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Environmental and internal control of seasonal growth in seaweeds

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Abstract

Elaborate control systems activate and inactivate growth meristems of certain seaweeds so that their seasonal phases of growth and rest are exactly synchronized to the environmental annual course. Recent tank experiments with kelp species demonstrate the existence of endogenous, circannual clocks which are synchronized to the period of the natural year by the annual course of daylength.

Introduction

Many seaweeds exhibit distinctive seasonal growth cycles. The question, 'which environmental factor drives these cycles?', has fascinated phycologists since the turn of the century. Today we know for several seaweed species that no environmental factor directly causes these seasonal growth rhythms, but that they are produced by the plant itself, *i.e* they are endogenous rhythms. The role of the environmental signals is only to synchronize the period of the endogenous rhythms to exactly one year. How we came to this insight, that phycologists had much to learn from the zoologists in this respect, and which experiments on kelps were performed with which results, I would like to present in the following.

The phenomenon: start of growth in winter and growth reduction in summer

Many seaweed species start new growth in winter and stop or reduce growth rate in summer, often with easily detectable differences between previous and current growth, as will be illustrated by several examples. The arctic kelp *Laminaria solidungula* starts new blade growth in November, near to the onset of the arctic winter darkness, attains its maximum growth rate in May, and after this blade growth slows down during the arctic summer, all with little seasonal change of ambient seawater temperatures (Chapman & Lindley, 1980; Dunton, 1985). Each annual blade generation is separated by a constriction from the previous year's blade. The same is evident in the cold-temperate kelp *L. hyperborea* which sheds the old blade mostly in May. New growth of the blade starts in December, throughout the whole distribution range of the species from northern Norway to Portugal, and stops by June (Kain, 1963, 1979; Lüning, 1969), or July in first-year plants (Lüning, 1979).

Constrictions between the old and new terminal blade are also evident in *Pterygophora californica*, a northeast Pacific representative of the Alariaceae, whose members have their sporangia on special sporophylls (lateral blades). The meristem at the top of the perennial stipe produces a new terminal blade and several new sporophylls every year from February to June. The last year's terminal blade and sporophylls are shed in February to March of the following year (De Wreede, 1984, 1986; Hymanson *et al.*, 1990). Constrictions between the previous and current year's blade growth can also occur in *L. saccharina*, but are not to be seen in *L. digitata*. Both species occur in arctic-cold temperate regions. In both species, new blade growth begins in December (Parke, 1948; Pérez, 1969) with an increase in cell divisions and re-establishment of a new surface meristoderm in the dormant winter tissue (Conolly & Drew, 1985b).

A North Pacific digitate species with some outer resemblance to the North Atlantic *L. digitata* and again with no constrictions between old and new blade is *L. groenlandica*, which bears the name *L. bongiardiana* on the Asiatic side of the North Pacific (Petrov, 1972). New blade growth is initiated in January, with a seasonal peak of maximum blade size in April–May and a somewhat delayed seasonal peak in first-year plants (Druehl *et al.*, 1987).

Before leaving the phenomenon of seasonal growth in kelps, we should also call attention to the fact that the growth cycles are paralleled by the well-known seasonal cycles in content of reserve materials, with peaks of laminaran and mannitol in late summer and minima in spring, after being used up by respiration and growth in winter and early spring (Black, 1950; Haug & Jensen, 1954). There is also a minimum content of the growth hormone abscisic acid during the rapid-growing phase in *Laminaria hyperborea* and *L. digitata* and a maximum content during the slow-growing phase (Schaffelke, 1992).

Apart from kelps, a good example for new growth starting in winter and stopping in summer is found in *Desmarestia aculeata* with new, light-brown coloured regenerating branches arising from December onwards and senescing in summer (Chapman & Burrows, 1970, 1971). Another example is *Delesseria sanguinea* with tiny new blades at the turn of the year and growth stop in late summer (Kain, 1984, 1987).

Evolutionary advantage for seaweeds to start new growth in winter and reduce growth rate in summer

In the field, one observes the well-known coincidence between the seasonal cycles of the primary environmental factors and the growth cycles of seaweeds. Nutrient content of the seawater is high and temperature, light intensity, and daylength – as a possible environmental signal – are low in winter, when growth starts, and the environmental parameters reverse, when growth slows down in summer. We may ask why this macroalgal strategy developed. In the course of evolution the primary ecological factors exerted selection pressure on the organisms to perform certain activities at certain times of the year. Birds must have been selected to breed in spring in order to use the maximum food supply available at this season for the offspring (Gwinner, 1986). As for perennial seaweeds, it is advantageous to start growth from stored carbon in early winter, well ahead of the spring phytoplankton bloom, because of the wealth of nutrients in the seawater after remineralization of the plankton since the autumnal storms. In summer, when the bulk of annual light supply arrives, it is 'wise' to stop or reduce growth activity, even if sufficient nutrients were available, and allocate the assimilated carbon to reserve materials in order to survive the winter with its little light – and to start growth again, at first from reserve materials. One may imagine that only seaweeds adopting this strategy, called 'season anticipators' by Kain (1989), did well in polar and temperate regions in the deeper, light-poor water. In summary, one may say in an evolutionary sense that in fact the primary ecological factors, the 'ultimate factors' (Thomson, 1950), caused the evolution of the seasonal seaweed growth cycles, the annual nutrient cycle in the seawater being probably a main factor.

