REVIEW

STRESS TOLERANCE IN INTERTIDAL SEAWEEDS

Ian R. Davison[1] *and Gareth A. Pearson*

Department of Plant Biology and Center for Marine Studies, Deering Hall, University of Maine, Orono, Maine 04469-5722

ABSTRACT

Intertidal seaweeds are periodically exposed to air where they experience a variety of potentially stressful environmental conditions, including nutrient limitation, high light, high and low temperature, desiccation, and osmotic stress. This paper considers the current understanding of stress tolerance in intertidal seaweeds and discusses ways in which future research could increase our understanding of the role of environmental factors in the ecology and physiology of these algae. We believe research is required in at least three areas. 1) Laboratory physiological studies have established that correlations exist between stress tolerance and the vertical distribution of species. However, little information is available on the importance of stress in determining community structure in nature. Field experiments are essential to relate the impact of single or multiple stresses on the survival, growth, and reproductive output of macroalgae. In paticular, it is necessary to clarify the role of sublethal stress in determining the outcome of competitive interactions. 2) With the exception of obvious lethal effects or damage associated with extreme environmental conditions, such as unusually hot and dry weather, it is difficult to evaluate the occurrence and severity of stress in natural populations of seaweeds. There is a need to develop molecular and biochemical markers specific for individual stresses or groups of stresses to allow the unambiguous and direct determination of stress in situ. 3) Despite the apparent importance of stress in intertidal seaweeds, we are largely ignorant of the mechanistic basis of tolerance. The application of currently available tools of molecular and cell biology to the investigation of stress-induced transcriptional and translational changes could enormously increase our understanding of both the sites of, and pathways involved in, stress tolerance. In summary, there are numerous unanswered fundamental questions about the stress tolerance of intertidal seaweeds, providing opportunities for research ranging from field ecology to molecular biology and biochemistry.

Key index words: active oxygen; desiccation; freezing; intertidal seaweeds; nutrient limitation; photoinhibition; stress physiology

The communities of animals and plants that inhabit the intertidal zone of rocky seashores are freqently dominated by dense populations of macroalgae that exhibit distinct patterns of vertical distribution. These patterns have interested ecologists and phycologists for almost a century (e.g. Baker 1909, 1910, reviews by Zaneveld 1969, Chapman 1986, Norton 1986), and the mechanisms underlying species distributions and interactions are still the subject of considerable research (Lubchencho 1978, 1986, Chapman and Johnson 1990). The ecology of fucoid brown algae, a major component of many intertidal communities, has recently been reviewed by Chapman (1995). A major focus has been, and remains, the effect of the physical environment on intertidal organisms (which, with very few exceptions, are marine in origin). The reasons for this are clear; intertidal communities are relatively simple assemblages that exist in a habitat where a steep gradient of environmental variation occurs over a small (vertical) spatial scale, from marine to fully terrestrial. Tidal emersion exposes intertidal organisms to a variety of potentially adverse environmental conditions including high light, freezing, high temperature, desiccation, and osmotic shock, and also renders essential resources, such as nitrogen and phosphorus, obtained from seawater temporarily inaccessible. Here we review the literature on the effect of these factors, and focus on those areas that we believe provide new and important opportunities for research. Our review is necessarily subjective; there are undoubtedly many important avenues for future research that are not covered here. However, we will have succeeded in our objective if this paper stimulates wider interest in the physiology of marine macroalgae and results in an increase in our understanding of the mechanisms and ecological importance of stress tolerance. Wherever possible we refer to earlier reviews or original papers that provide good summaries of paticular topics. We have not attempted to incorporate predictions about stress into general models that seek to explain ecological interactions in the intertidal zone because we believe there is insufficient information to do so. However, we do argue for the incorporation of careful and quantitative determinations of stress into ecological investigations and attempt to identify those areas where more information is required.

WHAT IS STRESS?

It is clear that stress must be defined in terms of the response of an individual rather than the value of a particular environmental variable. For example, optimum temperatures for growth of tropical seaweeds may exceed the upper lethal limit of many Antarctic species. Grime (1989: p. 4) defined stress as "external constraints limiting the resource aquis-

---

[1] Author for reprint requests.

ition, growth or reproduction of organisms." Although broadly speaking we have retained Grime's definition in this paper, we have found it useful to differentiate between reductions in growth rate that occur because of an inadequate supply of resources (= limitation stress) and those which result from damage due to adverse conditions (or the allocation of resources to prevent damage), which we refer to as disruptive stress. Examples of limitation stresses include low light and insufficient levels of inorganic nutrients such as nitrate or phosphate. Disruptive stresses include conditions such as freezing or desiccation that either cause cellular damage or require the allocation of resources to prevent and/or repair damage. Note that a particular factor is defined as nonstressful, limiting, or disruptive by the response of the individual, population, or species, and not by the environmental variable itself.

There is some overlap between our definition of disruptive stress and Grime's (1979: p. 39) definition of disturbance ("mechanisms that limit the plant biomass by causing its partial or complete destruction"). The loss of tissue that results from frost damage to *Laminaria* is an example of a process that is both a disruptive stress and a disturbance. However, damage due to *Littorina* grazing is a disturbance but not a disruptive stress, whereas a reduction in growth due to severe desiccation that does not result in loss of tissue or biomass could be an example of disruptive stress that would not be recognized as a disturbance. The distinction is important because subtle effects of adverse environmental factors that reduce growth but do not result in obvious damage are likely to be particularly important in, for example, influencing the outcome of interspecific competition.

One problem with Grime's definition of stress is that it is comparative and requires information on performance under unstressed conditions. Measurements of performance (e.g. growth or reproductive output) at a single location can not establish if stress is occurring. Not only is information required on growth rates and reproductive output of the same species under other conditions, but one must also consider the possibility of genetic differences between populations. For example, suppose that individuals of the kelp *Laminaria digitata* grow more slowly in upper-shore rock pools than at the sublittoral fringe. There are two possible explanations: tide pool algae may be subject to greater limitation or disruptive stress or, alternatively, the difference in growth may reflect genetic differences between the two groups. Reciprocal transplant experiments could differentiate between the two possibilities. However, if these support the hypothesis that tide pool plants are subject to greater stress, it would be uncertain, and difficult to determine, which factors were responsible (e.g. high temperature, photoinhibition, C or N limitation). A further problem with Grime's definition is that growth rates cannot

be measured instantaneously (an important consideration for slow-growing species), and in many field situations it is difficult, if not impossible, to measure growth at all. This discussion indicates the difficulty ecologists face when considering the occurrence and consequences of stress. As discussed below, one of the challenges for seaweed physiologists is to develop unambiguous molecular or biochemical markers for specific stresses that can be used to determine the occurrence and severity of stress in nature.

The overall ability of an organism to cope with adverse conditions (commonly called stress resistance) is a function of stress tolerance at the cellular level and/or avoidance mechanisms that prevent stress affecting cells. Desiccation resistance of saccate seaweeds such as *Colpomenia* and *Halosaccion* is based on stress avoidance that depends upon central sacs of seawater that prevent cellular dehydration (Oates 1985, 1986, 1988). In contrast, desiccation resistance in the upper-shore species *Fucus spiralis* depends upon the ability to recover from a high degree of cellular dehydration (Dring and Brown 1982, Madsen and Maberly 1990). With the exception of a few seaweeds, such as young thalli of the saccate species noted above, the degree of resistance of most intertidal seaweeds to physical stress depends on tolerance of cellular stress. Although differences occur in the rates of water loss between different species due to thallus morphology, despite initial controversy (e.g. Dorgelo 1976), it is now established that the difference between desiccation resistant and susceptible species is not related to the rate of water loss during emersion (Dromgoole 1980, Schonbeck and Norton 1980a, Dring and Brown 1982, Lipkin et al. 1993). Similarly, freezing resistant and susceptible species freeze at the same temperature indicating that resistance is based on cellular tolerance rather than avoidance (Pearson and Davison 1993).

