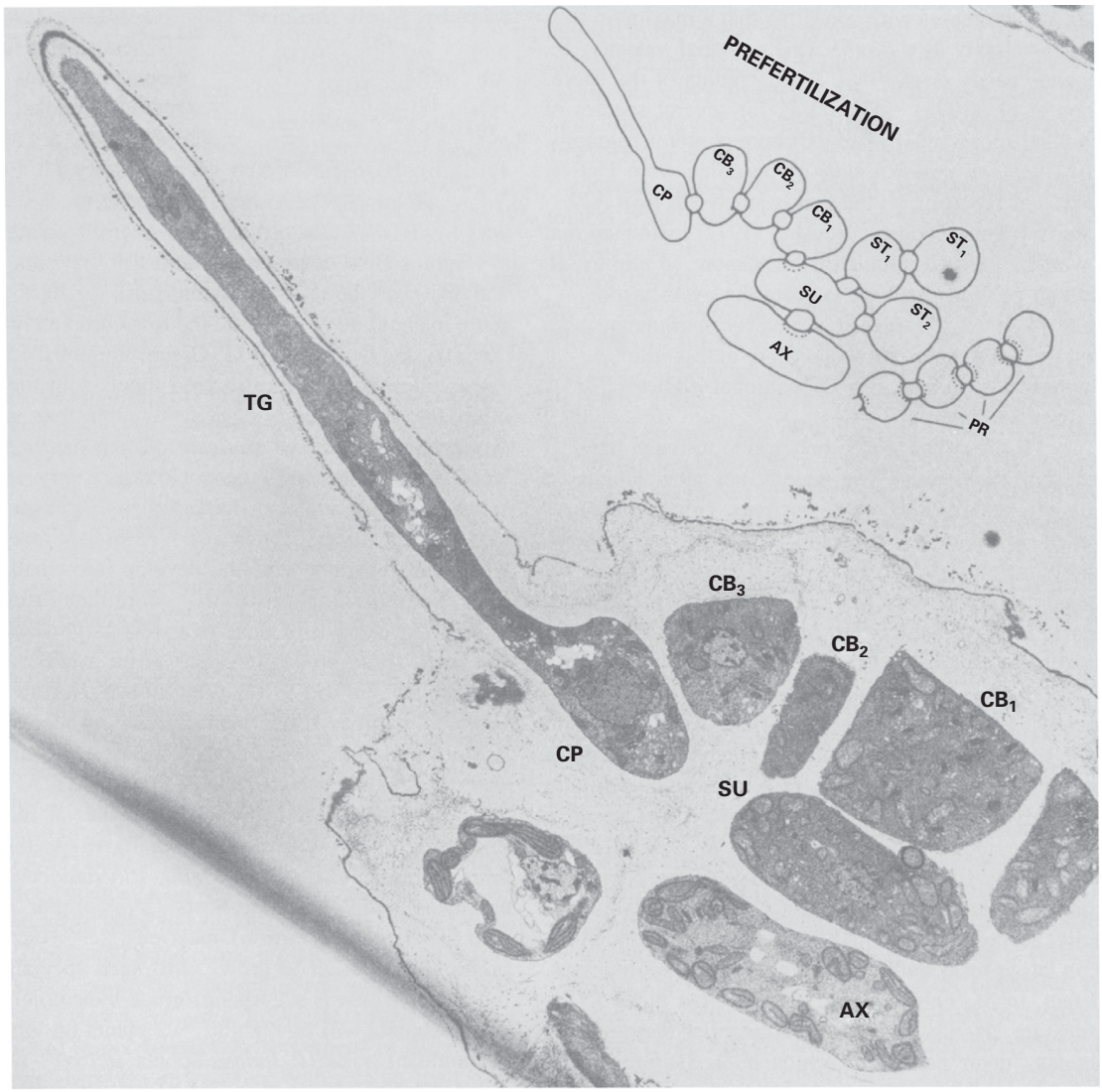


Figure 2.16 Carpogonial branch of *Neosiphonia harveyi* (previously *Polysiphonia harveyi*). Electron-micrographic section and diagram of prefertilization appearance. AX, auxiliary cell; $CB_{1...3}$, carpogonial branch cells; CP, carpogonium; PR, pericarp; $ST_{1,2}$ sterile cells; SU, support cell; TG, trichogyne. (From Broadwater and Scott 1982; with permission of *Journal of Phycology*.)

formed after about 40 h, and at around 72 h were diploidized; that is, the original haploid nucleus was partitioned off into a foot cell, and a diploid nucleus from a carpogonium daughter cell was transferred via a connecting cell. Some key aspects of this process, such as spermatial release and attachment to the trichogyne, have been elucidated for a few species (Pickett-Heaps and West 1998; Santelices 2002; Wilson *et al.* 2003). The events following a mass release of spermatia from *Bostrichya* leading to fertilization

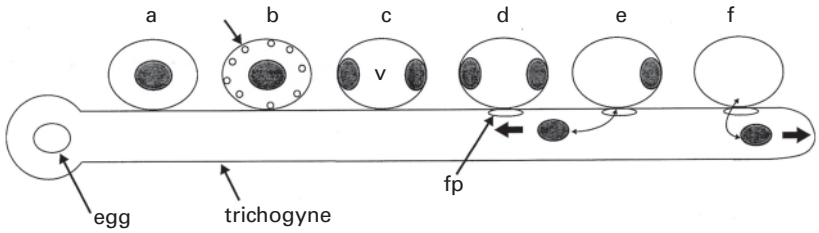


Figure 2.17 A diagrammatic representation of the events leading up to fertilization in red algae. Each spermatium represents a sequential stage from a to f. (a) Spermatium with a prophase nucleus binds to the surface of a trichogyne; (b) a few minutes after gamete binding, the spermatium develops small vacuoles (arrow) at the cell periphery; (c) at 20–30 min after gamete binding, the spermatial nucleus resumes mitosis and is usually complete within 15 min. Cytokinesis does not occur and a binucleate spermatium results. At this time much of the spermatium's intercellular space is occupied by a vacuole (v); (d) within 45 min of male nuclear division the walls separating the two gametes erode away until cytoplasmic continuity between the spermatium and trichogyne is achieved (gametes are connected by a fertilization pore, fp); (e) approximately 15 min after the fertilization pore has formed, the first nucleus enters the trichogyne and travels either towards the trichogyne tip or the base of the carpogonium which bears the female nucleus; (f) soon after, the second nucleus enters the trichogyne cytoplasm and travels in the direction opposite to the first migrating male nucleus. (From Wilson *et al.* 2003, reproduced with permission.)

(Fig 2.17) were documented using time-lapse video. The sperm adhere to the fragile filaments of the trichogyne, and within 30 min a mitotic division of the sperm nucleus occurs. At 50–80 min after attachment, the sperm membrane seals with the trichogyne and two nuclei move from the spermatia into the trichogyne. These two nuclei are differentiated and have different fates: one migrates down the trichogyne to the egg, the other stops moving and remains in the trichogyne. The mechanism of movement is unknown, but for *Aglaothamnion* and *Murrayella periclados* actin filaments are involved (Kim and Kim 1999; Wilson *et al.* 2003). The generality of these findings to other red algae is unclear, but time-lapse video microscopy has opened new avenues of investigation.

2.5 Settlement, attachment, and establishment

Once the reproductive cells have been released from the parent generation they must get to a surface and stick to it, prior to germination. The following scheme was developed by Fletcher and Callow (1992) to track the fate and development of spores that are released into the water column: (1) "Settlement" or "encountering the substrate". Settlement is the mechanism by which they move from the water column to the seabed. (2) "Attachment" which involves an initial weak attachment to the substrate, followed by permanent adhesion. (3) "Establishment" in which cells acquire a cell wall and polarity, then germinate to produce an apex and rhizoid.

2.5.1 Settlement

The spores and gametes of many seaweeds are motile and show directional responses to environmental cues such as light and gravity. Even more are non-motile, i.e. red algal spores, green- and brown algal aplanospores, and multicellular propagules, as in *Sphacelaria* and *Sargassum muticum*. In a turbulent water column, however, the movement of propagules is controlled by water movement; the small size and low Reynold's number (see Chapter 8) of motile propagules means that their powers of motility are limited, similar to flagellated phytoplankton. However, water velocity and turbulence decreases towards the seabed, and at the seabed surface there are slow-moving (1–10 mm s^{-1}) and non-moving layers of water that form around all submerged objects (Denny 1988; Amsler *et al.* 1992; sec. 8.1.3). Estimates of the thickness of the viscous region (viscous sub-layer) of the velocity boundary layer are 5–150 μm for various surfaces, whereas red

algal spore sizes, for instance, are 15–120 μm (Coon *et al.* 1972; Neushul 1972). In order to get into the "safe zone" of the boundary layer where they have time to attach, cells must travel through moving water.

Non-motile cells get to the seabed by strictly physical forces (Coon *et al.* 1972; Amsler *et al.* 1992; Stevens *et al.* 2008). Gravity tends to pull cells downward at ever-increasing speeds, but drag also increases with speed, so that a maximum (terminal) velocity is reached. This terminal velocity, V_t, depends partly on the density and radius of the spore (i.e. Stokes law). Coon *et al.* (1972) measured V_t for several species of red algal spores using time-exposure photomicrographs. *Sarcodiotheca gaudichaudii* carpospores were fastest, sinking at 116 μm s^{-1}, but that is much less than typical water-current velocities. Neushul (1972) estimated that it would take a *Cryptopleura* carpospore 10 min to fall through perfectly still water from the cystocarp on the adult to the seabed. Taylor *et al.* (2010) examined the effect of turbulence on settlement and attachment rates of eggs and zygotes of five species of fucoid algae. In still water, unfertilized eggs sank at a rate that was correlated to their size: the smallest eggs were of *Durvillaea antarctica* (29 μm diameter) and these had the slowest sinking rate (0.029 cm s^{-1}), the largest eggs were produced by *Cystophora torulosa* and *Pelvetiopsis limitata* (~100 μm) and sank at ~0.062 cm s^{-1}, whereas those of *Hormosira banksii* and *Fucus distichus* (previously *Fucus gardneri*) were intermediate in size and sinking rates. However, egg density was not related to sinking rate, with the eggs of *Durvillaea* having the greatest density, and they concluded that the stickiness and mucus characteristics of eggs are important in explaining differences amongst species. For fucoids, egg buoyancy appears to be the most important factor controlling sinking rates, and this is dependent on zygote properties such as the density of the mucus coat and how fast this dissolves (Stevens *et al.* 2008). The newly liberated spores of the red seaweed *Laurencia* are surrounded by an acid polysaccharide, which upon hydration, may increase the weight of the spores and their rate of deposition (Bouzon and Ouriques 2007). Unequal distributions of heavy (e.g. plastids and nuclei) and light (e.g. lipid bodies) organelles within the cells might result in specific cell orientation in the water column, and "also influence propagule behaviour" (Amsler *et al.* 1992).

Swimming speeds of motile cells are "puny in relation to the [current and wave] forces that beset them" (Norton 1992), and motility probably has little influence on the ability of cells to move from the mainstream seawater to the boundary layers at the seabed. Suto (1950) reported speeds of 0.13–0.30 mm s^{-1} for various zooids, and similarly *Ulva* zoospores swim at 0.2 mm s^{-1} (Granhag *et al.* 2007) and *Laminaria* at 0.16 mm s^{-1} (Fukuhara *et al.* 2002). The energy required to sustain swimming is derived from photosynthesis for some species, for example, the motile spores of *Macrocystis pyrifera* and *Pterygophora californica* can swim for up to 120 h in the light, and only 72 h in the dark (Reed *et al.* 1992). This ability differs among species, however, and for *Saccharina japonica* (previously *Laminaria japonica*) swimming was greater in the dark and there was no effect of photosynthesis on swimming. In this case the energy reserves may come from energy stores, such as the lipid stores observed for *Macrocystis pyrifera* and *Pterygophora californica* (Brzezinski *et al.* 1993; Reed *et al.* 1999). While in the water column, spores and gametes are part of the phytoplankton, and Graham (1999) reported between 1360 to 18 868 spores L^{-1} depending on season. The length of time spent in the plankton varies between species and this is determined by environmental factors but also their life-history stage and strategy (Fletcher and Callow 1992).

Several seaweeds have evolved interesting means of improving the chances of spores reaching substratum (Fletcher and Callow 1992). *Nereocystis* blades float far above the seabed, but the entire sorus, which sinks readily, is shed (reviewed by Springer *et al.* 2010). Sorus shedding takes place for a few hours around dawn, giving the spores the best chance for photosynthesis and survival. Spore release begins before sorus abscission, continues as the sorus sinks, and is completed within about 4 h (Amsler and Neushul 1989a, b). *Postelsia*, which grows in very high-energy intertidal habitats, releases its spores when the first waves of the incoming tide splash over the seaweed; water and spores flow down channels in

the drooping blades and drip onto the rock and parent seaweed's holdfast (Dayton 1973). *Fucus* releases its eggs still held together in the oogonium; the mass of eight eggs sinks faster than would a single egg. The invasive seaweed, *Sargassum muticum*, has a very effective settling mechanism in which the eggs released from the conceptacles remain attached to the outside of the receptacle, where they are fertilized and develop into small germlings, usually without rhizoids, before they drop to the seabed. As a result of their relatively large size (mean 156 μm), these propagules sink at an average rate of 530 μm s^{-1} in still water (Deysher and Norton 1982), some 5–10-times faster than unicellular spores. Once rhizoids start to grow out, they increase the drag and slow the sinking rate (Norton and Fetter 1981).