Do the primary ecological factors directly drive the growth cycles?

The question is then, not why, but how seaweeds manage to start growth in winter and reduce growth rate in summer. It may seem quite natural that phycologists putting up hypotheses to explain this and looking for factors to drive the seaweed growth cycles, at first paid attention to the primary environmental factors themselves.

The discussion became complicated due to the fact that for many years only field observations on growth cycles of the larger macroalgae were available. Decisions on the question which primary ecological factor exactly would be the main driver were difficult if not impossible, since in the field several factors change at the same time. The development of hypotheses in regard to annual cycles of temperature or nutrients as possible drivers of the growth cycles will be discussed in the following. The role of the annual cycle of daylength, which is a driver of photoperiodic systems and a synchronizer for circannual systems (Farner, 1985; Gwinner, 1986), will be treated after presentation of the circannual rhythms in kelps.

Temperature cycle as a driver of the growth cycle?

Temperature was proposed to be the main factor by Kniep (1914) and Harder (1915). These investigators performed short-term measurements of respiration and photosynthesis at different water temperatures in spring, e.g. in *Laminaria saccharina*. They found that, with increased temperature, respiration increased more than photosynthesis, and assumed, erroneously, that growth rates might become reduced in summer because of too high respiration values and a lack in photosynthetic surplus during the warm season. Low temperatures in winter, correspondingly, were thought to start new algal growth because of favourable low respiration rates and a maximum net gain of photosynthesis. Later it was found for several kelp species that, first, there is seasonal adaptation of respiration (Kanwisher, 1966; Lüning, 1971), second, respiration occurs at a higher rate in growing than in non-growing thalli, and third, the main net gain of photosynthesis at temperate and polar latitudes is in fact attained in summer and carbon is then allocated mainly to storage, when the bulk of the annual light supply arrives (Lüning, 1971; Hatcher *et al.*, 1977; Johnston *et al.*, 1977). Enzymatic acclimation and almost constant photosynthetic rates at growth temperatures between 5 and 20 °C were noted in *L. saccharina* by Davison (1987) and Davison & Davison (1987).

Nutrient cycle as a driver of the growth cycle?

Many years after the hypothesis of Kniep and Harder on temperature control of seasonal growth cycles in seaweeds the cycles of nutrient availability in the seawater were thought to exert direct control on seasonal growth cycles, with emphasis on nitrate. Feeding natural populations of *Laminaria longicruris* with additional nitrate from June to October increased the growth rate until August, but could not prevent growth reduction from September to October (Chapman & Craigie, 1977). Similarly, Conolly & Drew (1985a, b) were able to halt the late spring decline in growth rates of *L. saccharina* and *L. digitata* by artificial fertilizing with nitrate and phosphate. This demonstrated that the nitrate content of the seawater may temporarily control the amplitude of growth rate, just as favourable or adverse temperatures or light intensities as other primary ecological factors do.

The question remained open, however, whether the environmental nitrate cycle in the seawater – as an external signal – can direct the complete annual growth cycle and allocate the assimilated carbon to either growth or storage. Would the growth cycles also continue at constant high nitrate content of the seawater and otherwise constant conditions of temperature in experimental seawater tanks? Buggeln (1978), employing short-term tank experiments, could not prevent reduced summer growth in *Alaria esculenta* under cold, nitrate-enriched conditions in tanks at ambient solar radiation and photoperiod. Nitrogen was only a growth-limiting factor from April to early June. Moreover, regression analysis in a field study by Druehl *et al.* (1987) on *L. groenlandica* revealed that seasonal changes in growth rate were most closely associated with temperature, followed by salinity, and then light intensity, neither nitrate nor phosphate being significantly associated with growth rate. Different species and different age classes of the same species may behave differently under a given set of environmental conditions. This enforced the difficulties involved in interpreting field data.

It was clear that these complications could only be overcome by long-term cultivation of laminarian sporophytes of different species under controlled conditions in the same seawater tank system in a laboratory. This approach lead to the discovery of endogenous circannual growth rhythms in kelps, as we will see below. Zoologists had laid the ground for the discovery of endogenous annual clocks in animals about 30 years ago, with a steadily increasing number of successful experiments and much theoretical progress since then. Several basic principles of circannual rhythms in animals were re-discovered in kelps, so a short excursion to the zoological field of circannual rhythmicity will help to understand the basic findings in kelps to be reported after the zoological interlude.

Circannual rhythms in animals: the circannual clock as a driver

Organisms with an endogenous circannual clock or rhythm continue their annual cycles in the laboratory in constant conditions, e.g. of temperature, food or nutrient supply, and daylength. The period (*i.e.* the time span for a whole cycle) of the free-running rhythms deviates as a fixed value in most cases from 12 months (as indicated by the prefix 'circa'). Because of the continuation of circannual rhythms without concurrent periodic changes in the environment, circannual rhythms behave like physical oscillators in the absence of periodic driving agents and represent autonomous oscillating systems (Gwinner, 1986). Free-running rhythms may occur also in nature under special conditions, e.g. in rodent animals in darkness during their hibernating phase, or in organisms during the arctic polar night, or in organisms near to the equator at constant daylength.