THE IMPORTANCE OF STRESS IN THE INTERTIDAL ZONE

Disruptive Stress

Most research on stress in intertidal algae has focused on stresses that are disruptive in the species studied, including high light (Henley et al. 1992), high temperature (Bell 1993, Kübler and Davison 1993), freezing (Pearson and Davison 1993, 1994), and desiccation (Lipkin et al. 1993) (earlier references can be found from these papers); desiccation has received by far the most attention. The ability to withstand emersion is a major determinant of whether an alga can occur in the intertidal zone, and sublittoral algae are excluded because they have a very limited resistance to desiccation and freezing when compared to intertidal species (e.g. Dring and Brown 1982, Davison et al. 1989). The key question is: To what extent are interspecific differences in stress tolerance important in structuring communities within the intertidal zone? In the case of the

upper shore species *Pelvetia canaliculata* and *Fucus spiralis*, it is apparent that the upper distributional limits observed in nature are at, or very close to, the limits of the fundamental niche (see Chapman 1986). Plants close to the upper limit exhibit visible damage during hot dry spells and plants transplanted higher on the shore die (Schonbeck and Norton 1978). This work has been extended by Chapman and Johnson (1990), who employed transplant experiments involving both adult and early post-settlement stages (three-day-old germlings). However, it is important to realize that, although transplant experiments may provide support for the hypothesis that physical factors are responsible for the observed upper distributional limits of certain species, they do not test alternative hypotheses such as the failure to recruit higher on the shore (see Underwood 1986, Underwood and Fairweather 1989). Future studies should incorporate tests of alternative hypotheses that cannot be rejected by simply finding stress limitation. Another example of lethal stress controlling upper distributional limits is the effect of freezing on sublittoral fringe species of *Laminaria* (Todd and Lewis 1984). These studies emphasize the importance of considering the effect of seasonal changes in environmental factors on the ecology of species. For example, in Maine *L. digitata* and *L. saccharina* are essentially summer annuals in the lower intertidal zone, with sporophytes recruiting from the perennial population of subtidal plants in the spring and surviving until the first severe frosts in the fall (pers. obs.).

The importance of the episodic nature of stress over smaller temporal scales was demonstrated by Brawley and Johnson (1991) and Davison et al. (1993). Zygotes of *Pelvetia fastigiata* displayed age-specific survival patterns (Brawley and Johnson 1991). Furthermore, survival in the habitat where successful recruitment occurs (a red algal turf) was dependent upon the occurrence of "recruitment windows," when emersion coincided with benign (nondesiccating) climatic conditions. Until recently, ecologists and physiologists interested in the intertidal zone have focused almost exclusively on the behavior of adult seaweeds. Although recruitment is now an active area of research, there is a need for more information on the physiological responses of juvenile stages and their potential role in determining the distribution of adults. Because of their small size, microscopic stages of seaweeds can occupy distinct microhabitats at a single tidal elevation (e.g. Brawley and Johnson 1991), a factor that needs to be considered when designing physiological experiments. Emersion stress is likely to be particularly important in propagules and juvenile stages of macroalgae because 1) they settle over a wider range than that occupied by the adults (e.g. Schonbeck and Norton 1979b), 2) they are more susceptible than adults, and 3) stress tolerance changes during early development (Brawley and Johnson 1991, Davison

et al. 1993). For example, Brawley and Johnson (1991) found that 6-h-old zygotes and 1-wk-old embryos of *P. fastigiata* exhibited greater survival than 24–48-h-old embryos during natural emersion. Furthermore, even those *P. fastigiata* zygotes and embryos that survived natural emersion experienced reductions in photosynthesis on reimmersion indicating that sublethal disruptive stress may be important (Davison et al. 1993). Sublethal stress delays rhizoid development in *P. fastigiata*, possibly increasing the susceptibility of zygotes to dislodgement by water motion (Vadas et al. 1992).

Interactions occur between acclimation processes and developmental stage. An example is provided by some of our recent unpublished work on freezing tolerance of *Fucus* spp. Adult plants grown at 5° C were more freezing tolerant than plants grown at 15° C. However, the opposite was true for germlings, with 15° C grown germlings being more freezing tolerant than germlings of the same age grown at 5° C. The explanation is that freezing tolerance increases rapidly during the first 48 h of development, and that development, and hence the acquisition of tolerance, occurs more rapidly at 15° C. In addition to the intrinsic importance of the physiological responses of juvenile stages to stress, they are a more tractable experimental system than adult plants in which to address some of the fundamental questions outlined below. For example, we are able to separate proteins extracted from *Fucus evanescens* germlings using SDS-PAGE but are unable to do so with adults plants, presumably because of interference from large quantities of mucus and/or polyphenolics.

Although pointed out by Schonbeck and Norton (1978) and repeatedly since then (e.g. Hawkins and Hartnoll 1985, Chapman and Johnson 1990), it is worth reemphasizing that the evidence that upper distributional limits are controlled by lethal stress applies only to a few upper- and lower-shore species such as *Pelvetia canaliculata* and *Laminaria digitata*, and there is no evidence that this is a general mechanism controlling the upper limits of all, or even most, species in the intertidal zone. This does not necessarily mean that stress is unimportant but rather that its ecological importance depends upon sublethal rather than lethal effects. A key question in intertidal ecology concerns the importance of sublethal stress and the costs and benefits of stress tolerance in interspecific competition. Most intertidal seaweeds are able to survive and grow below their lower distributional limit provided that lower-shore species are removed (see Chapman and Johnson 1990). However, with some exceptions, such as upper-shore species *Pelvetia canaliculata* and *Fucus spiralis*, this is also true for upper distributional limits, i.e. algae are able to grow higher on the shore provided upper-shore species are removed (see Hawkins and Hartnoll 1985, Chapman and Johnson 1990). Although other explanations are possible (e.g. propagule dispersal, settlement, and herbivory) and need

to be tested, the available data are consistent with the hypothesis that competitive exclusion controls both upper and lower limits of many intertidal algae. Furthermore, the outcome of the presumed competition may be mediated by sublethal stress. For example, one of the few studies of competition between intertidal macroalgae indicated that *F. vesiculosus* is the competitive dominant over *F. spiralis* in the midshore region dominated by the former species (Chapman 1990). However, *F. spiralis* appears to be the dominant species higher on the shore; *F. vesiculosus* can only colonize and grow in the *F. spiralis* zone if *F. spiralis* is removed (Hawkins and Hartnoll 1985, Chapman and Johnson 1990). *Fucus spiralis* is less susceptible to sublethal stress from desiccation and freezing (Dring and Brown 1982, Davison et al. 1989), suggesting that the reversal of the competitive outcome in the *F. spiralis* zone may occur because more frequent and prolonged emersion reduces growth to a greater extent in *F. vesiculosus* than in *F. spiralis*. A similar mechanism (i.e. the regulation of competitive interactions by sublethal stress) has been proposed for the interaction between the red algae *Chondrus crispus* and *Mastocarpus stellatus* (Dudgeon et al. 1989).

Our understanding of the role of physical stress in the ecology of intertidal macroalgae would be greatly enhanced if future investigations more closely integrated physiological measurements with field manipulations. Well-designed field experiments can be used to identify the role of factors such as herbivory, competition, and recruitment in controlling community structure (see Chapman and Johnson 1990). However, it is often necessary to make inferences about the occurrence and severity of stress, and it is difficult to determine what physical factors are most important because several environmental factors covary from high to low shore. Knowing if stress is due to, for example, desiccation or nutrient limitation is important because different types of stress induce different physiological responses and may have different ecological outcomes. On the other hand, although laboratory physiological experiments provide very precise information on the effect of specific physical factors, it is often difficult to apply the results to the natural environment because insufficient attention has been paid to subjecting plants to ecologically relevant conditions; the authors of this review are by no means blameless in this regard. The following discussion, together with that on photosynthesis in air (see section on limitation stress), provides some indication of the problems with the ecological interpretation of physiological studies of stress tolerance. We should emphasize that we are not criticizing the papers we cite but rather are using them to 1) illustrate the types of problems that occur when relating the data from laboratory studies to the situation in nature, and 2) to indicate where further research is necessary.

Wiltens et al. (1978), Dring and Brown (1982),

and others (Smith and Berry 1986, Brown 1987, see Zaneveld 1969 for a review of earlier work) demonstrated that a direct relationship exists between the effect of desiccation on photosynthesis and the vertical distribution of species, with upper-shore seaweeds having the highest desiccation tolerance. Although some differences occur in the degree of inhibition following desiccation, the primary difference between tolerant and susceptible species is in the extent to which photosynthesis recovers when algae are reimmersed in seawater. For example, the percentage water loss that could be tolerated with complete recovery of photosynthesis increased from 55% in the sublittoral fringe species *Laminaria digitata* to 90% in the high-shore species *Pelvetia canaliculata* and *Fucus spiralis* (Dring and Brown 1982). In most cases, photosynthesis is significantly inhibited immediately following desiccation, but complete or partial recovery to control (nondesiccated) rates occurs rapidly (within 2 h). Where photosynthetic rates do not regain control values within 2 h, little subsequent recovery occurs over the following 14 to 22 h (Dring and Brown 1982, Brown 1987). Similar patterns of recovery have been described for HPO_4^{2-} uptake following desiccation and photosynthesis following freezing (Hurd and Dring 1991, Pearson and Davison 1993). The prevailing paradigm is that physiological differences in desiccation and freezing tolerance are ecologically important because they are correlated with vertical distribution patterns. While this is a reasonable hypothesis, it requires rigorous testing. In most cases, the different species used in experiments were collected from different tidal elevations and thus had different histories of stress exposure. Therefore, it is not clear if the physiological differences are the cause or a consequence of vertical distribution patterns. It is also unclear if sublethal effects reported in physiological experiments are important in nature. The transient reduction in photosynthesis and nutrient uptake and the loss of cell contents (organic nitrogen, amino acids, HPO_4^{2-}) immediately following sublethal desiccation or freezing stress (Moebus et al. 1974, Dring and Brown 1982, Davison et al. 1989, Dudgeon et al. 1989, Hurd and Dring 1991, Pearson and Davison 1993, 1994) probably occur in nature, as similar responses have been measured for plants exposed to emersion stresses *in situ* (e.g. Davison et al. 1993). The question is, are they important? Clearly, reductions in primary productivity, impairment of nutrient uptake, or loss of cell contents could potentially affect growth or reproductive output and hence competitive interactions between species. However, to assess their importance, we need more information on the frequency and severity of sublethal effects in nature and to consider them in the context of the overall physiology of the plant. For example, transient reductions in photosynthesis following emersion may be relatively unimportant in the overall carbon budget because productivity is

regulated by fluctuations in other factors such as light.