Significant numbers of propagules can reach the seabed in the fecal pellets of grazers (Santelices and Paya 1989). Herbivores ingest vegetative and reproductive tissues, sometimes preferring the latter (Santelices *et al*. 1983), and spores and tissue fragments often survive passage through their guts. Such fragments can form swarmers or protoplasts that will give rise to new individuals, especially in opportunistic algae like *Ulva*. Cells in fecal pellets have several advantages: The pellets are heavy, sinking 8–22-times faster than *Sargassum* propagules and 40–100-times faster than algal spores; the stickiness of pellets greatly improves attachment; the pellets provide protection against desiccation in the intertidal zone, giving sensitive germlings a chance to establish; and nutrient availability may be higher in the pellets.

Although swimming ability is ineffective compared to the waves and currents of mainstream flow, mobility is important in keeping cells at the water surface, or locating the seabed. A range of tactic responses have been observed for motile cells. Laminarian zoospores, for example, swim randomly, changing direction frequently. These zoospores do not have an eyespot and chemotactic responses to nitrate and phosphate guide them to the substratum (Amsler and Neushul 1989b, 1990; Fukuhara *et al*. 2002). Some motile cells can orient with respect to light, indicating the presence of a photoreceptor (sec. 1.3.3); some of these are negatively phototactic and swim toward the seabed but others are positively phototactic and continuously swim upwards which may aid dispersal (Amsler and Searles 1980; Hoffman and Camus 1989; Clayton 1992). Swarmers of *Scytosiphon* exhibit either phototactic or thigmotactic swimming: of 34 swarmers released, 22 had a spiral phototactic swimming pattern, 10 swam in a circular path near to the coverslip surface, characteristic of thigmotactic swimming, and two were initially thigmotactic but became phototactic (Matsunaga *et al*. 2010). The zoospores of *Feldmannia irregularis* (previously *Hincksia irregularis*) from North Carolina, USA, were positively phototactic, whereas those from the Florida panhandle were negatively phototactic; Greer and Amsler (2004) caution that these could be different cryptic species. For *Monostroma grevillei*, and also in *Ulva lactuca*, gametes are initially positively phototactic which may enhance fertilization success (see below); they become negative upon pairing facilitating movement towards the seabed (Kornmann and Sahling 1977). Zoospores, which must move back to the intertidal, are positively phototactic.

For green seaweeds, there may be a link between the type of sexual reproduction, the possession of a photoreceptor and the location within the water column in which fertilization occurs (Togashi *et al*. 2006). For isogamous or slightly anisogamous green seaweeds such as *Ulva*, male and female gametes each have two flagella and a single eyespot. They are positively phototactic, and migrate to the water surface where fertilization takes place. This migration is thought to be advantageous particularly in shallow water because the water surface is essentially a two-dimensional plane in which the chances of gametes of the opposite sign meeting is enhanced (compared to a three-dimensional water column). The resulting planozygote has four flagella and two eyespots (one pair of flagella and one eyespot from each gamete), and is negatively phototactic, allowing the zygote to move to the seabed where it will settle. Green seaweeds, such as *Bryopsis*, *Caulerpa*, and *Halimeda*, possess "markedly anisogamous" gametes and in their case only the female gametes have an eyespot and exhibit phototaxis – the

male has none and its movements are random (Togashi *et al.* 2006). The male does not, therefore, migrate to the water surface, and the motile female is attracted to the male via pheromonal cues. The planozygote inherits the single eyespot from the female gamete and, again, is negatively phototactic (Miyamura *et al.* 2010). In other markedly anisogamous seaweeds (e.g. *Udotea*, *Derbesia*) neither gamete has an eyespot, and in this case pheromonal attraction and fertilization takes place near the substratum so that the planozygotes are ideally placed to follow chemical signals to the seabed (Togashi *et al.* 2006).

Motile cells can "choose" their settlement site (Callow and Callow 2006). The behavior of *Ulva* spores changes from random movements to a "searching pattern" as spores near the seabed. In pre-settlement behavior, spores of *Ulva* "probe" the substrate with their flagella, and make repeated contacts with the apical papilla upon which they "spin like tops" (at 240 rpm) as the spore senses the chemical, physical, topographic, and biological characteristics of the surface (Callow and Callow 2006; Michael 2009). Spores are attracted to chemical signals released from surface biofilms (Joint *et al.* 2000) and the bacterial quorum sensing signal molecules *N*-acylhomoserine lactones (AHLs) are involved in this response (Joint *et al.* 2002; Fig. 2.18). For *Ulva flexuosa* and *U. lactuca* (previously *U. fasciata*), glycoconjugates on the surface of the zoospore flagella, apical dome, and anterior surface show molecular compatibility with biofilms that are suitable for settlement (Michael 2009).

Self-Assembled-Monolayers (SAMs) have been used to illustrate how *Ulva* zoospores choose their settlement site. These are synthetic surfaces which can be manufactured to have the same physical (e.g. topographical) and chemical properties, but differ in their wettability, also termed "surface energy" or "surface tension". High-energy surfaces are hydrophilic (wettable), and low-energy surfaces are hydrophobic. *Ulva* spores can sense the hydrophobic regions of the SAMs upon which they preferentially settle, and the signaling molecule nitric oxide (NO) triggers spore settlement (Thompson *et al.* 2010). They also choose surfaces with the most complex topographies on a

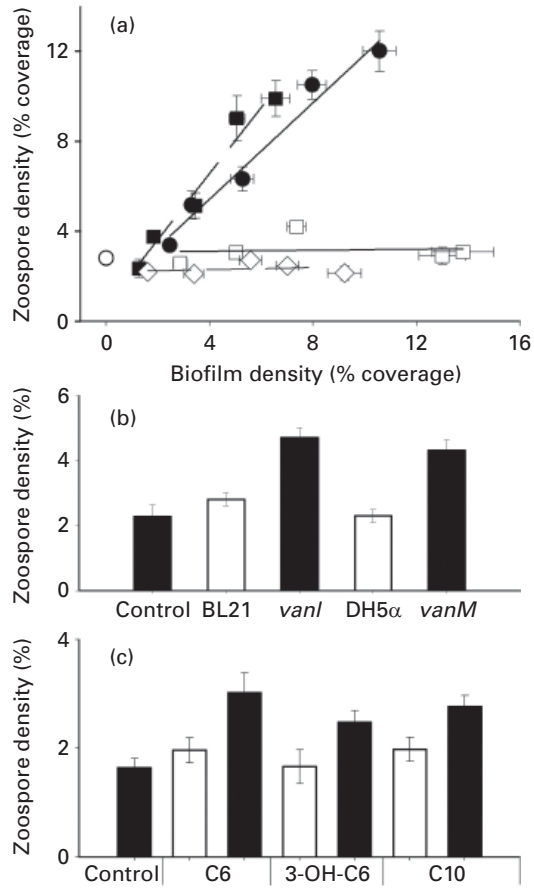


Figure 2.18 (a) Settlement of *Ulva* (previously *Enteromorpha*) zoospores on wild-type *Vibrio anguillarum* and mutants, expressed as percentage of surface covered. ○, control surface (clean cover glass); ●, wild type; ❑, *vanM* mutant; ■, *vanI* mutant; ◇, *vanIM* mutant. (b) Attachment of zoospores to *Escherichia coli* strains BL21 and DH5α with and without the insertion of plasmids expressing *vanI* – producing 30, C_{10}-HSL – and *vanM* – producing both C_6-HSL and 30H, C_6-HSL, respectively. There was no enhancement of attachment to either strain containing the vector plasmids alone. (c) Settlement of zoospores is not enhanced in the presence of three AHLs that have been treated to open the lactone ring structure (white bars) but is restored when the ring is closed (black bars). All error bars indicate ±2 SE. (From Joint *et al.* 2002, reproduced with permission.)

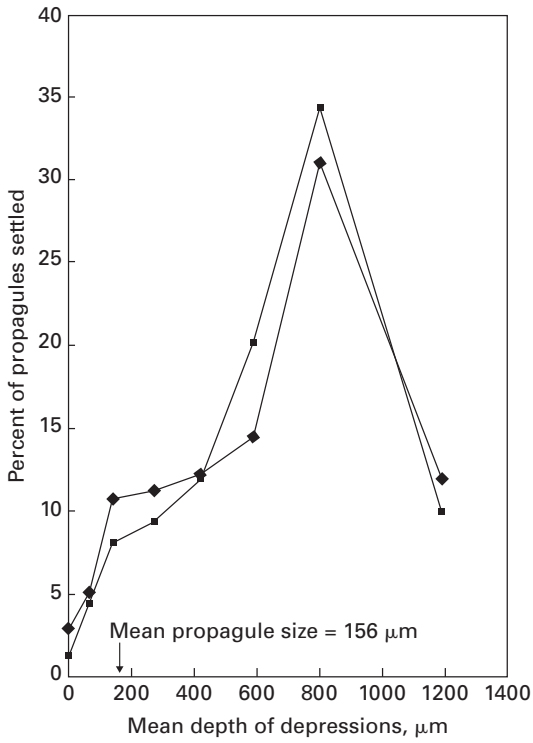


Figure 2.19 Effect of substratum roughness on settlement of *Sargassum muticum* propagules. The substratum consisted of sand-coated microscope slides on a surface with a jet of water flowing over it. Two independent experiments were run; within each experiment, several water velocities were used, and the results were pooled. (Drawn from data in Norton and Fetter 1981, with permission of Cambridge University Press.)

microscopic scale, which provide a greater surface area for the adhesive to stick to than a smooth surface; spores will chose depressions and crevices, which have a higher energy surface. Furthermore, they settle gregariously, responding to yet unidentified cues from each other, which may be beneficial in terms of space and post-germination factors like protection from desiccation and UV-R (Callow *et al.* 1997).

Surface roughness on the slightly larger scale of millimeters to centimeters is also important in affecting the passive settlement of motile and nonmotile propagules. Clean glass slides (a favorite experimental surface in the past) are unnatural surfaces, to which macroalgal cells do not adhere well (see Fig. 2.18). Natural surfaces, in contrast, usually are rough. Evidence from a number of experiments shows that surface roughness is an important factor in settlement (Vadas *et al.* 1992). Essentially, cells are deposited by eddies, in the same way that sand grains are deposited on the lee side of a sand dune. Norton and Fetter (1981) built a "waterbroom" to study the effect of surface roughness on settling of *Sargassum muticum* propagules in moving water. They found that settlement of *Sargassum* propagules was best on a surface with a mean depression depth of ~800 μm (Fig. 2.19), no matter what the water speed (range of 0.22–0.55 m s^{-1}). The propagules attached not because of sinking but because of turbulent deposition. There was a low rate of attachment in the depression of largest size because it was sufficiently large to be swept clean by water flow, rather than creating depositional eddies. Algal turfs also provide a rough surface, and they facilitate the settlement of reproductive cells, for example the zygotes of *Silvetia compressa* (previously *Pelvetia fastigiata*; Johnson and Brawley 1998). Of course, where cells can settle, so can sediment which can have a negative effect on recruitment (see sec. 8.3.2).

2.5.2 Attachment

Once a suitable substratum has been selected, the cells must stick to it. The ability of cells (i.e. their mucilage) to stick to a surface depends on the surface energy, which in turn depends on the nature of the substratum, including any coatings. Any material submerged in the ocean will quickly be coated by a biofilm of bacteria and their associated mucilage, which increases the surface energy and makes the surface much more suitable for macroalgal settlement (Fletcher *et al.* 1985; Dillon *et al.* 1989). In contrast, treatment of surfaces with hydrophobic coatings, such as silicone elastomers, in conjunction with engineered microtopographies, reduces algal settlement and is an effective antifouling technique (e.g. Fletcher *et al.* 1985; Callow and Callow 2006; Schumacher *et al.* 2007).

Zoospore or gamete ultrastructure and settling have been studied in a few species (Fletcher and Callow

1992), including *Ulva* species (= *Enteromorpha intestinalis*) (e.g. Evans and Christie 1970; see Callow and Callow 2006), *Scytosiphon* (Clayton 1984), a variety of Laminariales (Henry and Cole 1982), and several red seaweeds (Bouzon *et al.* 2006; Bouzon and Ouriques 2007). All these cells initially lack a cell wall and have among their organelles numerous cytoplasmic vesicles that contain adhesive material (Fig. 2.20a). Attachment first takes place by the tip of the anterior flagellum in kelps and *Ulva*. For *Ulva*, an initial, elastic bond is made with the substrate, but this bond is weak and the algae are easily removed, leaving the "blob" behind. For fucoid zygotes and red seaweed spores, neither of which have flagella, the initial attachment is via sticky mucilage (e.g. Ouriques *et al.* 2012). Once cells commit to settlement, vesicles containing adhesive material are trafficked to the cell surface and enormous quantities of adhesive are released (Fig. 2.21). This process is regulated by Ca^{2+} signaling in *Ulva* and *Fucus* (Roberts *et al.* 1994; Thompson *et al.* 2007). Following exocytosis, the cell membrane of *Ulva* is "dynamically recycled" to prevent it from stretching and expanding (Thompson *et al.* 2007). Newly settled spores of *Ulva* and *Cladophora surera* undergo rapid morphological changes including the adsorption of the flagellar axonemes and the shape changing from pear-shaped to round, and cell wall formation begins (Callow *et al.* 1997; Cáceres and Parodi 1998; Fig. 2.20b).