Since the early 1960s circannual rhythms have been demonstrated with strong evidence in about 40 animal species in laboratory culture under constant conditions. Examples are mostly migrating birds (e.g. cycles of molt and testicular size), mammals (e.g. hibernation, body weight, milk production, breeding activity, antler replacement), and a few reptiles, fish, molluscs, arthropods and coelenterates (Gwinner, 1986). Strong evidence for circannual rhythms requires that at least two free-running cycles were measured and that the period of the free-running rhythm deviates from 12 months. With periods close to one year there is the danger that some environmental cycle has caused the observed rhythmicity. The periods of most circannual rhythms were found to be shorter than a year, often around 10 months (Gwinner, 1986), as if the internal oscillator would anticipate what is up-coming.

A special problem resides in the fact that free-running circannual rhythms in certain species – and it should be emphasized again that the free-run is the only good proof of the endogenous nature – may have the tendency to hide themselves from the eyes of the investigator. Which constant light-dark regime shall we choose in the laboratory for, say, three years to discover a free-running circannual rhythm? 24 h light per day would be ideal, but not all organisms flourish in this condition, for example several algal species do not tolerate continuous light. Equally important, it has been found that the expression of the free-running circannual rhythms are often confined to a narrow constant daylength regime. The well-investigated European starling *Sturnus vulgaris* provides an extreme case: In nature, testicular size increases in spring and decreases in autumn, followed by the molt. In the laboratory, the free-running circannual rhythms of molt and testicular size are only expressed at constant daylengths of 11 or 12 h light per day (Gwinner & Wozniak, 1982; Gwinner, 1986, 1989; Gwinner *et al.*, 1989). At shorter constant daylengths than 11 h light per day testicular width never decreases and the gonads remain continually active – no rhythm develops. At daylengths longer than 12 h light per day the gonad size goes through one cycle, and gonads subsequently remain small with no future size increase. So if the investigator chooses the wrong constant daylength regime for a certain species, he may never find the free-running rhythm – a good starting-point may be 12 h light per day.

As another remarkable aspect, most zoologists regarded the case of the European starling 30 years ago as a photoperiodic example, like all other animal rhythms known to be circannual today. The increase of testicular width exactly every 12 months in spring and the decrease in autumn, followed by the molt, were thought to be driven by subsequent passages of critical daylengths. Only long-term rearing of the birds at 12 h light per day over several years revealed the circannual nature of the reaction. So this is what may happen with some or maybe many of the 50 or so known photoperiodic reactions in seaweeds (see enumerations in Dring, 1984; Lüning, 1990; Kain & Norton, 1990), if phycologists become sufficiently patient to observe the development of photoperiodic organisms in future longer than in the usual experiments on photoperiodism.

The period of the internal oscillator of a circannual organism must be synchronized to exactly 12 months – otherwise the organism would be lost already in the second year, doing the wrong thing at the wrong time. The most powerful synchronizing or entraining factor (also called 'zeitgeber' in chronobiological terminology), is for all circannual systems the annual cycle of daylength, in nature and in the laboratory (Gwinner, 1986). The biological rhythm easily adapts to the period of 12 months, but also to shorter periods, e.g. 6 or 3 months, or even 2.3 months in the case of molt and testicular size in the European starling (Gwinner, 1981, 1986). In terms of physical oscillator theory, the forcing or driving oscillator is the annual course of daylength, and the driven oscillators are the biological circannual rhythms. As in circadian systems, it is possible that other strong zeitgeber cycles besides the photocycle, or weaker ones, may also be used by circannual systems, for example the temperature cycle, but little is known in this respect so far (Gwinner, 1986).

Circannual rhythms secure a sequential order of development, and may be especially important where there are few periodic changes in the environment, e.g. in tropical regions. A tropical bird, the African stonechat, exhibits circannual rhythms, e.g. of testicular width, as ascertained for an investigation span of 7.5 years, the longest circannual reproductive rhythm yet documented (Gwinner & Dittami, 1990). This suggests that phycologists should also search for circannual rhythms in tropical environments, where the annual course of daylength is lacking as a zeitgeber.

Circannual rhythms in higher plants and dinoflagellates

As for the plant kingdom, observations of early botanists indicated the possibility of annual rhythms persisting under constant conditions, e.g. in tree growth periodicity at tropical latitudes (see reviews by Sweeney, 1969; Bünning, 1973; Borchert, 1973; Gwinner, 1986). Compared to zoological evidence, however, there are so far only few botanical examples for free-running circannual rhythms. Examples are germination capacity of seeds, with an in-built resting phase of several months (e.g. Bünning, 1949; Spruyt *et al.*, 1987, 1988), dry matter production in the duck weed, *Lemna minor* (Bornkamm, 1966), or light-induced hook opening and leaf expansion of bean seedlings (Spruyt *et al.*, 1983).