An additional problem is that many apparent examples of sublethal stress in laboratory studies may be artifacts of experimental design and may not occur in nature. For example, the partial recovery of photosynthesis in susceptible species following desiccation or freezing (e.g. Dring and Brown 1982, Pearson and Davison 1993) is clearly sublethal stress within the context of the experimental protocol (i.e. single exposure to stress followed by a 24-h recovery period), but what would happen if plants experienced multiple exposures to stress as occurs in nature? If repeated exposure to a suite of environmental conditions ultimately results in death, then data on the physiological response to a single exposure is ecologically irrelevant. Despite this, with the exception of Hodgson (1981), relatively little attention has been paid to subjecting algae to ecologically relevant multiple exposures to a particular stress. Recently we discovered that the partial recovery of photosynthesis in *Fucus distichus* following freezing stress is associated with the death of a portion of the cells in the thallus, suggesting that repeated exposure to the same stress would be lethal unless the thallus could replace dead cells (Pearson and Davison 1994).

The problems outlined above do not mean the physiological studies are of no value. Laboratory studies are required to determine the mechanistic basis of stress tolerance and potentially can provide valuable insights into the importance of stress in nature. However, it is essential that physiologists design ecologically relevant experiments. For example, although LT_{50} values for freezing (the temperature which kills 50% of the sample) indicate freezing tolerance is correlated with vertical distribution patterns (e.g. Frazer et al. 1988), the results are difficult to apply to the natural environment where the major difference between the high and low shore is not air temperature but the frequency and duration of exposure and freezing rate (Dudgeon et al. 1990, Pearson and Davison 1993). Wherever possible, physiological experiments in the laboratory should be based upon measurements of physical factors encountered on the shore during emersion. This consideration also applies to studies on limitation stress; for example, as pointed out by Hurd and Dring (1991), many experiments on nutrient uptake have used unnaturally high concentrations that make the data difficult to apply to nature. An alternative is to perform *in situ* physiological measurements on plants in the natural environment (e.g. Huppertz et al. 1990, Henley et al. 1992, Davison et al. 1993). These studies can be performed on seashores adjacent to marine laboratories or make use of the impressive array of physiological equipment (fluorometers, infrared gas analyzers, etc.), designed primarily for crop plants, that can be used in the field. Physiological investigations in the field should be combined with careful monitoring of environmental conditions, paying particular attention to microhabitat effects. It is important to emphasize that such investigations should be experimental, incorporating treatments that allow specific hypotheses concerning environmental factors to be tested. For example, spraying emersed plants with seawater could be used to separate the effects of photoinhibition and desiccation. Ideally, with the development of appropriate molecular markers for stress, samples from *in situ* treatments could be analyzed to determine the effects of particular environmental variables.

Limitation Stress

In addition to disruptive stress, tidal emersion isolates algae from the source of essential nutrients such as nitrogen and phosphorous and exposes them to a very different environment for photosynthesis. Considerable attention has been paid to the effect of emersion on photosynthesis (see Maberly and Madsen 1990, Lipkin et al. 1993), and it is clear that the response to emersion is not simply a matter of tolerating disruptive stress, but that under certain circumstances intertidal seaweeds achieve high rates of photosynthesis in air. Although initial studies found that lower-shore species exhibit lower rates of photosynthesis in air than in water, with the opposite being true for upper-shore species (Johnson et al. 1974, Quadir et al. 1979), this is not a general pattern; rather, as noted by Lipkin et al. (1993: p. 517), "there seems to be no causal relationship between photosynthetic performance of various species during emergence and the level at which they grow." Two points are worth noting here. First, the apparent lack of a general relationship between emersed photosynthesis and vertical distribution does not preclude this being an important factor in the interaction between a specific species pair, such as the brown algae *Hesperophycus harveyanus* and *Pelvetia fastigiata* (Oates and Murray 1983). Although photosynthetic rates of these species are similar in water, photosynthetic rates of fully-hydrated *H. harveyanus* in air are four times those of *P. fastigiata* which occurs lower on the shore. Furthermore, although both species exhibit a similar percentage reduction in photosynthesis when subjected to the same percentage desiccation, *H. harveyanus* loses water more slowly than *P. fastigiata* because of its smaller surface area : volume ratio. Second, it is important to differentiate between physiological differences between sublittoral fringe or subtidal species and eulittoral species, and differences among eulittoral species. Although Dring and Brown (1982) found no relationship between the photosynthetic performance in air and position on the shore for four *Fucus* spp., net photosynthesis in air declined more rapidly in the sublittoral fringe species *Laminaria digitata* because respiration was desiccation sensitive in *Fucus* but not *Laminaria*. Physiological processes that

exclude susceptible species from the intertidal are not necessarily important in structuring communities within the eulittoral.

Several studies have compared photosynthetic light use characteristics (PI parameters; see Henley 1994) of submerged and fully-hydrated plants in air. Although PI parameters do appear to vary between air and water, as with light-saturated photosynthesis discussed above, no general patterns are evident and these data will not be discussed further (Oates and Murray 1983, Johnston and Raven 1986, Madsen and Maberly 1990). However, this is an area which may require more study. Although dark respiration and light harvesting efficiency (α: photosynthesis per incident photon) and possibly quantum yield (Φ: photosynthesis per absorbed photon) are different in air and water (e.g. Madsen and Maberly 1990), the ecological significance and mechanistic basis of these changes are not understood.

Regardless of the absolute rates of photosynthesis in air compared with water, most workers have reported an increase in photosynthesis following moderate desiccation (compared to the fully hydrated emersed state) (Brinkhuis et al. 1976, Quadir et al. 1979, Johnston and Raven 1986, Lipkin et al. 1993), presumably because of a reduction in the aqueous diffusion barrier for CO_2. The response of photosythesis to inorganic carbon concentration in air (CO_2) and water ($CO_2 + HCO_3^-$) has been studied by several authors (see Madsen and Maberly 1990). In general, the results suggest that photosynthesis is limited to a similar extent by inorganic carbon availability under ambient conditions in both air and water; the greater substrate affinity for CO_2 in air appears to compensate for the lower concentrations of inorganic carbon relative to water (Kerby and Raven 1985, Johnston and Raven 1986, Madsen and Maberly 1990). Surif and Raven (1989) discovered that the affinity for inorganic carbon was greater in intertidal fucoids than in subtidal or sublittoral fringe members of the Laminariales, with photosynthesis in the former, but not the latter, being saturated for inorganic C in ambient seawater. This may be related to differences in extracellular carbonic anhydrase (CA) activities, which is involved in HCO_3^- uptake in seaweeds (Haglund et al. 1992); CA activities were higher in intertidal fucoids. Carbon limitation may be potentially more important in the intertidal than the sublittoral because more light is available. However, as with most physiological studies on intertidal seaweeds, it is not known if the differences in CA represent genetic adaptations that may control zonation patterns or phenotypic acclimation in response to differences in carbon availability associated with vertical zonation. The latter is possible because it is known that the affinity for photosynthetic carbon assimilation can be influenced by the availability of inorganic carbon and growth irradiance (Johnston and Raven 1990, Kübler and Raven 1994). An example of the possible

interaction or tradeoff between the increased availability of inorganic carbon for growth and emersion is provided by the intertidal red alga *Acanthophora najadiformis*, which has a relatively low affinity for HCO_3^- relative to most seaweeds (Einav and Beer 1993 cf. Reiskind et al. 1989) and is absent from the sublittoral zone. Affinity for CO_2 in air was found to be much higher and emersed rates of photosynthesis were five times greater than those in water. However, *A. najadiformis* is intolerant of desiccation, which confines it to a narrow zone in the lower eulittoral where it has access to CO_2 but where wave action prevents desiccation (Einav and Beer 1993).