Fucoid eggs are initially covered by a mucilaginous layer of alginates and fucoidan that attaches them to the oogonium wall. The eggs are expelled from the conceptacle still enclosed in this layer, which is called the mesochiton. In *Fucus* and *Himanthalia* the mesochiton soon breaks down, and the zygotes attach to the substrate by the sticky zygote wall. In *Pelvetia canaliculata* the mesochiton persists, probably to protect zygotes from drying out in the very high shore habitat of this species (Moss 1974; Hardy and Moss 1979). The mesochiton, rather than the zygote wall, attaches the pairs of *Pelvetia* zygotes to the substratum. Within 24 h of settling, the *Pelvetia* zygote develops a firm alginate wall inside the mesochiton. Each zygote divides once or twice and then pushes out a group of up to four rhizoids, each from a single cell. These rhizoids grow down into the substratum, entering minute crevices, if these are available, and the mesochiton splits open. The time between fertilization and the formation of rhizoids in this species is about 1 week. For various seaweeds, the surface energy of the substratum affects the morphology of the germlings, especially their rhizoids. Many species (but not all), when on the preferred high-energy surfaces, form compact, well-attached basal filaments or rhizoids, whereas on low-energy surfaces the filaments or rhizoids spread widely and are poorly attached (Fletcher *et al.* 1985).

The composition of the adhesive substances is thought to vary widely between species. The precise chemical composition of adhesives and the nature of the bonds made with the substratum is incompletely understood, but an area of active research (see Vreeland *et al.* 1998; Callow and Callow 2006). Glycoproteins and/or sulfated polysaccharides are involved in initial attachment of red, brown, and green algal spores/zygotes (e.g. Ouriques *et al.* 2012). After spores have initially stuck to a surface, they begin to improve their adhesion by hardening the adhesive. Hardening (curing) of the attachment mucilage in various seaweeds involves the formation of crosslinks between polymer molecules, especially Ca^{2+} bridges between alginate chains or between sulfate ester groups of fucoidan. The rhizoid wall and the rest of the zygote wall have different alginates, as shown by antibody labeling (Boyen *et al.* 1988). *Fucus* embryos grown in sulfate-free seawater form normal rhizoids, but cross-linking cannot occur, and the rhizoids cannot adhere to the substratum (Crayton *et al.* 1974). Moreover, sulfation is necessary for intracellular transport of fucan (sec. 2.5.3). Attachment of single cells mechanically released from *Prasiola stipitata* thalli also requires sulfation of a cell wall polysaccharide; inhibitors of sulfation (such as molybdate) and of protein synthesis prevent attachment (Bingham and Schiff 1979). The protein may be complexed with the polysaccharide or may be an enzyme involved in the sulfation process. In brown seaweeds, phlorotannins are released at the same time as the initial adhesion and Vreeland *et al.* (1998) suggest that three adhesion precursors (a sulfated carbohydrate, a phenolic compound and a haloperoxidase), released together, form

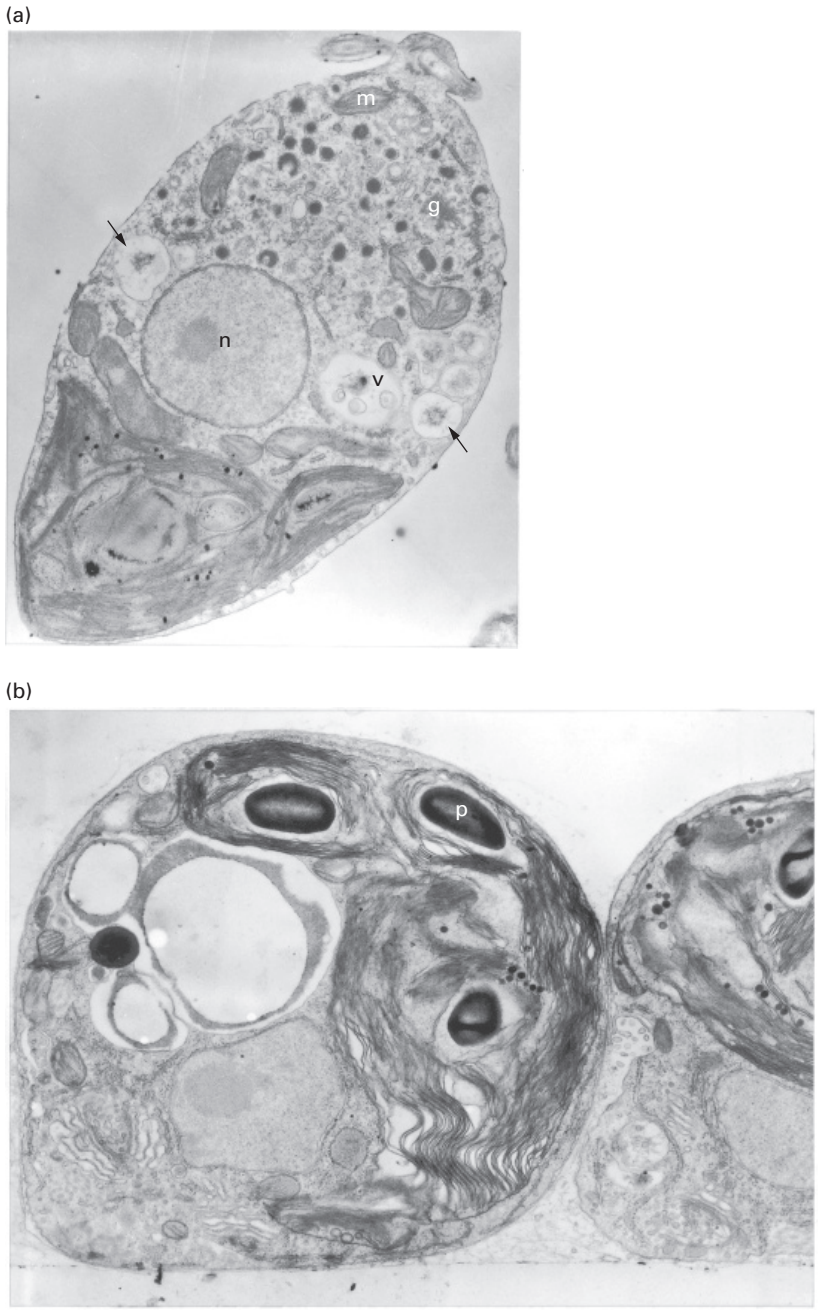


Figure 2.20 Ultrastructure of swimming (a) and newly settled (b) zoospores of *Ulva intestinalis*. In the anterior of the swimming cell can be seen numerous vesicles filled with adhesive (arrows). Also visible are part of the nucleus (n) and flagella bases, Golgi body (g), vacuole (v), and mitochondrion (m). (b) A mass of secreted adhesive lies in the triangle between the two cells, and there are virtually no vesicles remaining in the cell. (The attachment surface is parallel to the bottom of the photograph.) p, pyrenoid. Scales: (a) × 9000; (b) × 10 000. (From Evans and Christie 1970, with permission of the Annals of Botany Company.)

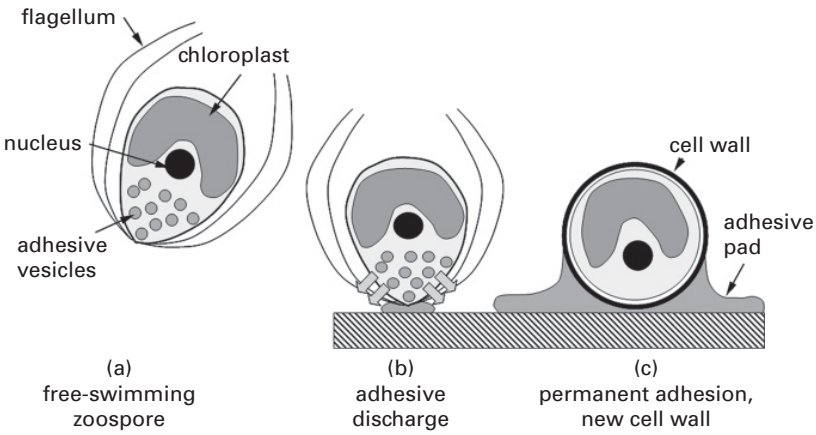


Figure 2.21 Cartoon depicting the course of events involved in the settlement and adhesion of *Ulva* spores. (From Callow and Callow 2006, © Springer-Verlag Berlin Heidelberg 2006.)

the glue. The phlroratannins stick to the substrate by "hydrogen bonding, metal complexation, and hydrophobic interactions". They suggest that this mechanism is common across the three seaweed lineages, although the form of carbohydrate, phenolic compound and haloperoxidase will vary between groups.

Attachment and hardening take time, and thus experiments designed to dislodge settled cells have shown that with a given water pressure, the numbers of cells that are washed away decrease the longer the cells have been allowed to settle (Christie and Shaw 1968). Other experiments have shown that the hydrodynamic force that a settled cell can withstand increases with time. *Ascophyllum* zygotes apparently cannot settle unless there is an adequate period of very calm water (Vadas *et al.* 1990). It takes 8 h for *Ulva* spores to be fully attached, at which time it is extremely difficult to remove them using hydrodynamic pressure. Also, spores that settle in groups are more resistant to hydrodynamic pressure (Callow and Callow 2006).

2.5.3 Establishment

After attachment, a cell wall is deposited around the spore (Fletcher and Callow 1992; Ouriques and Bouzon 2003; Fig. 2.22). Prior to germination, cells acquire polarity. In non-polar cells, for example an unfertilized egg, cellular components such as organelles and actin filaments are evenly distributed (Hable and Kropf 2000). A polarized cell (zygote or vegetative cell) has an asymmetrical arrangement of organelles, proteins, or cytoskeleton (Varvarigos *et al.* 2004). Fertilized eggs (zygotes) of fucoid algae are used as a model system for early developmental processes because of several advantages over terrestrial plants: (1) The eggs are free living and thus have no maternal influence on their polarity unlike angiosperm eggs which have maternally induced polarity prior to fertilization. (2) The eggs are easily accessible while those of angiosperms are contained within the ovule and are inaccessible. (3) The zygotes are relatively large (75–100 μm) facilitating micromanipulation, can be collected in quantity, aseptically, and they develop in synchrony in response to environmental cues. Their patterns of polar development and embryogenesis are morphologically similar to those of many algae (some exceptions will be discussed later) and angiosperms (Bisgrove and Kropf 2007; Bisgrove 2007)

Polarity is a key process that sets developmental patterning in algae, allowing the cell to respond to appropriate environmental cues that will ensure the correct orientation of the germling, juvenile, and adult.

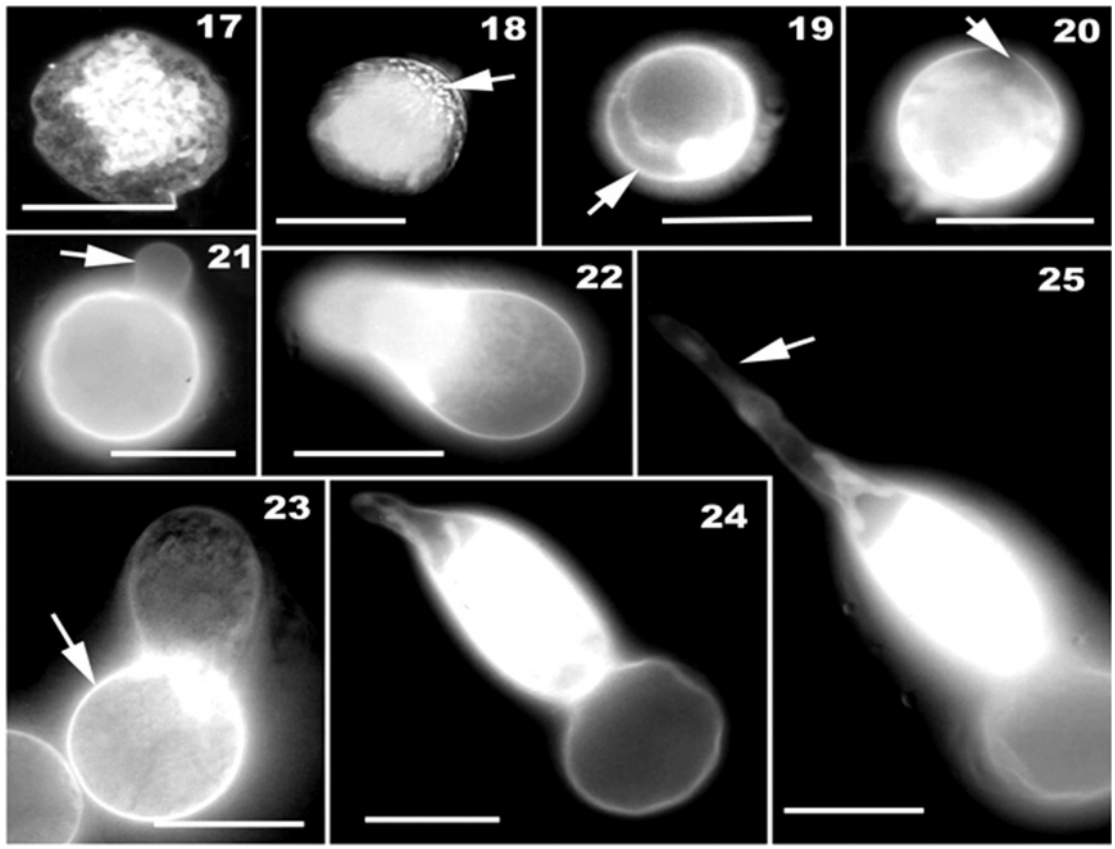


Figure 2.22 Tetraspores and tetrasporelings of *Gelidium floridanum* in different stages of development stained with calcofluor. (17) Non-germinated tetraspore without cell wall. (18) Beginning of cell deposition on a pole (arrow). (19) Tetraspore surrounded by the cell wall. (20) Polar disorganization of the cell wall signaling the start of the germination process (arrow). (21–23) Germ tube in varying stages of development covered by a thin cell wall. (24–25) Tetrasporelings showing a very thin cell wall on the rhizoids (arrow). Scale bar = 20 μm. (From Bouzon *et al*. 2006, reproduced with permission.)