For marine biologists it is interesting to note that the seagrass *Posidonia oceanica* exhibits circannual growth cycles (Ott, 1979), and, even more surprising, circannual clocks govern cyst formation and germination capacity of certain dinoflagellates, again with an in-built resting phase of several months in the resting stages, as in seeds (Anderson & Keafer, 1987; Costas & Varela, 1989). Also, growth rates of dinoflagellates exhibit circannual rhythmicity (Costas & Varela 1988, 1989). The finding of circannual clocks in dinoflagellates indicates the possibility of universal distribution of circannual clocks among eukaryotic organisms, like circadian clocks.

It may not be accidental that circannual growth rhythms in the plant kingdom have preferentially been detected so far in aquatic organisms, such as the duck weed, a seagrass and dinoflagellates. This may simply mean that cultivation under constant conditions for several years may be technically more difficult with terrestrial than with aquatic higher plants, or algae. Seaweeds are, for this reason, possibly good candidates for the detection of further circannual growth rhythms, and it may not seem surprising that first attempts with long-term cultivation experiments on kelp sporophytes lead quickly to the discovery of free-running circannual growth cycles, as will be seen in the following.

Free-running circannual growth cycles in the kelp *Pterygophora californica*

Long-term cultivation of the larger seaweeds under constant conditions (as constant as possible), requires nevertheless some technical efforts and should take place in seawater tank systems with continuous mixing of the water bodies of the individual, light-tight tanks in order to prevent nutrient and other chemical differences between the seawater in different tanks. With this in mind, a cultivation system consisting of 20 300-l-seawater tanks in 5 constant-temperature rooms was constructed in 1986 in the Central Laboratory of the Biologische Anstalt Helgoland. Continuous water exchange between all tanks was secured by continuous pumping, and macronutrients were held constant at around 40 μM nitrate and 4 μM phosphate (see Lüning, 1988, for further technical details). Illumination was rather weak, with only two fluorescent tubes inside each light-tight hood of each tank, this resulted in a photon fluence rate of 20–30 μmol m^{-2} s^{-1}.

A circannual growth rhythm was found in sporophytes of the northeast Pacific kelp *Pterygophora californica* grown in the laboratory at Hamburg from filamentous gametophytes to a length of several cm in glass beakers and transferred to the described tank system (Lüning, 1991). Figure 1 shows, as an example, the growth rhythms of the terminal blades of four individual sporophytes cultivated for somewhat more than two years. Every 44 weeks or 10 months on average, and with individual deviations, the basal meristem of the terminal blade was activated and formed a new light-brown coloured blade, clearly separated by a constriction from the old dark-brown blade. Maximum growth rates were attained around 14 weeks after the start of the new blade, whereafter growth rates declined and a resting phase with little or no growth began (Fig. 1), while the new blade became dark-brown.

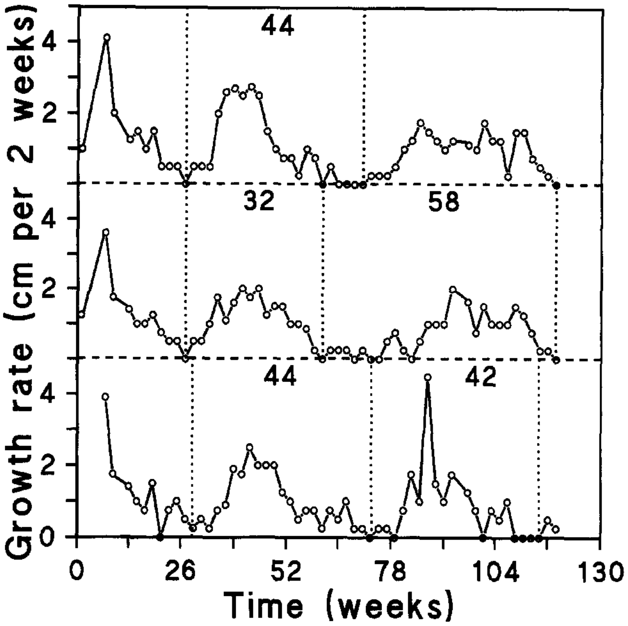

Fig. 1. Free-running circannual growth rhythms in terminal blade production of the kelp *Pterygophora californica* cultivated at 16 h light per day and 5 °C. Measuring points are 2 weeks apart. Vertical dotted lines indicate the first observation of a new blade, numbers within the graph indicate the period for each blade cycle in weeks (from Lüning & Kadel, 1993).

It should be stated here that the low light-levels of 20–30 μmol m^{-2} s^{-1} employed may have resulted in the fact that only two kelp species grew quite successfully in our tank system over several years and attained considerable total lengths of up to 50 cm, *i.e.* *Pterygophora californica* and *Agarum cribrosum* (see below). In nature, both are low-light and shadow-loving kelps (Lüning, 1990). It was not possible to obtain such big sizes in *Laminaria setchellii* (see below), and virtually impossible in several kelp species from the upper sublittoral. Attempts are under way to cultivate such presumably more light requiring kelp species at 100 μmol m^{-2} s^{-1} by means of metal halide lamps on top of the light-tight hoods of the seawater tanks.