Although laboratory studies on photosynthetic performance in air and water provide an indication of the probable physiological responses to emersion and immersion, they do not answer two critical questions: 1) to what extent do air and water contribute to the overall carbon budget in intertidal algae, and 2) do differences in productivity due to emersion play a role in regulating vertical distribution? Based on a very simple model, Oates (1985, 1986) estimated that emersed photosynthesis contributed between 17 and 32% of total productivity for *Colpomenia perigrina* and 60 to 85% of total productivity in *Halosaccion americanum*; in both cases productivity increased with increasing tidal elevation. Because both species are saccate it was not necessary to consider the effects of desiccation. By far the best estimate of the effect of emersion on the carbon budget of an intertidal seaweed is that of Maberly and Madsen (1990) on *Fucus spiralis*, which was based on a sophisticated model that incorporated the effect of desiccation. Desiccation had a major effect, with air contributing ca. 50 and 75% (April and July) of total productivity at the minimum levels of desiccation measured on the shore, but only ca. 20 and 40% (April and July) when desiccation was high. The model indicated that productivity increased lower down the shore, with maximum values occurring 1–3 m below the zone where *F. spiralis* occurs naturally. This is consistent with the observation of Schonbeck and Norton (1980b) that growth rates were highest in plants transplanted 0.85 m below their normal zone. It is therefore clear that the absence of *F. spiralis* from the lower shore is not controlled by carbon balance. Maberly and Madsen (1990) found that water type (clarity) had little effect on calculated productivity. In contrast, in the absence of desiccation effects in saccate seaweeds, calculated productivity declines lower on the shore because of the attenuation of light through the water column (Oates 1985, 1986, Matta and Chapman 1991). The role of light may become more important lower on the shore. For example, Huppertz et al. (1990) suggested that the lower-shore species *F. serratus* was light limited during immersion, while Dring (1987) suggested that fucoids were absent from the lower intertidal in the Bristol Channel because light levels were below the compensation point for growth due

to turbid water and large tidal amplitude. Other factors that need to be considered are the actual light environment experienced in nature, and in particular the effect of the canopy. The light environment during immersion is characterized by high frequency fluctuations due to wave focusing and movement of the canopy and these may increase productivity (Greene and Gerard 1990, Wing et al. 1993, Wing and Patterson 1993). The dense stands of species such as *Fucus vesiculosus* and *Ascophyllum nodosum* collapse during low tide; plants or sections of plants at the canopy surface may experience desiccation and photoinhibition (e.g. Huppertz et al. 1990) while those beneath the canopy are protected from desiccation but are in darkness or very low light.

There is good evidence that tidal emersion leads to nutrient limitation. Laboratory culture studies by Schonbeck and Norton (1979a) indicated that the brown algae *Pelvetia canaliculata* and *Fucus spiralis* grow more rapidly when immersed for 20 h than 4 h but that this difference disappeared when additional nutrients were added to the seawater. Transplanting the red alga *Gigartina pacifica* to higher tidal elevations led to a reduction in tissue nitrogen content and an increase in NO_3^- uptake rates and nitrate reductase activities suggesting that plants were more nitrogen limited higher on the shore (Thomas et al. 1987c). Recent work by Hurd et al. (1993) on the production of hyaline hairs by *Fucus* spp. is also consistent with more severe nutrient limitation higher on the shore. Hyaline hairs increase HPO_4^{2-} uptake and are produced during the late winter (January or February) and disappear in the fall when nutrient levels increase. The cessation of hair production is correlated with tidal elevation; hairs disappear from the low shore *F. serratus* before the midshore *F. vesiculosus*, which in turn stops hair production before the upper shore. *F. spiralis*. Despite the evidence that nutrient limitation is more severe higher on the shore, it is not known to what extent this influences vertical distribution, nor if interspecific differences occur in the response to limitation. If upper-shore species have an enhanced ability to acquire, and/or a lower requirement for, the limiting resource, it might enable them to outcompete and exclude low-shore species from the upper intertidal. This hypothesis is difficult to test because of the difficulty in determining limitation under field conditions. Demonstration of enhanced growth of plants in fertilized plots relative to unfertilized controls provides unambiguous evidence of limitation by a specific nutrient (e.g. Chapman and Craigie 1977). However, experiments to measure competitive interactions in the field in fertilized and unfertilized treatments with adequate replication would be extremely labor intensive. A large number of laboratory studies have defined critical tissue concentrations of specific elements that are indicative of limitation (see Wheeler and Björnsäter 1992). Unfor-

tunately, critical concentrations have not proved useful for indicating limitation under field conditions (e.g. Fujita et al. 1989), presumably because, as pointed out by Lapointe and Duke (1984), factors such as light modify the nitrogen requirements for growth. The same problem precludes the use of many biochemical components of seaweeds as indicators of nutrient limitation. For example, although photosynthetic pigments respond to nitrogen limitation (e.g. Lapointe and Duke 1984), they are also affected by light and temperature (Henley and Ramus 1989, Davison et al. 1991). There is a need to develop specific indicators of limitation stress. For example, alkaline phosphatase has been used as an indicator of P limitation (Weich and Granéli 1989), while the ratio of *in vivo* nitrate reductase activity measured with and without the addition of NO_3^- can be used as an indicator of N limitation where NO_3^- is the major source of nitrogen (Davison et al. 1984). Bacteria, cyanobacteria, and eukaryotic unicellular algae produce nutrient stress proteins in response to sulfur, iron, phosphorus, and nitrogen limitation that are specific for the limiting element (Coleman et al. 1988, Atkinson and Fisher 1991, LaRoche et al. 1993). To date there is little information on nutrient stress proteins in macroalgae. The iron stress protein flavodoxin has been reported in the red macroalga *Chondrus crispus* (Fitzgerald et al. 1978). Flavodoxin does not contain iron and replaces Fe-containing ferrodoxin in the photosynthetic electron transport chain of iron-limited phytoplankton (LaRoche et al. 1993). However, in *Chondrus* it is not known if flavodoxin is constitutive or only expressed under iron limitation. A systematic search for nutrient limitation proteins in seaweeds can be justified by the considerable benefits that would result if such proteins were identified. Not only would information on the physiological function of stress proteins significantly increase our understanding of the metabolic response of macroalgae to limiting nutrients, but antibodies for such proteins or cDNA probes for the transcripts encoding them could form the basis of specific assays for nutrient limitation that could be used in ecological studies.

There is no obvious correlation between uptake kinetics for NO_3^- and HPO_4^{2-} and vertical distribution patterns (Thomas et al. 1987a, Hurd and Dring 1990). Desiccation enhances the uptake of NO_3^- and NH_4^+ in *Fucus distichus* and has a similar effect on HPO_4^{2-} uptake when P limitation is induced by N enrichment (Thomas and Turpin 1980). Photosynthetic rates in air are generally enhanced by moderate desiccation (see Madsen and Maberly 1990), and studies on the activities of a variety of enzymes suggest that emersion produces a general stimulation of several aspects of metabolism (Murthy et al. 1986, 1988, Murthy and Sharma 1989). The increase in N uptake following emersion may be a mechanism to counteract an N deficit that occurs

when metabolically active plants are isolated from their source of nutrients. Thomas et al. (1978a) found that there was a correlation between the stimulation of N uptake following emersion and vertical distribution patterns. Upper shore species 1) exhibited a greater stimulation of N uptake following desiccation, 2) achieved maximum uptake at higher levels of desiccation, and 3) were able to take up NO_3^- following severe desiccation that inhibited uptake in low-shore species. It is tempting to speculate that these differences in the ability to acquire an essential resource following emersion is a causal factor controlling vertical distribution patterns. However, as with the response of photosynthesis to desiccation and freezing, the critical question is, to what extent do the observed differences in NO_3^- uptake reflect rather than contribute to the observed distribution of plants in natural communities? In the case of the red alga *Gracilaria pacifica*, rates of NO_3^- uptake, internal NO_3^- content, NO_3^- reductase activities, and the response of uptake to desiccation were related to tidal elevation and could be modified by transplanting algae up or down the shore (Thomas et al. 1987b). Changes in desiccation, freezing, and high temperature tolerance induced by acclimation to moderate levels of stress have also been reported in intertidal seaweeds (e.g. Schonbeck and Norton 1979a, Dudgeon et al. 1990, Kübler and Davison 1993, Pearson and Davison 1994), as have seasonal changes in stress tolerance (Parker 1960), and it is premature to presume that physiological differences between upper- and lower-shore species are necessarily adaptive. Wherever possible, physiological responses should be compared for plants collected at the same tidal elevation (e.g. Dudgeon et al. 1989), or where this is not possible on plants transplanted to the same tidal height or grown in the laboratory under common garden conditions (e.g. Dudgeon et al. 1990). Conversely, the physiological differences that are frequently reported between members of the same species from the upper and lower parts of their distribution range (e.g. Parker 1960) are not necessarily due to phenotypic acclimation but may be due to genetic differences between high- and low-shore members of the same species (e.g. Innes 1988). There are several interesting questions that could be addressed about intraspecific genetic differences associated with tidal elevation: 1) How widespread are such genetic differences? 2) Do genetic differences represent stable ecotypes that result from strong selection pressure and limited gene flow between high- and low-shore populations of the same species? 3) Do the genetic differences result from differential selection pressures associated with the vertical gradient of stress acting on each generation of juvenile plants? Genetic differences are unlikely to occur in species (e.g. *Pelvetia canaliculata*) with a narrow vertical distribution range but may be common in species that occupy a wide vertical zone (e.g. *Fucus vesiculosus* or *Ascophyllum nodosum*). Inherited genetic differences might occur in species with self-fertilization and/or limited dispersal distance, whereas they are more likely to be the result of selection on a single generation in species in which successful recruitment occurs over a wide area.