For members of the Fucales, polarization begins upon fertilization of the egg (Fig. 2.23). The entry of the sperm into the egg triggers an actin/Arp2/3 network at the entry site. In the absence of any subsequent environmental stimuli such as a light gradient, blue light, temperature and pH, the site of sperm entry is the rhizoid pole. However, the polarity of the zygote remains labile for 8–14 h after fertilization and environmental cues perceived by the zygote trigger the disassembly of the sperm-induced actin/Arp2/3 network and its reassembly at a new location (Hable and Kropf 2000). Following fertilization, the zygote begins cell wall synthesis and secretes mucilage around itself, which facilitates attachment to the substratum. Once attached, each side of the cell will receive different environmental stimuli, for example the attached side will be shaded while the upper side will receive light. Such a differential light stimulus triggers the relocation of the rhizoid pole to the shaded side of the zygote. The mechanisms for sensing the environmental stimuli are unknown but it is likely that light is sensed by a rhodopsin-like protein (Gualtieri and Robinson 2002).

About 4 h after fertilization, axis amplification occurs. Ca^{2+} and H^{+} ion transporters accumulate at the actin/Arp2/3 network that is located at the rhizoid pole, and an ionic concentration gradient forms across the cystol (Pu and Robinson 2003). The Ca^{2+} gradient is dependent on a Reactive Oxygen Species (ROS; see secs. 7.1 and 7.8) gradient which forms simultaneously and may act to modulate the intracellular Ca^{2+} signal (Coelho *et al.* 2008). Axis fixation occurs about 10 h after fertilization, and the polarity is no longer labile (Hable *et al.* 2003). Germination quickly follows, with the rhizoid tip emerging from the now pear-shaped cell. Soon thereafter, the first cell division occurs, resulting in the apical cell that will become the thallus and reproductive structures, and rhizoid cells that will develop into rhizoids and holdfasts. Numerous extensions of the nuclear membrane project toward the rhizoidal pole, and there is an accumulation of vesicles at the pole (Fletcher and Callow 1992). The vesicles, derived from the Golgi apparatus, are filled with a highly sulfated fucoidan (F2) that is deposited in the cell wall at the pole and serves to anchor the cell to the substratum: the apical cell does not produce F2 nor the associated binding proteins (Fowler and Quatrano 1997). Negative charges on the sulfate ester groups or perhaps on the vesicle surfaces may be needed to draw the material toward the positively charged pole. Sulfation of the fucan requires new enzyme synthesis; if synthesis is prevented by cycloheximide, or if SO_4^{2-} is lacking, there is no movement of fucan to the rhizoidal pole.

The fucoid algae have served as a model system to such an extent that germination in other seaweeds has received little attention, except for morphological studies. At the morphological level there is great variety. Even within the Fucales, *Himanthalia* shows a different pattern, which Ramon (1973) suggested was not oriented by light. Not all germinating spores or zygotes first divide parallel to the substratum (or perpendicular to the light gradient). Horizontal germination is common, in which a single filament (germ tube) or basal crust is formed. In some species, such as *Coelocladia arctica* (Dictyosiphonales), the protoplast migrates into the germ tube, leaving the spore wall empty (Pedersen 1981). Other brown algal zoospores push out several lobes that are then cut off as cells; this stellate kind of germination leads to a monostromatic crust. Five patterns of germination have been described for the Rhodophyta although many do not fit into these general groups (reviewed by Murray and Dixon 1992). Recent studies have visualized aspects of red algal germination in *Gelidium floridanum* (Bouzon *et al.* 2005, 2006; Fig. 2.22) and *Laurencia arbuscula* (Bouzon and Ouriques 2007). In *Porphyra spiralis* var. *amplifolia* germination starts with the differentiation of a vacuole, which then pushes the cellular organelles into the developing germ tube (Ouriques *et al.* 2012). Spores of Corallinaceae divide in patterns characteristic for particular species (tetraspores and carpospores show the same pattern) (Chamberlain 1984). For carpospores of *Bangia fuscopurpurea* unipolar germination occurred when the day length was greater than 12 h, whereas germination was bipolar when it was less than 12 h (Dixon and Richardson 1970).

2.6 Thallus morphogenesis

The process by which unspecialized cells differentiate into cells with a specific role within a seaweed body and then multiply to acquire the familiar architectural form of the adult, has been studied classically using microscopic examination to follow each cell division (e.g. Figs. 1.4 and 1.5). More recently, the cellular developmental patterns of many (20–50) replicate individuals have been followed so that the robustness, or plasticity, of a particular developmental sequence can be determined statistically (Le Bail *et al.* 2008). Such data are also used in computer model simulations in which strict set of developmental "rules" are applied, that dictate the number and plane of cell divisions or the effect of a new cell on the development of a neighboring cell. These simulations "build" adult seaweed with an architecture that is characteristic of a particular species (e.g. Corbit and Garbary 1993; Lück *et al.* 1999; Billoud *et al.* 2008).

Bisgrove and Kropf (2007) ask the question "what factors determine the developmental fate of newly formed cells?" and suggest three mechanisms (that

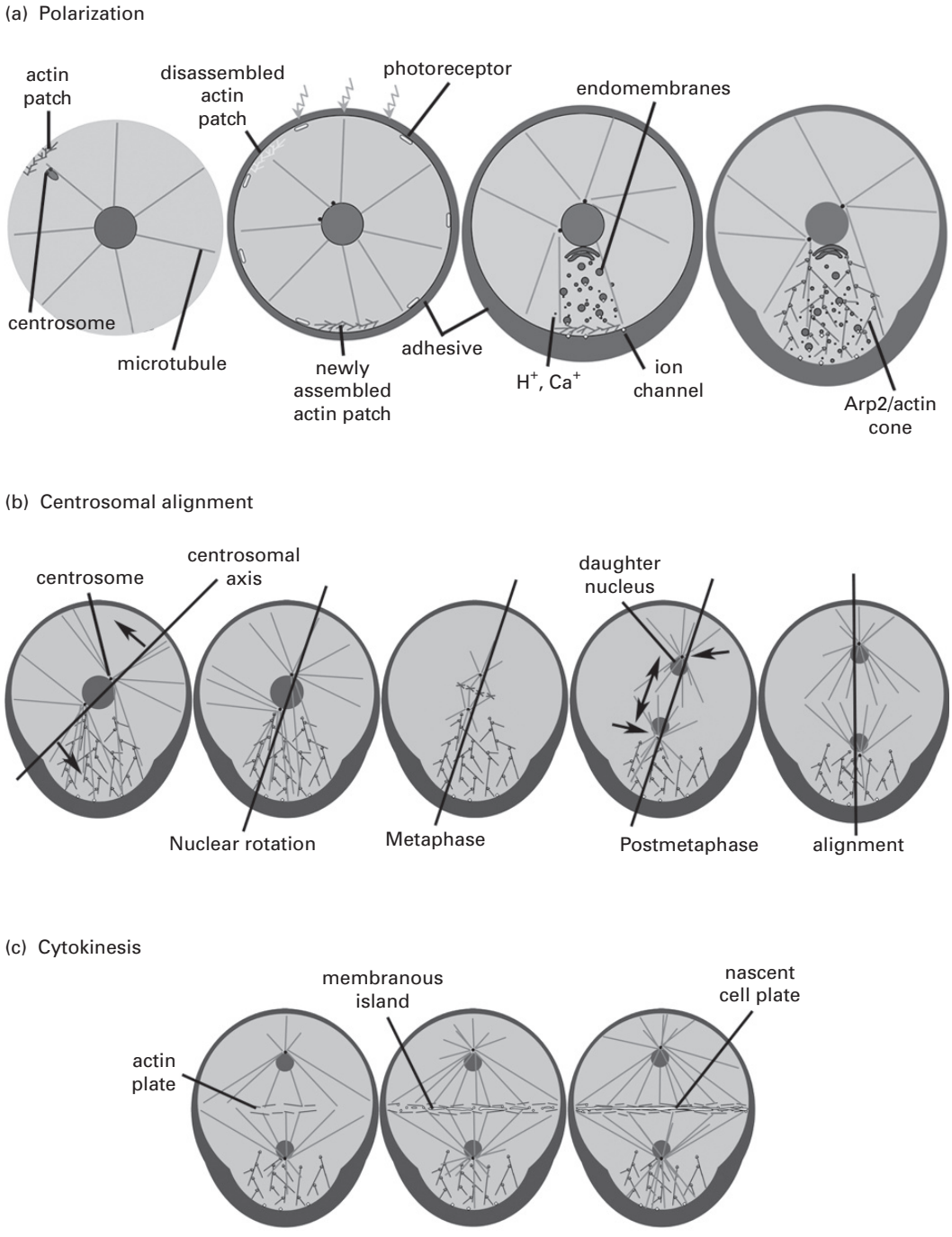


Figure 2.23 Mechanism of asymmetric cell division in zygotes of fucoid algae. (a) Fertilization induces formation of a cortical actin patch that marks the rhizoid pole of a default axis. Photopolarization causes disassembly of the sperm-induced patch and

are not mutually exclusive) by which cells acquire their own specific identities and roles within a plant or seaweed body: (1) "Intrinsic" or "cell-autonomous" development occurs when each new cell receives a different set of "cytoplasmic instructions". (2) "Extrinsic" or "non-cell-autonomous" development describes the influence of the cell's environment on its development. The "environment" here can include signaling from neighboring cells (e.g. by phytohormones; sec. 2.6.3), or external environmental signals (e.g. light, sec. 2.3.3). (3) Size and/or shape differences between the daughter cells can affect their developmental pathway. For the fucoid algae, all three of these processes affect developmental patterning as follows: Extrinsic signals, including sperm entry and light, set the initial polarity of the zygote (sec. 2.5.3). The first asymmetric cell division gives rise to daughter cells that are morphologically and cytologically distinct, and these give rise to the rhizoid and apical cell. During this first division, there is also an asymmetric allocation of mRNA to the daughter cells, indicating intrinsic signaling.

In Chapter 1 we reviewed the fundamental information on thallus construction and cell growth, and further details are found in van den Hoek *et al.* (1995) and Graham *et al.* (2009). In the following sections we review some classical and recent studies on cell differentiation, then consider how the development of the adult form is affected by abiotic (e.g. light, nutrients, temperature) and biotic (e.g. grazing), and how key developmental and physiological processes are regulated by seaweed growth substances (phytohormones).

2.6.1 Cell differentiation

The classical study of Ducreux (1984) follows the influence of the apical cell on the developmental fate of neighboring cells of the morphologically simple *Sphacelaria*. Here the apices have large cells with clearly defined functions. Organelles in the apical cell are concentrated near the tip, and the nucleus is also in the distal half of the cell. In many species the apical cell undergoes regular mitosis to form a symmetrical subapical cell. This cell, in turn, divides to produce two cells with different morphogenetic potentials: The upper one of the pair (the nodal cell) will branch, but the other (internodal) cell will not (Figs. 1.4a and 2.24). The asymmetry of the apical cell apparently is essential to its role as an apical cell and is also dependent on contact with older cells, as shown by regeneration experiments. If the apical cell is cut off, the subapical cell will become polarized and take over as a new apical cell (before or after dividing) (Fig. 2.24 b, c). An isolated subapical cell will form a new axis (Fig. 2.24 d), whereas an isolated apical cell will retain its polarity and continue to divide as before (Fig. 2.24 e). The polarity and shape of *Sphacelaria* apical cells is controlled by the cortical actin filaments of the cytoskeleton (Rusig *et al.* 1994; Karyophyllis *et al.* 2000).

Ectocarpus is "architecturally plastic" in response to environmental stimuli and there is also substantial morphological variation within populations grown in the same culture conditions. Le Bail *et al.* (2008) followed 20–50 *Ectocarpus* individuals, from two geographically isolated regions, from germination to the

Caption for Figure 2.23 (*cont.*) assembly of a new patch at the shaded pole. Endomembrane cycling then becomes focused to the rhizoid pole as the nascent axis is amplified, and cytosolic ion gradients are generated. At germination, the actin array is remodeled into a cone nucleated by the Arp2/3 complex. During early development the paternally inherited centrosomes migrate to opposite sides of the nuclear envelope and acquire microtubule nucleation activity, but microtubules play only an indirect role in polarization. (b) Centrosomal alignment begins with a premitotic rotation of the nucleus that partially aligns the centrosomal axis (defined by a line drawn through the two centrosomes) with the rhizoid/thallus axis. When the metaphase spindle forms it is partially aligned with the rhizoid/thallus axis. Postmetaphase alignment brings the telophase nuclei into almost perfect register with the rhizoid/thallus axis. *Arrows* indicate directions of nuclear movements.(c) Cytokinesis is positioned between the two daughter nuclei. A plate of actin assembles in the midzone between the nuclei, then membranous islands are deposited in the cytokinetic plane. The islands consolidate and cell plate materials are deposited in the division plane. All of these structures mature centrifugally, beginning in the middle of the zygote and progressing outward to the cell cortex. (From Bisgrove and Kropf 2007, © Springer-Verlag Berlin Heidelberg 2007.)