Synchronization of growth by the annual cycle of daylength

The growth rhythm of *Pterygophora californica* could be easily synchronized (entrained) to a period of 12 months by exposing the experimental algae to an annual cycle of daylength as a zeitgeber, e.g with the period of 12 months (Fig. 2a). New blade growth started after the minimum of daylength, that is after subjective December, and attained a maximum before maximum daylength occurred in subjective June (Fig. 2a). Figure 3 presents the photographs of one of the 10 sporophytes used for each experimental group whose

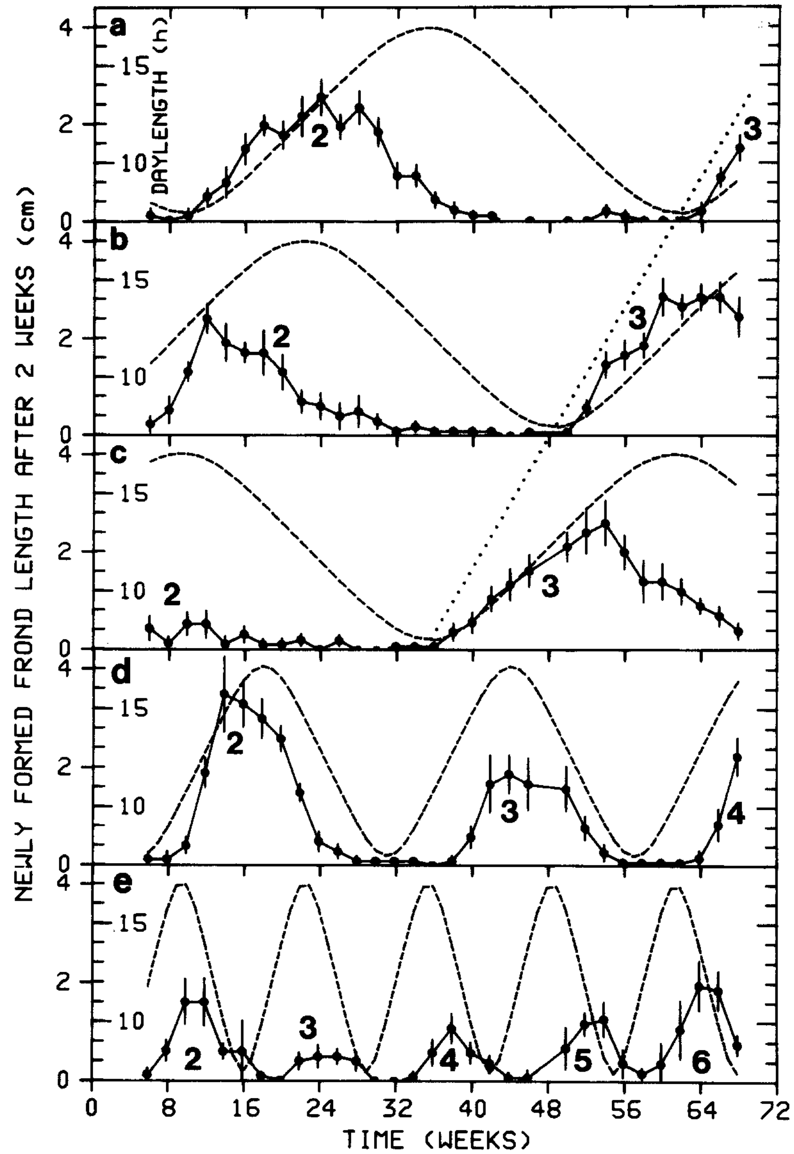

Fig. 2. Growth rhythms (solid curves with standard deviations; $n = 10$) in terminal blades of *Pterygophora californica* cultivated at 10 °C and synchronized (entrained) by sinusoidal changing cycles of daylength (broken lines; daylength for latitude 54°). Numbers within the graphs indicate successive blade generations. In the upper 3 diagrams (a–c) the period of the entraining rhythm was 12 months, but the phase was successively shifted by 3, or 6 months, as indicated by the oblique dotted line. In (d) the period of the entraining rhythm was 6 months, in (e) 3 months (from Lüning, 1991).