MECHANISMS OF STRESS TOLERANCE

We have a reasonable mechanistic understanding of the physiological response of the photosynthetic metabolism to desiccation, freezing, and high temperature stress (e.g. Wiltens et al. 1978, Smith et al. 1986, Nultsch et al. 1987, Bose et al. 1988, Dudgeon et al. 1989, Kübler and Davison 1993) and of the response of seaweeds to photoinhibition (e.g. Herbert and Waaland 1988, Herbert 1990, Franklin et al. 1992, Benet et al. 1994, Franklin 1994). In contrast, we have a very limited understanding of the mechanisms of tolerance at the cellular/molecular level or the control of tolerance. Light-limited photosynthesis appears to be more susceptible to emersion stress than light-saturated photosynthesis (Pearson and Davison 1993), possibly because desiccation and freezing both disrupt the transfer of energy from antenna pigments to the photosystem II (PSII) reaction center (Smith et al. 1986, Dudgeon et al. 1989). Intertidal seaweeds are potentially susceptible to photoinhibition because they alternate between a submerged environment where light levels may limit photosynthesis (Dring 1987) and full sunlight when emersed in air. In most intertidal seaweeds the photochemical apparatus operates to optimize photosynthesis at low light levels associated with immersion, with the result that emersed plants are exposed to a large excess of light energy. This could be exacerbated by desiccation and freezing, which reduce the ability to utilize light, requiring a corresponding increase in processes that dissipate excess solar energy if damage is to be avoided. In desiccated *Porphyra* there is a transfer of excitation energy away from PSII (thereby presumably avoiding photoinhibitory damage of the D_1 reaction center protein) towards photosystem I (PSI) where it appears to be dissipated as heat associated with cyclic PSI electron transport (Fork and Öquist 1981, Öquist and Fork 1982, Fork et al. 1986). Huppertz et al. (1990) suggested that desiccation may confer protection against photoinhibition in *Fucus serratus*. Unfortunately, because of the nature of the experimental design, which involved monitoring fluorescence in a natural population, other explanations of the data of Huppertz et al. (1990) are possible (see Henley et al. 1992), and a more detailed examination of the interaction between desiccation and photoinhibition is required. A recent study on the desiccation-resistant fern *Polypodium polypodioides* suggested that desiccation may confer protection against photoinhibition, with desiccated plants in which all photochemical activity had ceased being less damaged than partially desiccated plants in which PSII charge separation, but not electron transport, was

functional (Muslin and Homann 1992). Desiccation has also been reported to increase high-temperature tolerance in *Porphyra* (Smith 1984), possibly because PSII has a greater thermal stability in plants that are dry (Havaux 1992). Caution is necessary when using changes in quantum efficiency of photosynthesis (Φ), frequently measured as the ratio of variable to maximum fluorescence (F_v/F_m), as an indicator of photoinhibition. As discussed by Long et al. (1994), reductions in Φ may either reflect damage to the photochemical apparatus or the operation of mechanisms, such as the quenching of excess energy via the xanthophyll cycle or inoperative PSII reaction centers, that direct energy away from functional PSII centers to avoid damage. The relative importance of damage and protective mechanisms in contributing to reductions in F_v/F_m can be assessed by monitoring changes in F_o and F_m (e.g. Henley et al. 1992).

Although photosynthetic metabolism appears to be an important determinant of the ability of intertidal seaweeds to survive emersion stress, other processes are involved. Differences in desiccation and freezing tolerance of high- and low-shore species are not dependent on light and occur when plants are subject to stress under low light or in darkness (Smith and Berry 1986, Davison et al. 1989). With the exception of the data on photosynthetic metabolism discussed above, we have very little information on the mechanisms that confer tolerance to desiccation and other emersion stresses in intertidal seaweeds. For example, we have no idea why some members of the genus *Fucus* (e.g. *Fucus spiralis*) are highly tolerant of levels of desiccation or freezing stress that would kill lower-shore members of the same genus (e.g. *Fucus evanescens*). This is a fundamental question in seaweed biology that has important implications for both physiology and ecology. Although macroalgae have been largely neglected, they do represent a useful system for studying freezing and desiccation tolerance. 1) In most species, stress resistance depends upon tolerance at the level of cellular physiology, and tolerance mechanisms can be studied in the absence of complicating factors such as stomatal conductance. 2) Intertidal seaweeds inhabit a relatively simple and predictable environment, whereas most terrestrial plants are simultaneously exposed to two different environments in air and soil. 3) Congenerics (e.g. *Porphyra* spp. and *Fucus* spp.) occur at different tidal heights and exhibit different degrees of tolerance, allowing stress physiology to be studied in closely related species and offering potential insights into the evolution of tolerance. 4) Upper-shore seaweeds have a high degree of tolerance to desiccation and freezing (e.g. *Porphyra linearis* can survive emersion for three weeks; Lipkin et al. 1993) and are perhaps unique in that they recover very rapidly from a frequent and predictable exposure to stress once or twice each day (e.g. Bell 1993). Perhaps the major disadvantage

of intertidal macroalgae as model systems for the study of stress tolerance is their reputation as difficult subjects for physiological, biochemical, and molecular research. Although seaweeds do present technical problems, most can probably be overcome, and our general lack of knowledge of the basis of stress physiology in intertidal macroalgae is undoubtedly due to neglect rather than the intractable nature of the problem.

Elucidating the mechanisms responsible for stress tolerance would be of benefit to ecologists because it would allow the costs as well as the benefits of stress tolerance to be considered. There is a general tendency towards decreasing plant size with increasing tidal elevation. For example, individuals of the upper-shore species *Pelvetia canaliculata* and *Fucus spiralis* are smaller than fucoids from the mid- and low shore. There are at least three possible explanations (these are not mutually exclusive): 1) Upper-shore species may be smaller because more frequent and severe stress reduces productivity (e.g. Madsen and Maberly 1990). 2) The energetic and capital cost of the greater degree of stress tolerance reduces resources available for growth. 3) There is an adaptive advantage in small stature for high-shore species. Although the first possibility may also be important, the observation that upper-shore fucoids grow more slowly than low-shore species under common garden conditions (e.g. Schonbeck and Norton 1980a), despite having similar rates of photosynthesis (Dring and Brown 1982, Davison et al. 1989), provides indirect support for the hypotheses that growth rates are genetically constrained either because of the cost of stress tolerance (hypothesis 2) or because small stature confers an adaptive advantage (hypothesis 3). However, it is also possible that reduced growth rates of high-shore species in common garden experiments occurs because immersion acts as a stress. The upper-shore species *Pelvetia canaliculata* died when transplanted lower on the shore or immersed for 50% of the time in laboratory culture, although disease rather than immersion per se appeared to be responsible (Rugg and Norton 1987). However, there is no evidence that immersion is a stress for other intertidal seaweeds; isolated individuals are often found well below their normal zone, and they may grow more rapidly below their normal range (Schonbeck and Norton 1980b).

Some studies have shown that upper-shore species maintain a permanent high level of stress tolerance, whereas lower-shore species exhibit acclimation, modifying the degree of tolerance depending upon prevailing conditions, although they are always less tolerant than upper-shore species (e.g. Schonbeck and Norton 1979a), Dudgeon et al. 1990). These strategies are presumably adaptive given the difference in frequency and severity of emersion stress encountered at different tidal heights. Upper-shore species maintain a constant high degree of tolerance to cope with frequent yet stochastic exposure to se-

vere stress but achieve this at the expense of growth rate. Conversely, lower- and midshore species achieve faster growth by altering the allocation of resources between growth (during benign periods) and tolerance (e.g. during winter when exposed to freezing) but are susceptible to sudden or unusually severe stresses.