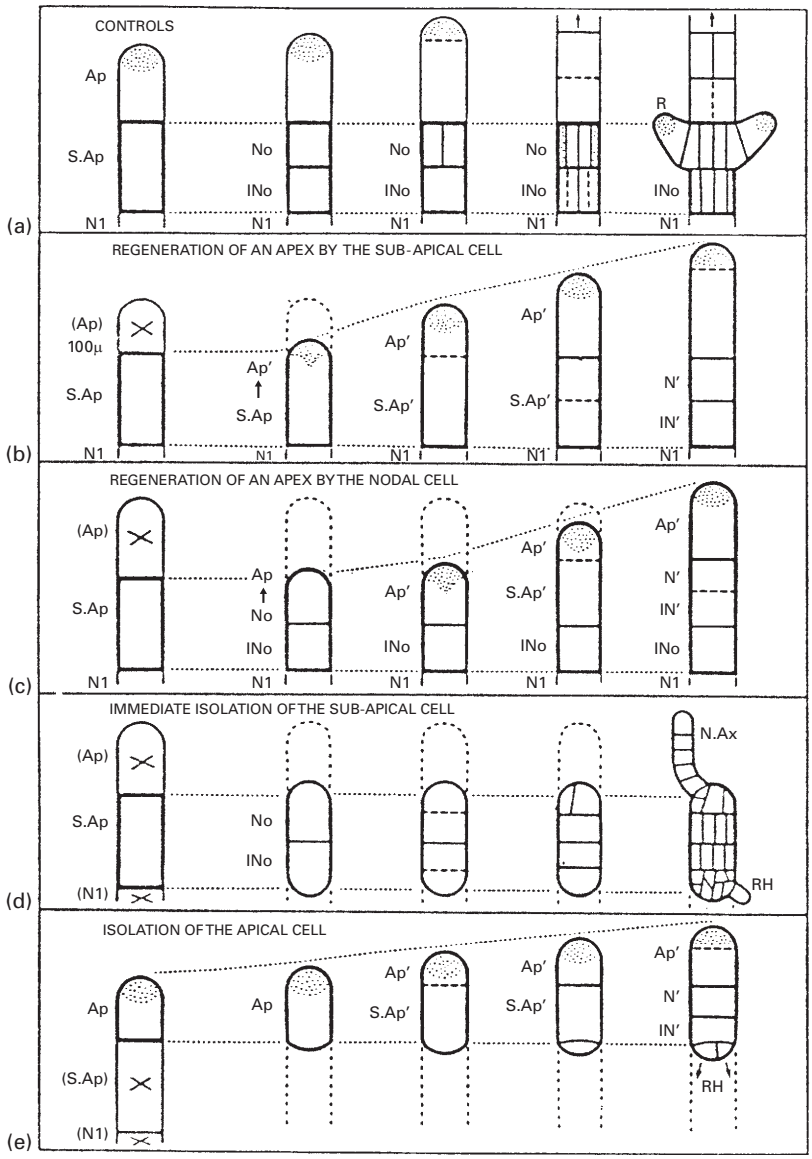


Figure 2.24 Development of apical (Ap) and subapical (S.Ap) cells in *Sphacelaria cirrosa*. (a) Normal ontogenesis of the subapical cell on control axes. (b, c) Regeneration of an apex after removal of the apical cell. If the subapical cell has formed recently (b), it transforms itself into an apical cell. If it is older (c), it undergoes a first division, and the nodal cell regenerates the apical cell. (d) Development of the subapical cell when isolated immediately after formation: modified development sequence leads to formation of a complete new axis. (e) An isolated apical cell continues normal sequence of cell divisions, except for the development of rhizoid initials (RH). N.Ax., newly formed axis; No, nodal cell resulting from transverse partitioning of the subapical cell; INo, corresponding internodal cell; NI, IN 1, . . ., successive nodal and internodal segments; R, branch. Abbreviations in parentheses indicate cells removed. (From Ducreux 1984, with permission of *Journal of Phycology*.)

100 cell stage, and assessed the robustness of the developmental pathways. Two distinct cell types, round and elongate, were observed, with the elongate cells being the "default" type that differentiated into round cells as filament growth progressed. A picture emerges of a tightly regulated pattern of branching which "ensured a stereotyped architecture", in spite of the inherent morphological variability. In a continuation of this work, Le Bail *et al.* (2011) created a morphological mutant, *étoile* (etl), that was "hyperbranched". They found branching to be controlled by a single, recessive locus *ETL*, the expression of which causes increased production of the round, branch-initiating cells and relatively fewer elongate cells.

When thalli or cells are injured, cells may dedifferentiate or redifferentiate. Cells isolated from the parent individual may behave as zygotes or spores and may form a whole new organism; the ability of a cell to do this is called totipotency. Single cells from simple thalli such as *Prasiola* (Bingham and Schiff 1979) readily regenerate the whole thallus, but cells from complex algae, such as cortical cells from *Laminaria* (Saga *et al.* 1978), can also regenerate the thallus under appropriate culture conditions. There probably are few cells in algal thalli that are irreversibly differentiated; anucleate sieve elements of *Macrocystis* provides an obvious example. Yet cells released from constraints imposed by neighboring cells do not always grow into a seaweed of the same generation. Examples are seen in the phenomenon of apospory: The diploid sporophytes of kelp tissue raised in stagnant culture for 3–4 months became bleached, leaving only isolated epidermal cells alive (Nakahara and Nakamura 1973). A few of these epidermal cells germinated, giving rise to gametophytes, which were shown to be diploid. These early studies on totipotency gave rise to an important area of applied seaweed research: cellular biotechnology and the search for methods of propogating seaweeds with superior characteristics (e.g. agar quality, growth rate) for aquaculture. Early work focused on producing viable protoplasts, in which the cell walls are digested and cells regenerated: Polne-Fuller and Gibor (1984) working with *Porphyra*, Ducreux and Kloareg (1988) with *Sphacelaria*, Fujimura *et al.* (1989) with *Ulva*, and Butler *et al.* (1989) with *Laminaria*. Protoplasts have been an important tool in understanding cell polarization (e.g. Varvarigos *et al.* 2004) and cellular biotechnology (e.g. Reddy *et al.* 2008a; see sec. 10.11).

Although some cells from seaweeds, if isolated, are totipotent and can regenerate into a whole seaweed, others cannot. Blade cells of *Ulva mutabilis* are unable to form rhizoidal cells, but form vesicular thalli one cell thick (Fjeld and Løvlie 1976). However, isolated rhizoidal cells of this species can form the whole seaweed. A repressor is present in the thallus cells, as shown by Fjeld's study of a mutant, *bubble* (*bu*), that behaves like isolated blade cells (Fjeld 1972; also see Fjeld and Løvlie 1976). The mutant gene is recessive and chromosomal. Curiously, *bu* spores from meiotic sporangia on heterozygous algae *(bu +/bu)* develop a partly or completely wild-type phenotype in the first generation. When these are propagated asexually, subsequent generations are completely the mutant type. The explanation appears to be that there are repressor or rhizoid-forming genes in normal blade cells and *bubble* in the cytoplasm of *bu* spores, as well as in wild-type mutant cells and that this repressor is removed during sporogenesis, so that spores can form rhizoids when they settle. The *bu+* wild-type gene is thus responsible for removal of the repressor, and its transcription takes place before meiosis, so that the de-repressor is present in the cytoplasm of *bu* spores. (This substance is not diluted through many cell generations, because the number of rhizoidal cells is small.) The rhizoid-forming genes of both types are re-repressed early in development, but the mutant gene cannot de-repress them when it forms spores.

Although coenocytes such as *Caulerpa* and *Bryopsis* are technically single cells, there are differences between regions of their cytoplasm, allowing the same kinds of differentiation that occur in multicellular thalli. *Caulerpa mexicana*, for example, has four morphologically distinct thallus regions: a creeping stipe, rhizoids, erect blades, and petiole-like stalks (Fagerberg *et al.* 2010). The structure of the cytoplasm differs among the peripheral, central, and trabecular regions of the thallus, and there are at least three types of trabeculae (Dawes and Barilotti 1969; Fagerberg *et al.* 2010). The trabeculae are thought to have a key role in

providing mechanical support and controlling developmental processes including cell shape.

2.6.2 Development of the adult form

The morphological form developed by a seaweed is strongly affected by their abiotic and biotic environment. Seaweeds exhibit considerable morphological plasticity which can vary over small spatial scales (e.g. *Ascophyllum*, Stengel and Dring 1997) and seasonally (e.g. *Ecklonia radiata*, Wernberg and Vanderklift 2010). Morphological plasticity is thought to be a mechanism by which seaweeds (and terrestrial plants) optimize resource acquisition and allocation to growth and/or reproduction in a spatially and temporally heterogeneous environment. Understanding how organisms interact with their environment is one of the "grand challenges" in organismal biology (Schwenk *et al*. 2009), and the effect of phenotypic plasticity on ecological organization is an emerging research field (Miner *et al*. 2005). The degree of phenotypic versus genotypic response of seaweeds to the environment, and evidence for incipient speciation, can be assessed by reciprocal transplant experiments (e.g. *Ecklonia radiata* Fowler-Walker *et al*. 2006; *Rissoella verrucosa*, Benedetti-Checchi *et al*. 2006), common garden experiments (e.g. *Eisenia arborea*, Roberson and Coyer 2004), and molecular studies (e.g. *Pelagophycus porra*, Miller *et al*. 2000). Morphological variation within a species can be so great that some specimens have been considered separate species, for example the kelp *Ecklonia brevipes* was found to be an extreme morphological variant of *Ecklonia radiata* (Wing *et al*. 2007; and see Essay 1, Fig. 1). Similarly, *Macrocystis* exhibits considerable morphological and reproductive plasticity across is wide geographic range, but evidence suggests it is just one species (Demes *et al*. 2009a). The propensity for phenotypic versus genotypic influence in response to environmental change seems to vary largely depending on species (Stengal and Dring 1997), and the ability to adapt and evolve to an environment may depend on if it is unitary or modular (Monro and Poore 2009a).

Branching is a characteristic developmental step in many algae and is important in establishing the final morphology of a seaweed (e.g. Coomans and Hommersand 1990; Waaland 1990). Branching patterns are consistent enough in many species (e.g. among Ceramiales) to be used as taxonomic criteria. In other species branching patterns are variable, and under apical control. For instance, when *Pterocladiella capillacea* loses its apical meristem due to wave action, the branching pattern changes from 1–3 orders to 2–5, that is, the seaweed becomes bushier (Scrosati 2002b). The effect of water motion on seaweed morphology is explored in Chapter 8.

The formation of erect uniaxial or multiaxial thalli from crustose germlings or microthalli requires that one or a number of erect filaments have their tips converted into meristems (reviewed by Murray and Dixon 1992). Formation of the meristems (macrothallus initials) and the outgrowth of the erect thalli are separate events, and in *Dumontia contorta (D. incrassata)* these events are controlled by different environmental cues (Rietema 1982, 1984). Production of the initials in this species depends solely on photoperiod: Short days (long nights) are required. Outgrowth of the initials also requires short days, but in addition the temperature must be 16ºC or lower.

The amount of light received can have marked effects on branching and elongation patterns. Two modular seaweeds, *Asparagopsis* (Rhodophyta) and *Caulerpa* (Chlorophyta), have very similar morphological responses to light quantity. In low-light environments, they are sparsely branched with long stolons, and in high light they form short, densely branched stolons and highly branched ramets (Peterson 1972; Collado-Vides 2002b; Monro and Poore 2007, 2009a, b). This response to light environment is also typical of modular terrestrial plants, and is considered a phenotypic adaptation that allows plants to "optimally forage" for resources (i.e. maximize resource acquisition) or "escape" competition. The sparsely branched "guerrilla" types allow individuals to invade new habitats, while the dense "phalanx" form is highly competitive and typical of densely populated environments. Another modular seaweed, *Codium fragile*, also possesses two distinct morphologies, "filamentous" and "spongy" (Nanba *et al*. 2005). In laboratory culture, vegetative fragments of filamentous thalli can give rise to spongy thalli and vice versa, and the type of thallus that is formed depends

Table 2.3 Non-photosynthetic effects of blue light on marine macroalgae

Description of response	Genus
1. Photo-orientation responses:	
Induction of polarity in germinating zygotes	*Fucus*
Negative phototropism of haptera	*Alaria*
Negative phototropism of rhizoids	*Griffithsia*
Plastid displacement	*Dictyota, Alaria*
2. Effects on carbon metabolism and growth:	
Stimulation of protein synthesis and mobilization of reserves	*Acetabularia*, *Dictyota*
Stimulation of dark respiration	*Codium*
Stimulation of uridine diphosphate glucose phosphorylase	*Acetabularia*
3. Effects on vegetative morphology:	
Induction of two-dimensional growth	*Scytosiphon*
Induction of hair formation	*Scytosiphon*, *Dictyota*, *Acetabularia*
4. Effects on reproductive development:	
Stimulation of cap formation	*Acetabularia*
Induction of egg formation	*Laminaria*, *Macrocystis*
Stimulation of egg release	*Dictyota*
Inhibition of egg release	*Laminaria*
5. Photoperiodic effects:	
Blue light alone effective as night break	*Scytosiphon*
Blue and red light effective as night break	*Ascophyllum*, *Rhodochorton*
Blue light effective as day extension	*Acrosymphyton*

Source: (From Dring 1984b, with permission of Springer-Verlag, Berlin.)

on the interactive effects of irradiance and water velocity. Spongy thalli were formed from filamentous thalli under high irradiances (50 and 100 μmol quanta m^{-2} s^{-1}) and at flows of 10 cm s^{-1} whereas filamentous thalli arose from spongy thalli at all irradiances tested (10, 50, 100 μmoles quanta m^{-2} s^{-1}) but only under no or slow flows (0 and 3 cm s^{-1}). This example also fits with the guerrilla (spongy, dense thalli in high light) and phalanx (diffuse thallus, low light) framework for optimal foraging.