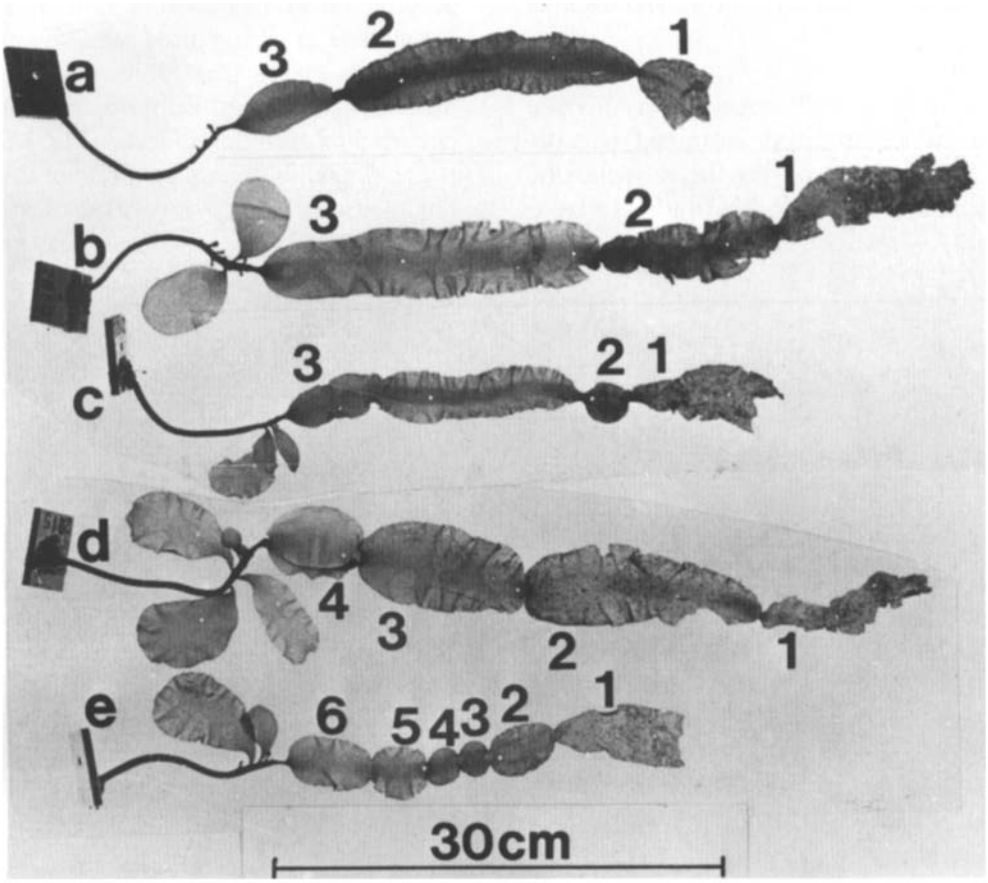

Fig. 3. Examples of sporophytes of *Pterygophora californica* entrained by artificial cycles of daylength; the growth curves of the sporophytes are illustrated in Fig. 2. Numbers indicate successive blade generations. Plants a–c were entrained by an annual cycle of daylength with a period of 12 months and formed, correspondingly, a new blade every 12 months. Plants d and e experienced entraining cycles of daylength with periods of 6, or 3 months, and formed a new blade every 6, or 3 months, correspondingly. Deciduous sporophylls are to be seen as lateral blades in b-e and were produced during the rapid-growing phases (from Lüning, 1991).

growth curves are represented in Fig. 2. Constrictions between successive blades clearly marked the annual blade generations (e.g. Fig. 3a).

What happens, if sporophytes which are just about to start a new blade cycle in subjective winter are abruptly transferred to the spring or summer phases of a daylength cycle, at otherwise constant conditions? The results in this respect are shown in Figures 2b-c and 3b-c. A 3-months-advance of the zeitgeber curve resulted in a shorter blade no. 2 because of shorter duration of the ascending part of the zeitgeber curve, and an earlier start of blade no. 3 (Figs 2b, 3b), if compared to the controls without a phase-shift of the zeitgeber curve (Figs 2a, 3a). A 6-months-advance meant a 'catastrophy' for blade no. 2 (Fig. 3c) because of the sudden exposition to long days in subjective summer (Fig. 2c), and a correspondingly earlier start of blade no. 3 (Fig. 2c).

The fact that the growth curves easily followed phase-shifts of the entraining daylength cycles, as indicated in Fig. 2a–c, means that there is now a simple method to produce the successive, vegetative growth phases of *Pterygophora californica* (and other seaweeds; see below) at the same time in the laboratory, at constant temperature and identical nutrient levels, by exposing the algae in parallel light-tight tanks or cabinets to different phases of the entraining annual cycle of daylength.

Synchronization by 'shortened years' and time-lag of the biological rhythm

The growth curves of *Pterygophora californica* also followed entraining daylength cycles with shorter periods, e.g. 6 months (Fig. 2d), or 3 months (Fig. 2e). The blades generations were again easy to detect by constrictions. The longest blades were produced at a period of 12 months (Fig. 3a), shorter or very short blades at periods of 6, or 3 months respectively (Fig. 3d, e).

Although the biological growth curves followed 'like slaves' the entraining daylength cycles with periods of 12, 6, or 3 months, the biological rhythms lagged behind in that measure, in which the period of the entraining rhythm became shorter (Fig. 2). Maximum growth rate occurred well before the maximum of daylength at a period of 12 months, both maxima were near to each other at a period of 6 months, and the growth maximum appeared well after the daylength maximum at a period of 3 months (Fig. 2a, d, e). These phase-relationships between the entraining and the biological curves remained fixed from cycle to cycle. They reflect a well-known rule of oscillator theory according to which the maximum of the driven oscillator lags behind the maximum of the driving oscillator (zeitgeber) in that measure, as the period of the driving oscillator becomes shorter. This rule has often been confirmed in circadian rhythms (Aschoff & Pohl, 1978; Aschoff, 1981) and circannual rhythms, e.g. also in the case of the European starling cited above (Gwinner, 1986).