Hypotheses that relate competitive ability to stress tolerance are difficult, if not impossible, to test in the absence of information on the mechanisms of stress tolerance. In theory, it is possible to calculate the costs of stress and stress tolerance from a carbon budget (i.e. primary production = growth + reproduction + respiration + storage + loss of dissolved and particulate organic carbon + cost of tolerance mechanism). However, carbon budgets are difficult to balance (and require several assumptions) even in plants grown under nonstressful, defined conditions in laboratory culture, and this approach is unlikely to prove successful in natural communities. If stress tolerance has a high energetic cost and requires a considerable investment in fixed carbon, this should correspond to measurable differences in biochemical composition or metabolic activity between stress tolerant and susceptible species; our goal should be to identify these differences.

There is an extensive literature on the stress tolerance of higher plants, especially crop species, to physical stresses that are important in the intertidal (e.g. high and low temperature, desiccation and freezing) (e.g. see reviews by Guy 1990, Vierling 1991, Demmig-Adams and Adams 1992, Li and Christersson 1993, Close and Bray 1993, Long et al. 1994). It seems logical and expedient to use the higher plant literature as a starting point by addressing the question: To what extent are the stress tolerance mechanisms used by higher plants used by macroalgae? The danger in this approach is that potentially important processes unique to macroalgae may be ignored. The advantage is that is offers the best prospect for rapid progress. If it can be demostrated that stress tolerance depends upon the same mechanisms in intertidal macroalgae and higher plants, it would be possible to use antibodies and cDNA probes developed for higher plants to address ecological questions in seaweeds. The limited evidence available does suggest that macoalgae respond similarly to plants. For example, Vayda and Yuan (1994) discovered that Antarctic isolates of the red alga *Plocamium cartilagineum* produced mRNA for the 70 kDa heat shock protein when transferred to temperatures above those encountered in the natural environment, and unpublished data suggests that *Fucus* spp. contain dehydrins (Pearson, Li, and Brawley, unpubl.), highly conserved proteins involved in desiccation tolerance (Close and Lammers 1993). Similarly, photoinhibition appears to involve similar mechanisms in seaweeds as in higher plants (e.g. xanthophyll cycle, D_1 damage and repair) (Franklin et al. 1992, Benet et al. 1994). However, even in the unlikely eventuality that stress tolerance

in macroalgae is identical to that of higher plants, research on intertidal seaweeds would still be worthwhile in order to understand how these mechanisms function in a unique environment. For example, although many plants experience desiccation, few, if any, are exposed to severe desiccation on a twice-daily basis, although this is the situation experienced by many intertidal seaweeds. It is worth noting that, although there is an extensive literature on proteins produced by higher plants in responses to specific stresses (e.g. Guy 1990, Vierling 1991, Li and Christersson 1993, Close and Bray 1993), with the exception of heat shock proteins, the function of these proteins is not understood.

As one would expect, there is a positive correlation between the ability of species to tolerate different stresses, e.g. desiccation resistant upper-shore species are also freezing tolerant (Mathieson and Burns 1971 cf. Dudgeon et al. 1989, Dring and Brown 1982 c.f Davison et al. 1989). Recently we demonstrated that, as with higher plants, there is a mechanistic linkage between the ability to withstand those stresses that involve cellular dehydration (desiccation, osmotic dehydration, and freezing). For example, *Fucus distichus* grown at high salinity (51‰) exhibits an increased tolerance to freezing stress relative to plants grown in seawater at salinities of 8.5, 17, or 34‰ (Pearson and Davison 1994). Similarly, *F. vesiculosus* plants grown at 5° C exhibit increased freezing and desiccation tolerance relative to plants grown at 15° C, with the tolerance to both stresses increasing further in plants subjected to moderate daily freezing stress (Davison et al., unpubl.). Other examples of acclimation of stress tolerance are provided by Dudgeon et al. (1990) and Kübler and Davison (1993).

Because so little information is available on the mechanistic basis of stress tolerance in seaweeds, there is little point discussing in detail all of the mechanisms known to be important in conferring stress tolerance in higher plants. Instead, we have decided to focus on a single mechamism, active oxygen metabolism, that is the subject of ongoing research in Davison's laboratory. There are two reasons for this. First, based on information in the literature, we believe that active oxygen metabolism may be important in stress tolerance of intertidal seaweeds. Second, it outlines the steps we believe are required to demonstrate that any mechanism is important in conferring tolerance; correlations are no more indicative of causality in physiology than they are in ecology. All aerobic organisms generate active oxygen-containing species such as singlet oxygen ($^1O_2$), superoxide (O_2^-), and peroxides (e.g. H_2O_2), together with hydroxyl radicals (OH·) produced from O_2^- and H_2O_2 (Asada and Takahashi 1987, Bowler et al. 1992). Active oxygen-containing species and hydroxyl radicals are highly reactive and denature essential components of cells including proteins, membrane lipids, and nucleic acids, and are formed in both respiration and photosynthesis

(Bowler et al. 1992). For example, plants generate O_2^- in the Mehler reaction when electrons are transferred from PSI to O_2 (Asada and Takahashi 1987) and may also generate active oxygen and OH from PSII (Tschiersch and Ohmann 1993). Any factor that disrupts respiratory or photosynthetic metabolism can lead to the production of active oxygen, including desiccation, freezing, chilling, and high light, and active oxygen is a major cause of metabolic damage associated with these stresses (Wise and Naylor 1987, Schöner and Krause 1990, Bowler et al. 1992, Mishra and Singhal 1992, 1993, Mishra et al. 1993, Tschiersch and Ohmann 1993). Plants employ two basic mechanisms to prevent damage once active oxygen species are formed. 1) Antioxidants with a high affinity for active oxygen species (ascorbic acid (vitamin C), glutathione, and α-tocopherol (vitamin E)) efficiently scavenge them before they can react with susceptible components of the cell (Asada and Takahashi 1987, Bowler et al. 1992, Fryer 1992). 2) Enzymes, including superoxide dismutase (SOD), catalase, and the enzymes of the Halliwell-Asada pathway, convert active oxygen species into harmless products (Bowler et al. 1992). There is good evidence that the ability of higher plants and bryophytes to tolerate photoinhibition, high temperature, desiccation, and freezing as well as atmospheric pollutants (e.g. ozone) depends, in part, upon the ability to process active oxygen (Dhindsa and Matowe 1981, Wise and Naylor 1987, Malan et al. 1990, Dhindsa 1991, Tsang et al. 1991, Mishra and Singhal 1992, 1993, Seel et al. 1992a, b). The relative importance of the two mechanisms varies between species, with some plants depending primarily on antioxidants and others on enzymic defenses (e.g. Buckland et al. 1991). Recent work with transgenic plants containing additional copies of the gene for SOD has provided definitive evidence that this enzyme confers the ability to tolerate freezing and photoinhibition (Gupta et al. 1993, McKersie et al. 1993).

A limited amount of evidence suggests that seaweeds contain both the antioxidants vitamin C and E and the enzymes SOD and peroxidase (Jensen 1964, Liso and Calabrese 1974, Liso et al. 1978, Marsden et al. 1984, Jayasree et al. 1985, Munda 1987, Lindstrom 1988, 1993, Murthy and Sharma 1989). However, most of these studies either used enzyme polymorphism as a taxonomic tool or were concerned with the potential food value of seaweeds, and no specific information is available on the role of active oxygen metabolism in conferring emersion tolerance. Circumstantial evidence that active oxygen might be important in intertidal seaweeds is provided by several studies. 1) Peroxidase activities in *Ulva lactuca* increase during desiccation (Murthy and Sharma 1989) and similar increases in activities of active oxygen processing enzymes are believed to be involved in desiccation tolerance of bryophytes and higher plants (Bewley and Krochko 1981, Buckland et al. 1991). 2) Munda (1987) reported that

contents of ascorbic acid in brown algae in the Adriatic appeared to be correlated with species' vertical distributions, being higher in the eulittoral *Fucus viroides* than the sublittoral fringe *Cystosira compressa*, which in turn had more ascorbic acid than the sublittoral *Cystosira crinita*. There are also pronounced seasonal changes in ascorbic acid content, which is generally high in the winter, declining throughout the summer (Jensen 1964, Munda 1987). 3) Both freezing and desiccation cause membrane damage (Davison et al. 1989, Hurd and Dring 1991), which is consistent with damage due to lipid peroxidation by active oxygen species (Bewley and Krochko 1981).