Specific wavelengths of light also can affect morphogenesis. Blue light (BL) is a very important environmental signal in the marine environment because it is transmitted to the greatest depths through the water column, compared to red light which is absorbed in the top 4 m (see sec. 5.2.2). Many photomorphogenetic effects are due to blue light (BL) (Table 2.3; Lüning 1981b; Dring 1984b; Schmid 1984). Algae are equipped with a range of photoreceptors although studies on the biochemical and molecular mechanisms underlying BL-mediated responses are largely unknown (Hegemann 2008; sec. 2.3.3). In terrestrial plants, phototropin senses the blue region of the spectrum (390–500 nm) and mediates a range of responses including plastid movement and solar tracking that facilitate optimal light harvesting (Ishikawa *et al.* 2009). This protein has two "light-oxygen-voltage" (LOV) domains at the N-terminus that sense blue light (BL) and an effecter at the C-terminus, and has been identified in the unicellular green alga *Chlamydomonas*. The filamentous, mud-flat dwelling *Vaucheria* (Xanthophyceae) shows a range of BL-mediated responses including a morphogenetic response of preferential branching at the BL irradiated side of the thallus, in this case the receptor is aureochrome (Takahshi *et al.* 2007).

Red and blue light have differing photomorphogenetic effects. For *Saccharina japonica*, red-light-grown sporophytes had larger holdfasts and longer stipes than those grown in blue or white light (Mizuta *et al.* 2007). This supports earlier work on red or far-red light effects on the growth of kelp stipes *(Nereocystis*

leutkeana and *Saccharina japonica;* Duncan and Foreman 1980; Lüning 1981b). Red light causes specimens of the red alga *Calosiphonia vermicularis* to grow shorter and bushier than they do under white or blue light (Mayhoub *et al.* 1976).

A role of nutrition in morphogenesis has been shown in *Petalonia fascia* (Hsiao 1969) and *Scytosiphon lomentaria* (Roberts and Ring 1972). In *Petalonia* from Newfoundland, Canada, Hsiao (1969) found that zoospores from plurilocular sporangia on the blade could form protonemata (sparsely branched uniseriate filaments), plethysmothalli (profusely branched filaments), or *Ralfsia*-like crusts, any one of which could reproduce itself via zoospores or give rise directly to the blade. Protonemata and plethysmothalli survived in iodine-free medium, but formation of *Ralfsia-like* thalli or blades required iodine (5.1 mg L^{-1} and 0.51 mg L^{-1}, respectively). Plethysmothalli formed blades in progressively shorter times as the iodine concentration increased. Roberts and Ring (1972) found that changes in the proportions of filamentous and crustose microthalli correlated with nitrogen and phosphorus levels.

Some seaweeds develop hairs in response to a nutrient shortage, including *Acetabularia acetabulum, Ceramium virgatum* (previously *Ceramium rubrum*), *Fucus* sp. *Undaria pinnatifida* and *Codium fragile* (DeBoer 1981; Norton *et al.* 1981; Benson *et al.* 1983; Hurd *et al.* 1993; Pang and Lüning 2004). These hairs can enhance nutrient acquisition (secs. 6.8.1 and 8.2.1). For *Acetabularia acetabulum* blue light and red light directly affect hair formation (Schmid *et al.* 1990). If this species is grown in red light, no hairs form, and growth gradually slows. If a pulse of blue light is given and then growth in red light is continued, hair whorls are produced. Blue light induces the response; the red light is used solely in photosynthesis, and there is no evidence for a red/far-red receptor.

Temperature affects the morphological complexity of *Chondrus crispus* (Kübler and Dudgeon 1996). When *Chondrus* was grown at 20°C, a highly branched thallus formed compared to the more sparsely branched thalli produced by specimens grown at 5°C. The growth rate was also higher in the higher temperature treatment. The greater propensity for branching at 20°C may be an adaptation to increase the surface area of thallus that is available for light harvesting and nutrient uptake, enabling greater procurement of light and nutrients to fuel the higher metabolic demands at higher temperatures. The higher branching may also increase evaporative cooling when exposed to the atmosphere (Bell 1995).

Growth of kelp haptera is oriented by negative phototropism, not geotropism, with blue light being the most strongly orienting part of the spectrum (Buggeln 1974). Thigmotropism takes over when the elongating hapteron touches the substratum (Lobban 1978a). Many phototropic responses, both positive and negative, have been recorded (reviewed by Buggeln 1981; Rico and Guiry 1996). Orientation of unicellular rhizoids is more rapid and easier to interpret than orientation of multicellular haptera. Unilateral irradiance is detected by some pigment, possibly phototropin (see above). The information can be stored for several hours, with the response exhibited in subsequent darkness. However, gravity may be the stimulus for rhizoid orientation in *Caulerpa prolifera*. When rhizomes are inverted, rhizoid initiation is preceded by a movement (sinking) of amyloplasts toward the lower side, and rhizoid initials contain numerous amyloplasts (Matilsky and Jacobs 1983).

Morphogenesis depends partly on attachment, if only because orientation depends on consistency in the direction of environmental cues. Unattached seaweeds may remain in place as loose-lying individuals, with little change in morphology, if there is little water movement. If there is extreme water motion, seaweeds will be tossed ashore. Moderate water motion, if it tumbles the thalli, can lead to growth in all directions to form balls, a habit technically called aegagropilous (Norton and Mathieson 1983). Such algae are often morphologically distinct from their attached counterparts (see sec. 3.3.5 on salt-marsh *Fucus*). Aegagropilous forms of coralline algae ("rhodoliths") are harvested as maerl in Europe (Nelson 2009). Species such as *Chondrus crispus* and *Gracilaria tikvahiae* in cultivation tanks also form balls. Many filamentous algae form hemispherical tufts that are restricted by the substratum from growing downward; when free, these will easily form balls. As balls develop from

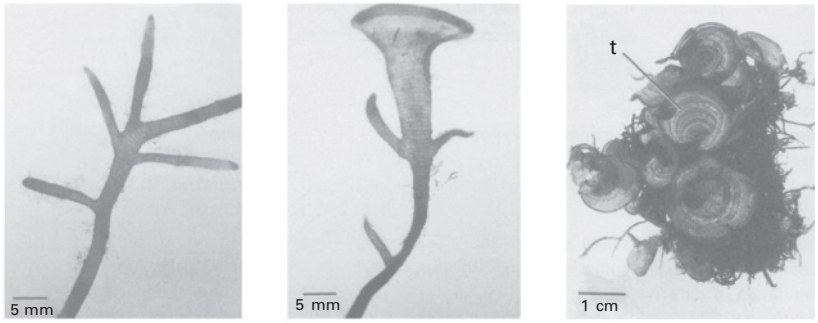


Figure 2.25 Morphological plasticity in *Padina jamaicensis*. (a) In heavily grazed areas, a prostrate, branching thallus with single apical cells forms a dense turf. (b) After grazing is reduced, the typical fan-shaped, calcified thallus begins to form; a row of apical cells develops along the tip of the thallus. (c) Foliose form on older turf after 8 weeks of reduced herbivory. The fan-shaped blades have produced concentric rings of tetrasporangia (t) on their upper surfaces. (From Lewis *et al*. 1987, with permission of the Ecological Society of America.)

fragments, abrasion and grazing damage will tend to increase their compactness by promoting regeneration and proliferation (Norton and Mathieson 1983).

A biotic factor, the presence of epiphytic bacteria, controls the development of the characteristic adult form of some thallose green seaweeds, including *Ulva lactuca*, *Ulva intestinalis*, and *Gayralia* spp. (Provasoli and Pintner 1980; Matsuo *et al*. 2005; Marshall *et al*. 2006). In the normal course of development of *Gayralia oxysperma* (previously *Monostroma oxyspermum*), the biflagellate swarmer produces a filament that divides in three planes to give a little sac, which subsequently ruptures, yielding a flat, monostromatic sheet (Tatewaki 1970). However, if placed in axenic culture, the germinating swarmer will form only a 2-cell thallus consisting of an apical cell (which will slough off cells during subsequent divisions) and a basal, rhizoidal cell. Normal morphology can be restored by addition of exudates of axenically cultured brown and red seaweeds, by growing *Gayralia* in bialgal axenic culture with a red or brown seaweed, and by extracts of seven marine bacteria (out of over 200 isolates tested) in the genera *Caulobacter*, *Cytophaga*, *Flavobacterium*, and *Pseudomonas* (Tatewaki *et al*. 1983). The morphogenetic inducer was isolated by Matsuo *et al*. (2005), who named it "Thallusin". Thallusin (a pyridine) concentrations in natural seawater are very low, and epiphytic bacteria growing on the seaweeds provide a continuous supply to the thallus.

Another example of biotic control of morphology is the morphogenetic switch induced by fish grazing that has been found in *Padina sanctae-crucis* (previously *Padina jamaicensis*; Lewis *et al*. 1987). When grazing is intense, they grow as uncalcified, straplike, creeping branches formed from a single apical cell. In the absence of herbivory, a marginal row of apical cells forms, and the typical erect, calcified, fan-shaped thallus develops (Fig. 2.25). Interestingly, this morphological switch also occurs in *P. boergesenii*, but the effect is dependent on season, indicating an interactive effect between herbivory and abiotic factors (Diaz-Pulido *et al*. 2007a).

2.6.3 Seaweed growth substances

Growth is an oriented process: polarities in cells and thalli are established from the start and are maintained throughout development. For the "accurate execution of developmental programs", communication between different cells and tissues of an organism is essential (Pils and Heyl 2009). Hormones are messenger molecules responsible for communicating between adjacent cells (paracine hormones), or for long-distance communication between different tissues (endocrine

hormones) of animals, terrestrial plants, and seaweed (Buchanan *et al.* 2000; Taiz and Zeiger 2010). Tarakhovskaya *et al.* (2007) list 10 hormones that are found in terrestrial plants which have been identified in seaweeds (see their Table 2). Note that some of the plant hormones listed by Tarakhovskaya *et al.* (2007) (polypeptides and jasmonic acid) are considered by others to be "signaling" or "elicitor" molecules rather than hormones (Buchanan *et al.* 2000; Taiz and Zeiger 2010). For terrestrial plants, the biosynthesis, regulation and action of plant hormones (phytohormones) and other growth substances is well understood, but there are few studies on algae (Tarakhovskaya *et al.* 2007; Stirk *et al.* 2009). The presence of phytohormones in algae suggests some metabolic roles similar to those in terrestrial plants, and recent studies on cytokinins and polyamines support this view. Nevertheless, there is much work to do before a complete picture is built of the biosynthetic pathways and integration of phytohormones in regulation of seaweed growth, reproduction, and physiology.

Early research on algal hormones involved the exogenous application of terrestrial plant hormones to algae, and examining any developmental or physiological response (reviewed by Bradley 1991); more recently such studies have been conducted in conjunction with other environmental variables such as day length or temperature (e.g. Lin and Stekoll 2007). The best-studied phytohormones in seaweeds are cytokinins, which are involved in signal transduction in terrestrial plants; cytokinin-based signal transduction evolved first in the ancestral green algae and is considered a key step in the colonization of terrestrial environments by plants (Pils and Heyl 2009). There are two major groups, "isoprenoid" cytokinins and "aromatic" cytokinins. Stirk *et al.* (2003) identified 19 types of cytokinins in 31 intertidal species from the green, brown, and red lineages. Interestingly, the cytokinin profiles of all 31 were very similar to each other, despite the different evolutionary lines and vertical positions on the shore. They also found little similarity between the cytokinin profiles of seaweeds and terrestrial plants, concluding that "different pathways for regulating cytokinin concentrations operate in macroalgae than in higher plants".

Seasonal patterns of phytohormones, and patterns relating to the zonal position on the shore, have been observed for seaweeds. iPRMP is a ribotide that higher plants use to synthesize cytokinins and for intertidal *Dictyota* and *Ulva*, a seasonal pattern was observed indicating a physiological function relating to higher growth rates in summer (Stirk *et al.* 2009). These findings support earlier work on *Macrocystis* in which increased "cytokinin-like" activity corresponded to higher seasonal growth rates (DeNys *et al.* 1990, 1991). Cytokinin activity was higher for *Ulva* from the high intertidal zone than low-shore *Dictyota*, which may indicate differences between species or a role in signaling environmental stress (Stirk *et al.* 2009). Abscisic acid (ABA) signals environmental stress in terrestrial plants and is a growth inhibitor, and appears to have similar roles in algae. ABA levels in high shore rock-pool *Ulva* were higher than those in low-shore *Dictyota* (Stirk *et al.* 2009). ABA has been detected in sporophytes of *Laminaria digitata*, *Saccharina japonica* and *L. hypoborea* (Schaffelke 1995a). For *L. hypoborea*, endogenous levels of ABA were inversely correlated to seasonal patterns of growth rate, and the external application of ABA inhibited growth under short-day but not long-day conditions. There is also a correlation between mannitol, laminarin, and ABA levels (Schaffelke 1995b).