The fact that new blade growth in *Pterygophora californica* starts after the annual minimum of daylength and culminates well before the longest days are encountered, in nature and in the experiment described (Fig. 2a–c), reflects thus a special situation dictated by the natural zeitgeber period of 12 months. The fact that the phase-relationships between the biological and the zeitgeber rhythms may be quite different with other zeitgeber periods, demonstrates that this kelp like other circannual organisms behaves as an oscillating system and is not bound to start growth when daylength is short or stop growth when daylength is long, like a photoperiodic organism.

The '6-months-year', which is easily produced by leaving out every second week in the zeitgeber cycle, may actually be a good growing condition for seaweeds in general, if they react similarly to *Pterygophora californica*. The blades obtained at a zeitgeber period of 6 months were still rather big (Fig. 3d), the sporophytes did not rest for half a year in the tanks, as with periods of 12 months (Fig. 2a–c), but re-started the new blade earlier (Fig. 2d). This reduced considerably the risk of losing plants due to epiphytes or herbivory during the resting phase.

Independence of vegetative and reproductive cycles

Deciduous sporophylls were formed as lateral blades by the meristem on the top of the stipe during rapid-growth phases (Fig. 3b–e), but only, for unknown reasons, by about half of the sporophytes. Sporangia were never observed on the sporophylls of the experimental sporophytes of *Pterygophora californica* in our tank system, and again the reasons are unknown. One hypothesis is that the high nutrient content in the tanks might prevent reproduction. Sanbonsuga & Hasegawa (1969) were able to retard growth and induce sporangium formation in fully laboratory-grown sporophytes of the kelp *Costaria costata* cultivated in 40-l-plastic tanks by using plain instead of enriched seawater.

The fact that the vegetative growth cycle of *Pterygophora californica* free-runs as a circannual system and is easily synchronized by the annual course of daylength, without any interference of reproductive structures, demonstrates the relative independence of the growth and reproduction cycles. Also sporophylls, as long as they are not covered by sporangia, can be regarded as vegetative structures obeying the basic growth cycle, since sporophylls were only produced during the rapid-growth phase, and the position of this was dictated by the annual cycle of daylength as a zeitgeber, or – at constant daylength – by the free-running circannual clock.

Continuous growth in constant short days

The constant condition of 8 h light per day seems to provide a strange situation for *Pterygophora californica* because the intercalary meristem in constant short days continuously produces new blade tissue, *i.e.* the circannual clock seems not to be able to provide the 'growth-off-signal' (Lüning, 1991). This is only possible at constant daylengths of 10–16 h light per day, while 24 h light per day is not tolerated over long terms for *P. californica* (Lüning & Kadel, 1993). Thus, only at 10–16 h light per day does the circannual clock of *P. californica* control the autonomous growth rhythms, while it is possibly arrested at short daylengths. Continuous growth at 8 h light per day has so far been detected also in several other kelp species in our seawater tank system (see below) and reminds us of the above-mentioned situation in the European starling, in which the rhythmic increase and decrease of testicular size takes place only at constant daylengths of 12 or 13 h light per day and the 'size-down-signal' cannot be provided by the circannual clock in short days (Gwinner, 1986, 1989). Hence, the 'critical daylength' known from photoperiodic studies revisits us through the back-door due to the restriction of the expression of circannual rhythms to certain constant daylength ranges in certain species.

Circannual growth rhythms in other kelps: *Laminaria setchellii*, *L. hyperborea* and *L. digitata*

Free-running growth cycles and synchronization of growth by daylength cycles as a zeitgeber have also been detected by tank experiments in sporophytes of the northeast Pacific kelp *Laminaria setchellii* grown in the laboratory from gametophytes (tom Dieck, 1991), and *L. hyperborea* and *L. digitata*, native species to the North Atlantic and also occurring near Helgoland (Schaffelke, 1992). The free-running periods were 11–17 months, so somewhat variable, in *L. setchellii* (tom Dieck, 1991), 9–10 months in *L. hyperborea*, and 6–8 months in *L. digitata* (Schaffelke, 1992). *L. setchellii* exhibited its circannual growth rhythm at 16 h light per day and grew continuously at 8 h light per day (tom Dieck, 1991), thus it is similar to *Pterygophora californica* (Lüning, 1991). The permitted daylength range seems to be narrow in *L. hyperborea*, since free-running growth cycles occurred only at 12 h light per day, and not at 8 h (continuous growth), or 16 h (almost continuous rest). *L. digitata* exhibited an unexpected behaviour, with continuous growth at 16 h light per day and free-running growth cycles – as opposed to the other kelp species investigated so far – at 8 h light per day (Schaffelke, 1992). Also closely related animal species may differ considerably in the conditions for expression of circannual rhythms (Gwinner, 1986), and the ecological significance of these differences remains to be resolved in the kelps under study.

Photoperiodic reactions in seaweeds: The annual cycle of daylength as a driver

What is the difference between a photoperiodic and a circannual system? A photoperiodically reacting organism, e.g. an alga at favourable conditions of temperature, nutrient supply and light intensity, waits for the annual course of daylength to surpass a certain critical daylength. Only then the effect occurs, e.g. the onset of growth or reproduction, but the organism requires this signal, otherwise the next developmental phase will not be entered. An organism with a circannual clock can autonomously enter the next phase of the developmental cycle, whose period is only 'stretched' or 'compressed' according to the period of the daylength cycle as a zeitgeber, as we have seen above.