The evidence outlined above clearly does not provide compelling support for the hypothesis that active oxygen metabolism is involved in emersion tolerance of intertidal macroalgae but does suggest that critical experiments are justified. There are several predictions, based on the hypothesis, that can be used to test if this or any other potential mechanism is important in immersion tolerance. 1) There should be a correlation between damage from active oxygen (e.g. malonyaldehyde release, a product of lipid peroxidation) during emersion stress and susceptibility to stress, with stress-susceptible, low-shore species suffering more damage than stress-tolerant, high-shore species. In fact, preliminary experiments indicate that this is the case, with severe desiccation resulting in lipid peroxidation in the rock pool species *Fucus distichus* but not in the upper-shore species *F. spiralis* (Grant and Davison, unpubl.). 2) There should be a correlation between susceptibility to stress and protective mechanisms against active oxygen, and upper-shore species should contain more antioxidants and/or protective enzymes than low-shore species. 3) Treatments that increase active oxygen production (e.g. D_2O or herbicides such as paraquat) or reduce contents of antioxidants or protective enzymes (Wise and Naylor 1987, Malan et al. 1990) should increase susceptibility to emersion stress. 4) Changes in stress tolerance induced by acclimation (e.g. Dudgeon et al. 1990, Pearson and Davison 1994) should be associated with corresponding changes in the ability to process active oxygen. Assuming the data from these experiments support the hypothesis, it would be appropriate to: 1) develop biochemical indicators of stress for use in ecological studies (e.g. activities of, or antibodies against, SOD or nucleic acid probes for transcripts encoding this enzyme), and 2) investigate how active oxygen metabolism responds to stress in nature (e.g. how do levels of antioxidants and enzyme activities change over a tidal cycle?)

SUMMARY

A perusal of the papers by Baker (1909, 1910) leads to the sobering conclusion that, despite 85 years of additional research, relatively little progress has been made towards elucidating the mechanisms that confer stress tolerance in intertidal seaweeds or

in understanding the role that physiological factors play in controlling ecological interactions within the intertidal zone. One major area where progress is needed is in the application of modern molecular, biochemical, and physiological techniques, not only to allow us to understand how seaweeds tolerate tidal emersion but to provide diagnostic probes that can be used to measure the occurrence of stress in the natural environment. There are opportunities for large numbers of innovative, exciting, and rewarding research projects. Hopefully, it will be possible for us to write another review on this topic in twenty-five years time and face the problem of a surfeit, rather than a deficit, of information.

Asada, K. & Takahashi, M. 1987. Production and scavenging of active oxygen in photosynthesis. *In* Kyle, D. J., Osmond, C. B., & Arntzen, C. J., *Photoinhibition.* Elsevier, New York, pp. 227–87.

Atkinson, M. R. & Fisher, S. H. 1991. Identification of genes and gene products whose expression is activated during nitrogen-limited growth in *Bacillus subtilis. J. Bacteriol.* 173: 23–7.

Baker, S. M. 1909. On the causes of zoning of brown seaweeds on the seashore. *New Phytol.* 8:196–202.

——— 1910. On the causes of zoning of brown seaweeds on the seashore. *New Phytol.* 9:54–67.

Bell, E. C. 1993. Photosynthetic response to temperature and desiccation in the intertidal alga *Mastocarpus papillatus. Mar. Biol. (Berl.)* 117:337–46.

Benet, H., Bruss, U., Duval, J-C. & Kloareg, B. 1994. Photosynthesis and photoinhibition in protoplast of the marine brown alga *Laminaria saccharina. J. Exp. Bot.* 45:211–20.

Bewley, J. D. & Krochko, J. E. 1981. Desiccation tolerance. *In* Lange, O. L, Nobel, P. S., Osmond, C. B. & Ziegler, H. [Eds.] *Encyclopedia of Plant Physiology, New Series, Vol. 12B. Water Relations and Carbon Assimilation.* Springer-Verlag, New York, pp. 325–78.

Bose, S., Herbert, S. K. & Fork, D. C. 1988. Fluorescence characteristics of photoinhibition and recovery in a sun and shade species of the red algal genus *Porphyra. Plant Physiol.* 86:946–50.

Bowler, C., van Montagu, M. & Inzé, D. 1992. Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:83–116.

Brawley, S. H. & Johnson, L. E. 1991. Survival of fucoid embryos in the intertidal zone depends on developmental stage and microhabitat. *J. Phycol.* 27:179–86.

Brinkhuis, B. H., Tempel, N. R. & Jones, R. F. 1976. Photosynthesis and respiration of exposed salt marsh fucoids. *Mar. Biol. (Berl.)* 34:349–59.

Brown, M. T. 1987. Effects of desiccation on photosynthesis of intertidal algae from a Southern New Zealand shore. *Bot. Mar.* 30:121–7.

Buckland, S. M., Price, A. & Hendry, G. A. F. 1991. The role of ascorbate in drought-treated *Cochlearia atlantica* Pobed. and *Armeria maritima* (Mill.) Willd. *New Phytol.* 119:155–60.

Chapman, A. R. O. 1986. Population and community ecology of seaweeds. *Adv. Mar. Biol.* 23:1–161.

——— 1990. Competitive interactions between *Fucus spiralis* L. and *F. vesiculosus* L. (Fucales, Phaeophyta). *Hydrobiologia* 204/205:205–9.

——— 1995. Functional ecology of fucoid algae: twenty three years of progress. *Phycologia* 34:1–22.

Chapman, A. R. O. & Craigie, J. S. 1977. Seasonal growth in *Laminaria longricruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol. (Berl.)* 40: 197–205.

Chapman, A. R. O. & Johnson, C. R. 1990. Distribution and organization of macroalgal assemblages in the Northwest Atlantic. *Hydrobiologia* 192:77–121.

Close, T. J. & Bray, E. A., Eds. 1993. Plant responses to cellular dehydration during environmental stress. *Current Topics in Plant Physiology* 10:296 pp.

Close, T. J. & Lammers, T. J. 1993. An osmotic stress protein of cyanobacteria is immunologically related to plant dehydrins. *Plant Physiol.* 101:773–9.

Coleman, L. W., Risen, B. H. & Schwartzbach, S. D. 1988. Preferential loss of chloroplast proteins in nitrogen deficient *Euglena. Plant Cell Physiol.* 29:1007–14.

Davison, I. R., Andrews, M. H. G. & Stewart, W. D. P. 1984. Growth regulation in *Laminaria digitata* (Huds.) Lamour: the use of *in vivo* nitrate reductase activities as an indicator of nitrogen limitation in field populations of *Laminaria. Mar. Biol (Berl.)* 84:207–17.

Davison, I. R., Dudgeon, S. R. & Ruan, H-M. 1989. The effect of freezing on seaweed photosynthesis. *Mar. Ecol. Prog. Ser.* 58:123–31.

Davison, I. R., Greene, R. & Podolak, E. J. 1991. Temperature acclimation of photosynthetic light harvesting in the brown alga *Laminaria saccharina* (L.) Lamour. *Mar. Biol. (Berl.)* 110: 449–54.

Davison, I. R., Johnson, L. E. & Brawley, S. H. 1993. Sublethal stress in the intertidal zone: tidal emersion inhibits photosynthesis and retards development in embryos of the brown alga *Pelvetia fastigiata. Oecologia* 96:483–92.

Demmig-Adams, B. & Adams, W. W. 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:599–626.

Dhindsa, R. S. 1991. Drought stress, enzymes of glutathione metabolism, oxidation injury, and protein synthesis in *Tortula ruralis. Plant Physiol.* 95:648–51.

Dhindsa, R. S. & Matowe, W. 1981. Drought tolerance in two mosses: correlated with enzymic defense against lipid peroxidation. *J. Exp. Bot.* 32:79–91.

Dorgelo, J. 1976. Intertidal fucoid zonation and desiccaton. *Hydrobiol. Bull.* 10:115–22.

Dring, M. J. 1987. Light climate in intertidal and subtidal zones in relation to photosynthesis and growth of benthic algae: a theoretical model. *In* Crawford, R. M. M. [Ed.] *Plant Life in Aquatic and Amphibious Habitats.* Blackwell, Oxford, pp. 23–34.

Dring, M. J. & Brown, F. A. 1982. Photosynthesis of intertidal brown algae during and after periods of emersion: a renewed search for physiological causes of zonation. *Mar. Ecol. Prog. Ser.* 8:301–8.

Dromgoole, F. I. 1980. Desiccation resistance of intertidal and subtidal algae. *Bot. Mar.* 23:149–59.

Dudgeon, S. R., Davison, I. R. & Vadas, R. L. 1989. Effect of freezing on photosynthesis of intertidal algae: relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus. Mar. Biol. (Berl.)* 101:107–14.

——— 1990. Freezing tolerance in the intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus*: the relative importance of acclimation and adaptation. *Mar. Biol. (Berl.)* 106:427–36.

Einav, R. & Beer, S. 1993. Photosynthesis in air and water of *Acanthophora najadiformis* growing within a narrow zone in the intertidal. *Mar. Biol. (Berl.)* 117:133–8.

Fitzgerald, M. P., Husain, A. & Rogers, L. J. 1978. A constitutive flavodoxin from a eukaryotic alga. *Biochem. Biophys. Res. Commun.* 81:630–5.