The auxin indole-3-acetic acid (IAA) has been identified in a range of seaweeds at concentrations similar to those of terrestrial plants. Auxins affect thallus branching, rhizoid development, and the establishment of cell polarity in *Fucus distichus* and *Ectocarpus siliculosus* (Basu *et al.* 2002; Le Bail *et al.* 2010), growth rate of *Pyropia* sporophytes (Lin and Steckoll 2007), growth and sorus formation in *Saccharina japonica*, *Undaria pinnatifida*, and *Alaria crassifolia* (Kai *et al.* 2006; Li *et al.* 2007), and the induction of cell division and reproductive structures (see Tarakhovskaya *et al.* 2007). The metabolic pathways and candidate genes that regulate IAA production, and its role in communicating cell–cell positional information and regulating developmental patterning, have been elucidated for *Ectocarpus* (Le Bail *et al.* 2010; Fig. 2.26). The red seaweed *Grateloupia americana* (previously *Prionitis lanceolata*) has a symbiotic relationship with a

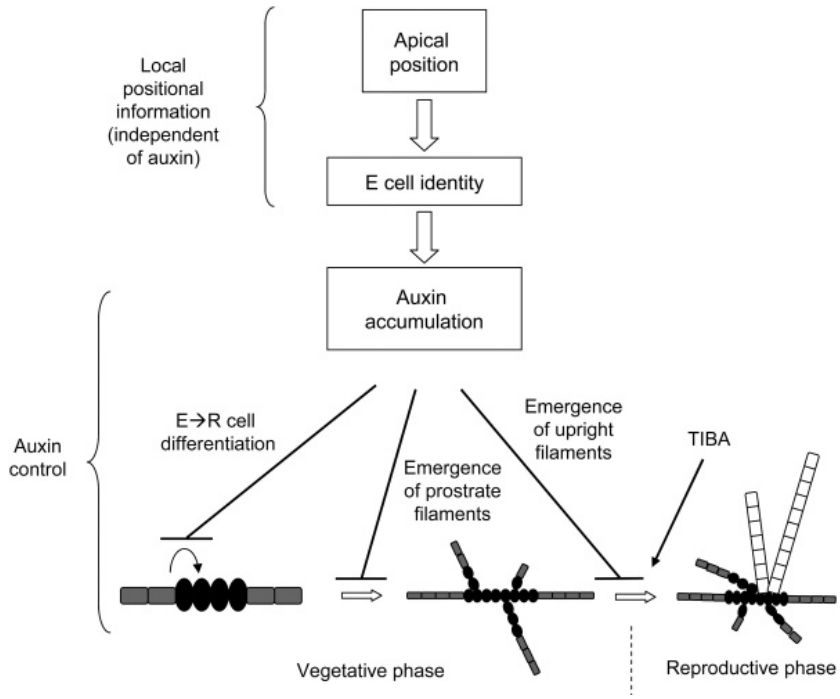


Figure 2.26 Model for the role of auxin in the development of the *Ectocarpus siliculosus* sporophyte. Cells positioned at the apices of the filament acquire the E identity. A higher concentration of auxin is present in these cells, which prevents them from differentiating into R cells and/or inducing branching. As the filament grows, subapical E cells get localized farther from the apex and perceive lower auxin concentrations, which progressively induce their differentiation into R cells as well as branching. Later, auxin maintains its control on the progession of the life cycle by negatively controlling the emergence of the upright filament and thereby the shift to the reproductive phase. Auxin control would then depend on active transport, allowing the apices to maintain control on distant tissues. (From Le Bail *et al.* 2010 ©American Society of Plant Biologists.)

gall-inducing bacteria, and within the galls levels of IAA were on average three-times greater than the surrounding tissue. Whether these high IAA levels in the galls are due to the symbiotic bacteria or host tissue is unclear (Ashen *et al.* 1999).

Polyamines (PAs) (predominantly putrescine, spermidine, and spermine) are low molecular weight aliphatic amines that modulate a wide variety of physiological processes from membrane stabilizations to senescence in all organisms (see Sacramento *et al.* 2004). In seaweeds, they are found in concentrations similar to those in terrestrial plants (Marián *et al.* 2000) and are synthesized from L-arginine via the L-ornithine decarboxylase (ODC) and arginine decarboxylayse (ADC) pathways (Sacramento *et al.* 2004). They are involved in cystocarp development and sporulation in *Grataloupia imbricata* with levels of PAs and ODC decreasing during the transition from infertile to fertile thalli, and the expression of the gene encoding for ODC (GiODC) follows the same pattern (Sacramento *et al.* 2004, 2007; García-Jiménez *et al.* 2009). Polyamines are also involved in stress responses to hyposalinity in *Grateloupia* and seven intertidal green seaweeds (Lee 1998; García-Jiménez *et al.* 2007). Internal levels of polyamine can be adjusted by *de novo* synthesis or by the mobilization of bound endogenous PAs. In *Grateloupia*, moderate hyposaline conditions (18 PSU) caused decreased activity of the enzyme TGase (responsible for binding free PAs), which triggered an increase in free PAs. When PAs

were applied exogenously, the maximum photosynthetic rates of the hyposaline treatment increased relative to the untreated control, indicating that PAs were involved in physiological adaptation to low salinity (García-Jimenez *et al.* 2007). PAs that are bound to the thylakoid membranes of plants and unicellular green algae protect the photosynthetic apparatus from UV-B damage, and this may also be the case for *Pyropia cinnamomea* (previously *Porphyra cinnamomea*) for which PA synthesis was upregulated upon exposure to UV-B radiation (Schweikert *et al.* 2011).

Ethylene is a gaseous hormone, found in the atmosphere in trace levels and is involved in the production and destruction of the ozone layer. In terrestrial plants, it has a well-known role in fruit ripening, but given its gaseous nature it was not considered a likely candidate for seaweed growth regulation. However, Plettner *et al.* (2005) demonstrate ethylene production by *Ulva intestinalis*, levels of which increased when low-light-grown samples were placed under stressful high-light conditions.

Several more phytohormones have been detected in seaweeds but their precise roles are not known (Tarakhovskaya *et al.* 2007). The oxylipins, jasmonic acid (JA) and methyl jasmonate (MJ), that regulate biosynthetic pathways of secondary metabolites in plants have been found in red, green, and brown algae (see Tarakhovskaya *et al.* 2007). Arnold *et al.* (2001) provide evidence that MJ triggers phlorotannin production in *Fucus vesiculosus* suggestive of a role in secondary metabolite induction. However, Wiesemeier *et al.* (2008) could not detect JA or MJ in *F. vesiculosus* nor in six other brown seaweeds, and the exogenous application of the hormones had no effect on the secondary metabolites studied: Any role of JA and MJ in brown seaweeds requires further clarification. Gibberellins are present in *Caulerpa* (Jacobs 1993) and *Fucus vesiculosus* (Table 2 in Tarakhowskaya *et al.* 2007), and have a growth-promoting effect on *Pyropia* sporophytes (Lin and Steckoll 2007). Rhodomorphin was found in *Griffithsia* (Waaland and Cleland 1972, see sec. 2.6.2), and lunularic acid, which signals environmental stress in liverworts, is found in *Enteromorpha* (Table 2 in Tarakhowskaya *et al.* 2007).

2.6.4 Wound healing and regeneration

Thallus damage is a fact of life for seaweeds and the major sources of injury are herbivores, parasites, epiphytes, sand abrasion, and wave forces. Seaweeds must be able to heal the injury and a different sequence of events takes place for wound healing in multicellular seaweeds compared to coenocytic seaweeds. In multicellular seaweeds there is no need for the cut cells to recover; instead, the wound is sealed, a process that involves changes in the underlying cells. Siphonous algae, however, risk lethal cellular hemorrhaging if a wound cannot be sealed immediately; wounding triggers a biochemical cascade that leads to the formation, within 2 min, of a gelatinous "wound plug" of cellular material that seals the cell, and under this plug a new cell wall can be assembled (Welling *et al.* 2009). Menzel (1988) presented a generic six-step sequence of events applicable to most siphonous green algae (Fig. 2.27): (1) repair of the cell membrane; (2) contraction of the cut edge of cytoplasm; (3) extrusion of the plug precursor material from vacuoles in which they are stored; (4) restoration of turgor pressure; (5) formation of a wound plug; and (6) formation of a new cell wall.

There are two general biochemical processes for wound plug assembly (step 5) in siphonous green algae: lectin-carbohydrate interactions (*Dasycladus vermicularis*, *Bryopsis*, *Microdictyon umbilicatum*, *Chaetomorpha*, and *Codium*) and protein cross-linking (*Caulerpa taxifolia*) (Welling *et al.* 2009). Lectins are proteins that bind reversibly with carbohydrates, and algal lectins differ from those of higher plants by having a lower molecular weight and high affinity for oligosaccharides and glycoproteins (rather than monosaccharides of higher plants). In 2005, Kim *et al.* isolated a novel lectin from *Bryopsis plumosa*, which they called bryohealin, which is more closely related to the fucolectins of some invertebrates than the lectins of higher plants (Yoon *et al.* 2008). When *B. plumosa* is injured, the cellular

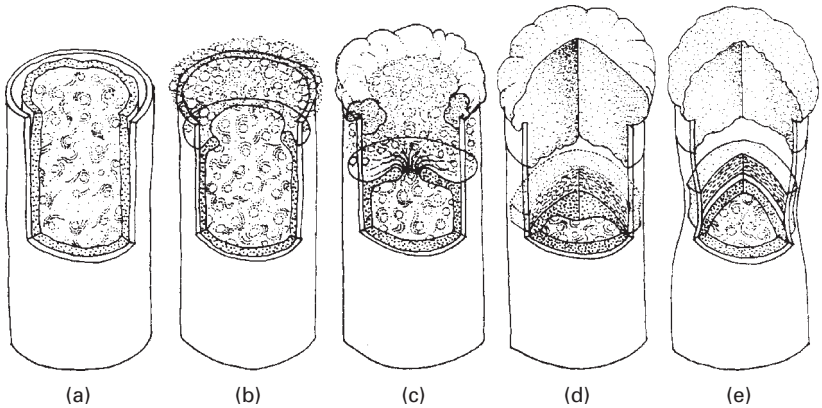


Figure 2.27 Wound healing in *Bryopsis*. Cutaway diagrams show changes in cell contents in the hour following a wound. (a) Undamaged siphon has a peripheral layer of cytoplasm and a large central vacuole filled with plug precursor material. (b) At 15–30 s after the siphon is cut, the cytoplasm begins to retract and form a concentric closure. Plug precursor is expelled; it swells and adheres to the edge of the cut wall. (c) After about 1 min, the cytoplasmic contraction is almost complete; the plug precursor coagulates, and the wound plug begins to form. (d) At 5–10 min after wounding, the wound plug begins to develop internal and external layers. (e) Within an hour, new cell wall has formed under the internal plug and begins expanding. (From Menzel 1988, with permission of Springer-Verlag, Berlin.)

contents spill out but the organelles then aggregate, and generate a new cell membrane around themselves, forming a protoplast which can eventually grow into a new individual (Kim *et al.* 2001b; Grossman 2005; Fig. 2.28). Bryohealin not only controls organelle aggregation, it also confers protection of the protoplast from bacterial contamination in a manner similar to the anti-pathogenic role of fucolectins in invertebrates (Pak *et al.* 1991; Kim *et al.* 2005; Yoon *et al.* 2008). In another example, the one-celled protoplasts formed following wounding of *Microdictyon umbilicatum* have two fates: 30% become mature *Microdictyon*, but the rest become quadriflagellate reproductive swarmers, and this could be a mechanism for dispersal (Kim *et al.* 2002). Similarly, protoplasts of *Chaeotomorpha aerea* develop into aplanospores or biflagellate swarmers (Klotchkova *et al.* 2003).

The biochemical signaling pathways for cell repair are being eludicated. When *Dasycladus vermicularis* is wounded, the cellular contents are extruded and within 1–2 min they form a sticky, protective gel. This solidifies at 10 min post-injury, and is fully "hardened" by 35–45 min (Ross *et al.* 2005a, b). The hardening process is preceded by a nitric oxide (NO) emission at 25 min post-injury, and followed by a burst of reactive oxygen species (ROS; oxidative burst; sec. 7.1 and Essay 5) at 45 min that causes the final cross-linking of plug constituents (Ross *et al.* 2006). NO is a common signaling molecule in higher plants and animals, but this was the first record of it in seaweed. In another first, Torres *et al.* (2008) show that for *D. vermicularis* and *Acetabularia acetabulum*, extracellular ATP (eATP) released upon wounding signals the production of ROS and NO.

Caulerpa taxifolia employs protein cross-linking to seal damaged cells. In this case, the secondary metabolite caulerpeyne (which is a mild anti-feedant) is deacetylated to oxytocin 2 (a 1,4-dialdehyde), 30 s after wounding. Oxytocin 2 is an aggressive protein cross-linker and proteins are recruited to it within a few seconds of wounding. The resulting polymer of proteins and secondary metabolites not only forms the foundation of the wound plug in *Caulerpa*, it also reduces palatability to herbivores possibly by reducing the food quality (Jung and Pohnert 2001; Weissflog *et al.* 2008).