There is quite a number of seaweed species with known growth synchronization by the annual cycle of daylength, but no circannual rhythms have been detected so far, as was the situation a few years ago for all seaweed species (Lüning & tom Dieck, 1989). Examples are the kelps *Agarum cribrosum*, *Pleurophycus gardneri*, *L. groenlandica* (= *L. bongardiana*), and *L. saccharina*, all with new blades in seawater tanks being formed right after the minimum of annual daylength. Another example is provided by the brown alga *Desmarestia aculeata* in which the whole developmental sequence from young to senescing regenerating branches can be synchronized at constant temperature of 5 °C by the annual course of daylength (Lüning & Kadel, 1993). All these examples must be interpreted as potentially photoperiodic systems, at least for the time being. Similar examples have been provided for Antarctic seaweeds cultivated under fluctuating Antarctic daylength by Wiencke (1990a, b). An example among algae for a transfer from the photoperiodic to the circannual lists of examples is new blade induction in *L. hyperborea* after short-day treatment, which was interpreted as a photoperiodic effect after short-term tank cultivation for 8 months (Lüning, 1986), but has been recognized as a circannual rhythm after tank cultivation for two years (Schaffelke, 1992).

Sorus production in kelps, as far as it is under daylength control, must also be regarded as a genuine photoperiodic effect so far. Transfer of vegetative sporophytes to short-day conditions induced sorus formation in sporophytes of *Laminaria saccharina* collected in the field in summer and transferred to the Hamburg seawater tank system (Lüning, 1988). Similar short-day effects were observed in fully laboratory-grown sporophytes of *L. setchellii* (tom Dieck, 1991), *Phyllariopsis brevipes* (Henry, 1987), and *Alaria crassifolia* (Nakahara, 1984). Although a circannual clock drives the growth cycle in *L. setchellii* at 16 h light per day, as mentioned above, only recurrent new blades appear under this condition, and no sorus was observed (tom Dieck, 1991). This is in contrast to the example of the European starling in which both the 'vegetative phase' (molt) and the reproductive phase (gonadal cycle) occur during the free-running circannual rhythms (Gwinner, 1986).

Interestingly, after the transfer to short-day conditions, a period of retarded growth preceded sorus formation, and a new blade was started soon after in *L. saccharina* (Lüning, 1988) and *L. setchellii* (tom Dieck, 1991). The abrupt stop of growth activity in a currently growing blade after a sudden transfer from long to short days and the subsequent formation of a new blade generation in continuing short-day conditions (although with continuous growth thereafter) has been observed also without interference of sorus formation in *L. setchellii* (tom Dieck, 1991) and *L. hyperborea* (Schaffelke, 1992). In laboratory slang, we call the sudden transfer to short days the 'short-day hammer' which hammers down the activity of a presently active meristem and starts a new blade shortly thereafter. Termination of growth and sorus induction may be independent processes, as suggested by tom Dieck (1991), the first process indicating a drastic synchronization effect on the circannual growth rhythm, and the second a genuine photoperiodic reaction.

Outlook

Algae with circannual rhythms may become important objects for research on basic chronobiological processes and molecular biological events underlying circannual rhythmicity, since algae are easier to cultivate than many more highly organized organisms. Apart from this, it seems possible that among the multicellular algae not only kelps may have a circannual clock, but also smaller seaweed species, since the circannual clock has now been found even in unicellular algae. Phycologists should search for circannual rhythms in perennial stages such as crustaceous thalli and microthalli. Algal cultivators have often found that material from their stock cultures sometimes grows and reproduces readily and sometimes not, so there may be circannual rhythms behind this which have to be still detected. Since many of the photoperiodic reactions in seaweeds relate to the induction of reproductive organs (Dring, 1984; Lüning, 1990; Kain & Norton, 1990), it will be interesting to see whether they too will have to be regarded as genuine photoperiodic reactions, or whether circannual systems are hidden behind. Fresh-water algae may be as important as subjects for observation, since there is evidence for an annual rhythm in growth rate and zygote formation in *Spirogyra insignis* under constant laboratory conditions, although followed only for one cycle so far (Costas & Lopez-Rodas, 1991).

Furthermore, studies on life histories of algae have been almost exclusively performed in constant daylength regimes, that means the investigators were possibly working sometimes or often with free-running rhythms, without thinking of it. Those cycles which went through all their successive stages autonomously, may be good candidates for further search of true circannual rhythms, but of course one must work longer than for just one cycle. Those cycles that became arrested at a certain stage may imply circannual rhythms that are free-running only in certain constant daylength regimes. As a rule, it would be better for life history researchers to use instead of constant daylengths the powerful seasonal cycle of daylength as a zeitgeber, *i.e.* to change every week daylength in their culture cabinets, in order to be sure that they will get the whole life history cycled through and do not end with a cycle arrested due to a wrong constant daylength regime for a free-running developmental cycle. For fast investigators the '6-months-year' or '3-months-year' may be recommended.

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