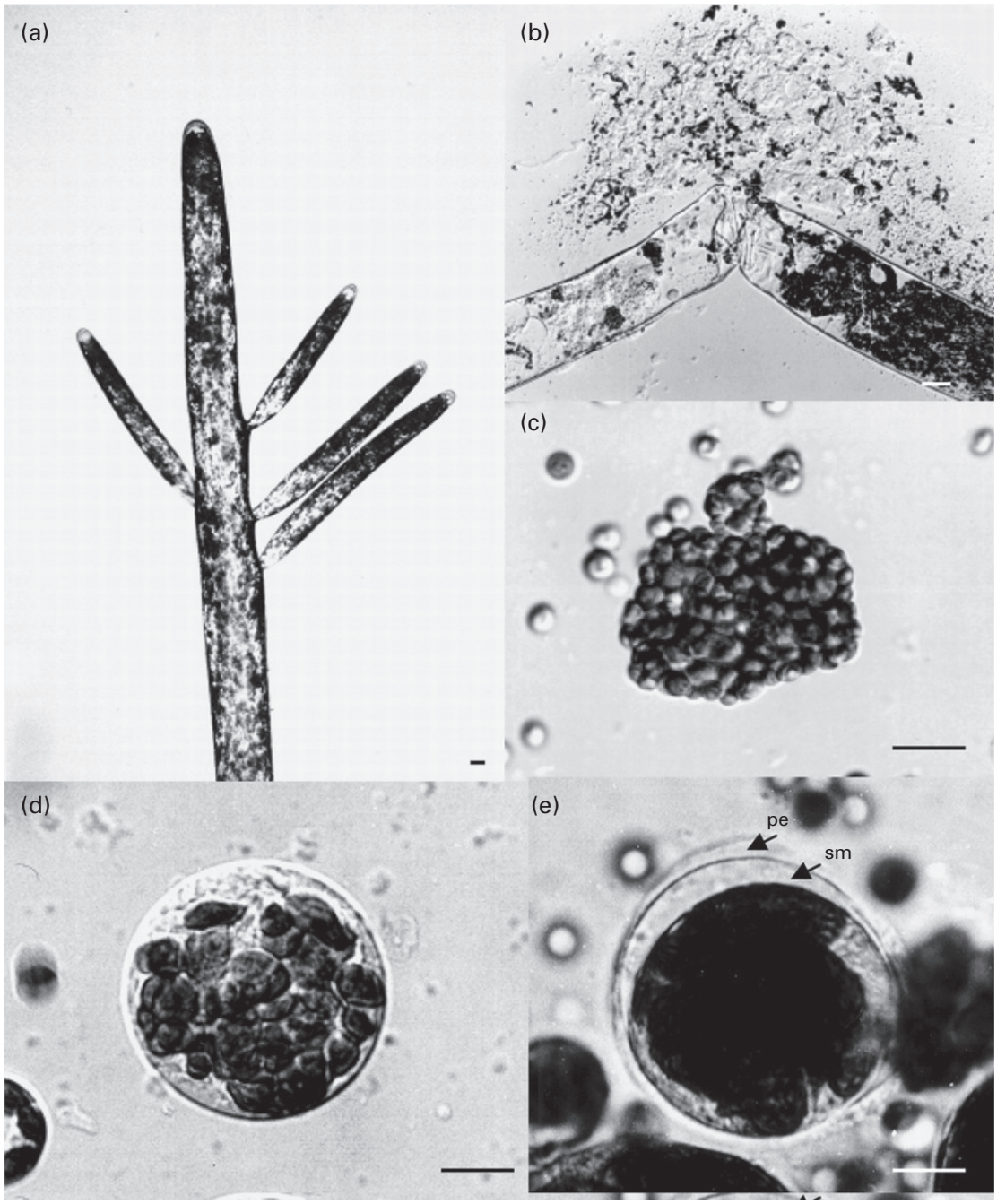


Figure 2.28 The sequential process of sub-protoplast regeneration from disintegrated cells of *Bryopsis plumosa*. (a) Vegetative plant with distichous branches. (b) Protoplasm comes out from the wounded cell and spreads in seawater. (c) Aggregation of the extruded cell organelles in seawater. (d) Regenerated sub-protoplast with a primary envelope 20 minutes after wounding. (e) The secondary lipid-based membrane inside the primary envelope 12 h after wounding (pe, primary envelope; sm, secondary membrane). Scale bars = 10 μm. (From Kim *et al.* 2001a © The Company of Biologists Ltd.)

A different process is seen in Siphonocladales, an order that is characterized by unique segregative cell division (e.g. *Ernodesmis, Boergesenia, Valonia)* (La Claire 1982a, b). In most cases, no plug is formed; rather, the cytoplasm retracts from the wound, again the work of actin microfibrils (La Claire 1989, reviewed by Shepherd *et al*. 2004), and then closes around the central vacuole in one or a few pieces, or breaks up into many protoplasts. The latter process looks much like segregative cell division and the production of gametangia.

Wound healing in multicellular algae has been most thoroughly studied in *Fucus vesiculosus* (Moss 1964; Fulcher and McCully 1969, 1971), *Sargassum filipendula* (Fagerberg and Dawes 1977), *Kappaphycus alvarezii* (previously *Eucheuma alvarezii*; Azanza-Corrales and Dawes 1989), and *Ecklonia radiata* (Lüder and Clayton 2004), and the processes for each of these seaweeds are similar. In the fucoids, the thin, perforated cross-walls of the medullary filaments are plugged after about 6 h with newly synthesized sulfated polysaccharide (presumably fucoidan). Later there is general accumulation of polysaccharide at the wound surface. Medullary cells adjacent to the damaged cells round off and become pigmented. After about a week they give rise to lateral filaments, which elongate and push through to the wound surface, where they branch repeatedly to form a protective layer. Cortical cells undergo longitudinal division (parallel to the wound surface), and the outer cells assume the cytological and functional characteristics of epidermal cells (e.g. they become pigmented). Cells of the medulla may also contribute to the formation of new epidermis. For *Ecklonia*, phlorotannins help seal cells by precipitating proteins, and also protect against microbial attack (Lüder and Clayton 2004). For *Kappaphycus*, cut cells lose their contents, while proteinaceous and phenolic substances accumulate at the pits of cortical and medullary cells just below the cut. After a few days, cellular extensions begin to grow from underlying cells, proliferate, and form a layer of new, pigmented cortical cells below the wound (Azanza-Corrales and Dawes 1989).

Wound healing is commonly followed by either regeneration or proliferation. The simplest kinds of regeneration involve uniseriate (branched or unbranched) filaments having apical growth. After the wound has healed, growth continues, as in the example of *Sphacelaria* (Fig. 2.24). There are well-documented cases of wound healing and regeneration in *Anotrichium tenue* (*Griffithsia tenuis*) and *Griffithsia pacifica* (Waaland and Cleland 1974; Waaland 1989, 1990). If filaments are severed, a rhizoid is produced from the base of the apical portion, and a new apical cell is regenerated on the basal portion (Fig. 2.29a). If, instead, an axial cell is killed, but the wall remains intact, the filament repairs itself (Fig. 2.29b). A regenerating rhizoid is produced by the apical fragment, and a special repair-shoot cell, not an apical cell, is produced by the basal fragment. This repair-shoot cell is induced by species-specific rhodomorphins, which diffuse out of the regenerating rhizoid. The repair-shoot cell grows toward and fuses with the regenerating rhizoid (Watson and Waaland 1983, 1986). Some seaweeds produce proliferations from cut surfaces; these are lateral outgrowths of cortical filaments, as in the red algae *Gigartina* (Perrone and Felicini 1976) and the brown *Dictyota* (Gaillard and L'Hardy-Halos 1990). The type of tissue produced in the reds – rhizoidal or bladelike – depends on the position of the wound with respect to the apex or base of the thallus. In other words, there is a correlation with an internal thallus polarity.

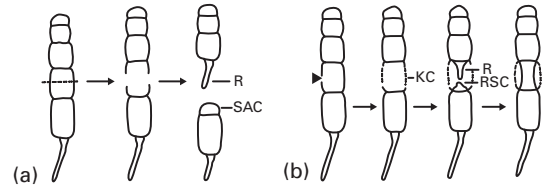


Figure 2.29 Cell regeneration (a) versus cell repair by cell fusion (b) in *Griffithsia*. When the filament is severed, a rhizoidal cell (R) and a new-shoot apical cell (SAC) form, and two separate filaments develop. If an axial cell is killed (KC), the rhizoidal cell fuses with a repair-shoot cell (RSC) and makes a new living link in the filament. (From Waaland 1989, with permission of John Wiley/Alan R. Liss Inc.)

Regeneration in *Caulerpa* is also correlated with cell polarity. Excised blade "leaves" regenerate rhizomes and rhizoids from the basal end and new leafy shoots

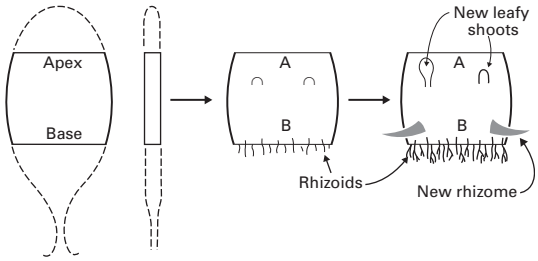


Figure 2.30 Regeneration from a portion of a "leaf" of *Caulerpa prolifera*. Leafy shoots form at the original apical end, and rhidoids form at the basal end. (From Jacobs 1970, with permission of the New York Academy of Sciences.)

from the apical end (Fig. 2.30). Rhizomes regenerate first rhizoids from the apical end, and later rhizoids from the basal end plus rhizome and leafy shoots from the apical end (Jacobs 1970, 1994). In Jacobs' experiments, "leaf" segments 30 mm long formed only rhizoids; if 40 mm long, half the specimens also formed a rhizome and a new leaf; if 50 mm long, all regenerated completely. However, leafy shoot production and rhizoid production from the rhizome of *Caulerpa* also respond to gravity, as shown by Jacobs and Olson's (1980) experiments, in which uninjured thalli were turned upside down. Rhizoids were produced from the new lower side, and leafy shoots from the new upper side (the rhizome did not twist, so polarity had been reoriented). Following wound healing, fragments of the tropical, calcifying Caulerpalean seaweed *Halimeda discoidea* also produce rhizoids; tiny pieces of 15 mm^2, that were cut on three of four edges, were able to produce rhizoids in 3 days, a mechanism of vegetative reproduction (Walters and Smith 1994).

The process of regeneration in *Fucus* is unusual in that distinct embryos, rather than lateral branches, are formed, although damaged holdfasts can regenerate adventitious shoots (McCook and Chapman 1992). During the process of wound healing in this genus, epidermal cells in certain regions of the wound begin to divide perpendicular to the wound surface, forming groups of branch initials (visible macroscopically after 4–6 weeks in culture), which develop directly into adventive embryos (Fulcher and McCully 1969, 1971). The midrib region of the thallus regenerates much more rapidly than the wings (Moss 1964), correlating with the abundance in the midrib region of medullary filaments, which are primarily responsible for formation of new epidermis. Regeneration from vegetative branches always gives rise to vegetative shoots. Regeneration of strips cut from the discolored frond beneath spent receptacles of the deciduous species *F. vesiculosus*, although extremely slow, results in branches with small receptacles at their tips. Branches regenerated from strips cut from male thalli bear male receptacles, and those from female thalli bear female receptacles (Moss 1964).

This concludes our survey of the seaweed life histories, modes of reproduction, and morphological development. In the following chapters we shall examine the communities and habitats in which seaweeds live, the biotic factors they face, and the ways in which they are affected by abiotic factors.

2.7 Synopsis

Seaweed life histories can follow several patterns, depending on the species and the environment. An alternation between two free-living stages – one a haploid gametophyte, the other a diploid sporophyte – is common, but many variations exist. Some seaweeds have dissimilar sporophytes and gametophytes; others have only one free-living stage. There is no direct relation between ploidy level and morphology and so many variations of life cycles are possible, including changes between microthalli and macrothalli of the same chromosome number.

The life of a seaweed is a complex sequence of interactions between its genetic information and its abiotic and biotic environment. Development, from the initial polarization of the spore or zygote to the production and release of reproductive cells, is a highly co-ordinated process. Light (quality and quantity), photoperiod (usually the length of uninterrupted darkness), and temperature are the principal environmental cues. In some seaweeds, the onset of reproduction is triggered as a "response" to the prevailing environment conditions. Others "anticipate" the seasons using an endogenous clock which is entrained

by environmental triggers (Zeitgebers; typically photoperiod). Most seaweeds exhibit short-day responses to photoperiod. Seaweeds also respond to different wavelengths of light but the nature of the receptor pigments and their mode of action are largely unknown. Several glycoproteins have been identified as regulatory factors that control the life-cycle progression of *Ulva*.

Sexual reproduction may be isogamous, anisogamous, or oogamous. "Broadcasters" liberate gametes into the water column and have external fertilization, whereas "brooders" have internal fertilization as the egg is retained on the female. Syngamy is regulated by cell recognition mechanisms on cell/flagella surfaces. Motile gametes in brown algae may be attracted to each other or to a stationary egg by volatile pheromones. In red algae, sexual reproduction often involves complex post-fertilization development of a carposporophyte for zygote amplification.

Settlement of spores or other reproductive structures depends a great deal on water motion (turbulence and eddies), notwithstanding the limited capacity of some cells for oriented swimming. Spores of *Ulva* can select their settlement site by responding to chemical cues from bacteria in the biofilm, and to surface energy. Cells then attach to the surface with adhesive substances. At first they are susceptible to being resuspended, but the chemical bond to the surface becomes stronger over time. Following attachment, cells acquire polarity and then germinate into rhizoidal cells and thallus-forming cells.

Erect thalli characteristically have an apico-basal polarity, which is expressed in the position and kind of regenerative outgrowths on wounded thalli and sometimes in apical dominance. Morphological development and the adult form depends on intrinsic factors such as phytohormones, extrinsic factors including light, temperature, and nutrients as well as grazing and epiphytic bacteria, and size/shape inequities between daughter cells. Some cells are totipotent and regenerate an entire thallus, whereas others cannot be regenerated. The morphological development of many seaweeds is plastic, and the genes responsible for regulating such development are being identified.

Wound healing is an important function in seaweeds, which are continually subjected to damage by grazers and abrasion. In siphonous algae, rapid plugging of the wound takes place to prevent cytoplasm loss. In multicellular algae, cut cells usually die, and wound healing is accomplished by the underlying cells. Regeneration commonly takes place from cut surfaces, with either frondlike or rhizoidlike tissue produced as a function of the distance from the dominant apex.