Fork, D. C. & Öquist, G. 1981. The effects of desiccation on excitation energy transfer at physiological temperatures between the two photosystems of the red alga *Porphyra perforata. Z. Pflanzenphysiol.* 104:385–93.

Fork, D. C., Bose, S. & Herbert, S. K. 1986. Radiationless transitions as a protection mechanism against photoinhibition in higher plants and a red alga. *Photosyn. Res.* 10:327–33.

Franklin, L. A. 1994. The effect of temperature acclimation on the photoinhibitory responses of *Ulva rotundata* Bild. *Planta (Berl.)* 192:324–31.

Franklin, L. A., Levavasseur, G., Osmond, C. B., Henley, W. J. & Ramus, J. 1992. Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta (Berl.)* 186: 399–408.

Frazer, A. W. J., Brown, M. T. & Bannister, P. 1988 Frost resistance of some littoral and sublittoral algae from southern New Zealand. *Bot. Mar.* 31:461–4.

Fryer, M. J. 1992. The antioxidant effects of thylakoid vitamin E (α-tocopherol). *Plant Cell Environ.* 15:381–92.

Fujita, R. M., Wheeler, P. A. & Edwards, R. L. 1989. Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. *Mar. Ecol. Prog. Ser.* 53:293–303.

Greene, R. M. & Gerard, V. A. 1990. Effects of high-frequency light fluctuations on growth and photoacclimation of the red alga *Chondrus crispus*. *Mar. Biol. (Berl.)* 105:337–44.

Grime, J. P. 1979. *Plant Strategies & Vegetation Processes.* John Wiley & Sons, New York, 222 pp.

——— 1989. The stress debate: symptom of impending synthesis. *Biol. J. Linn. Soc.* 37:3–17.

Gupta, A. S., Webb, R. P., Holaday, A. S. & Allen, R. D. 1993. Overexpression of superoxide dismutase protects plants from oxidative stress. *Plant Physiol.* 103:1067–73.

Guy, C. L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:187–223.

Haglund, K., Ramazanov, Z., Mtolera, M. & Pedersén, M. 1992. Role of external carbonic anhydrase in light-dependent alkalization by *Fucus serratus* L. and *Laminaria saccharina* (L.) Lamour (Phaeophyta). *Planta (Berl.)* 188:1–6.

Havaux, M. 1992. Stress tolerance of photosystem II *in vivo.* *Plant Physiol.* 100:424–32.

Hawkins, S. J. & Hartnoll, R. G. 1985. Factors determining the upper limits of intertidal canopy-forming algae. *Mar. Ecol. Prog. Ser.* 20:265–71.

Henley, W. J. 1994. Measurements and interpretations of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.* 29:729–39.

Henley, W. J. & Ramus, J. 1989. Photoacclimation and growth rate responses of *Ulva rotundata* (Chlorophyta) to intraday variations in growth irradiance. *J. Phycol.* 25:398–401.

Henley, W. J., Lindley, S. T., Levavasseur, G., Osmond, C. B. & Ramus, J. 1992. Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. *Oecologia* 89:516–23.

Herbert, S. K. 1990. Photoinhibition resistance in the red alga *Porphyra perforata*. *Plant Physiol.* 92:514–9.

Herbert, S. K. & Waaland, J. R. 1988. Photoinhibition of photosynthesis in a sun and shade species of the red algal genus *Porphyra*. *Mar. Biol. (Berl.)* 97:1–7.

Hodgson, L. M. 1981. Photosynthesis of the red alga *Gastroclonium coulteri* (Rhodophyta) in response to changes in temperature, light intensity and desiccation. *J. Phycol.* 17:37–42.

Huppertz, K., Hanelt, D. & Nultsch, W. 1990. Photoinhibition of photosynthesis in the marine brown alga *Fucus serratus* as studied in field experiments. *Mar. Ecol. Prog. Ser.* 66:175–82.

Hurd, C. L. & Dring, M. J. 1990. Phosphate uptake by intertidal algae in relation to zonation and season. *Mar. Biol. (Berl.)* 107:281–9.

——— 1991. Desiccation and phosphate uptake by intertidal fucoid algae in relation to zonation. *Br. Phycol. J.* 26:327–33.

Hurd, C. L., Gavin, R. S., Norton, T. A. & Dring, M. J. 1993. Production of hyaline hairs by intertidal species of *Fucus* (Fucales) and their role in phosphate uptake. *J. Phycol.* 29: 160–5.

Innes, D. J. 1988. Genetic differentiation in the intertidal zone in populations of the alga *Enteromorpha linza* (Ulvales: Chlorophyta). *Mar. Biol. (Berl.)* 97:9–16.

Jayasree, V., Solimabi, & Kamat, S. Y. 1985. Distribution of tocopherol (vitamin E) in marine algae from Goa, west coast of India. *India J. Mar. Sci.* 14:228–9.

Jensen, A. 1964. Ascorbic acid in *Ascophyllum nodosum, Fucus serratus* and *Fucus vesiculosus*. *Proc. Int. Seaweed Symp.* 4:319–25.

Johnson, W. S., Gigon, A., Gulmon, S. L. & Mooney, H. A. 1974. Comparative photosynthetic capacities of intertidal algae under exposed and submerged conditions. *Ecology* 55:450–3.

Johnston, A. M. & Raven, J. A. 1986. The analysis of photosynthesis in air and water of *Ascophyllum nodosum* (L.) Le Jol. *Oecologia* 69:288–95.

——— 1990. Effects of culture in high CO_2 on the photosynthetic physiology of *Fucus serratus*. *Br. Phycol. J.* 25:75–82.

Kerby, N. W. & Raven, J. A. 1985. Transport and fixation of inorganic carbon by marine algae. *Adv. Bot. Res.* 11:71–123.

Kübler, J. E. & Davison, I. R. 1993. High temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. *Mar. Biol. (Berl.)* 117:327–35.

Kübler, J. E. & Raven, J. A. 1994. Consequences of light limitation for carbon acquistition in three rhodophytes. *Mar. Ecol. Prog. Ser.* 110:203–9.

Lapointe, B. E. & Duke, C. S. 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *J. Phycol.* 20:488–95.

LaRoche, J., Geider, R. J., Graziano, L. M., Murray, H. & Lewis, K. 1993. Induction of specific proteins in eukaryotic algae grown under iron, phosphorus or nitrogen deficient conditions. *J. Phycol.* 29:767–77.

Li, P. H. & Christersson, L. 1993. *Advances in Plant Cold Hardiness.* C.R.C. Press, Ann Arbor, 332 pp.

Lindstrom, S. C. 1988. Protein electrophoresis of species of Palmariaceae. *J. Phycol.* 24:2.

——— 1993. Inter and intrapopulation genetic variation in species of *Porphyra* (Rhodophyta: Bangiales) from British Columbia and adjacent waters. *J. Appl. Phycol.* 5:53–62.

Lipkin, Y., Beer, S. & Eshel, A. 1993. The ability of *Porphyra linearis* (Rhodophyta) to tolerate prolonged periods of desiccation. *Bot. Mar.* 36:517–23.

Liso, R. & Calabrese, G. 1974. Research on ascorbic acid physiology in red algae. 2. Dehydroascorbic acid compartmentation in the cell. *Phycologia* 13:205–8.

Liso, R., Calabrese, G. & Chieppa, M. 1978. Research on ascorbic acid physiology in red algae. IV. On the seasonal variation of AA/DHA ratio in *Pterocladia capillacea* (Gmel.) Born. et. Thur. (Rhodophyta, Gelidiales). *Phycologia* 17:143–7.

Long, S. P., Humphries, S. & Falkowski, P. G. 1994. Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:633–62.

Lubchencho, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *Am. Nat.* 112:23–39.

——— 1986. Relative importance of competition and predation: early colonization by seaweeds in New England. *In* Diamond, J. & Case, T. J. [Eds.] *Community Ecology.* Harper & Row, New York, pp. 537–55.

Maberly, S. C. & Madsen, T. V. 1990. Contribution of air and water to the carbon balance of *Fucus spiralis*. *Mar. Ecol. Prog. Ser.* 62:175–83.

Madsen, T. V. & Maberly, S. C. 1990. A comparison of air and water as environments for photosynthesis by the intertidal alga *Fucus spiralis* (Phaeophyta). *J. Phycol.* 26:24–30.

Malan, C., Greyling, M. M. & Gressel, J. 1990. Correlation between CuZn superoxide dismutase and glutathione reductase and environmental and xenobiotic stress tolerance in maize inbreds. *Plant Sci.* 69:157–66.

Marsden, W. J. N., Callow, J. A. & Evans, L. V. 1984. A preliminary investigation of the electrophoretic characterization of enzymes from a range of macroscopic brown algae. *Bot. Mar.* 28:521–6.

Mathieson, A. C. & Burns, R. L. 1971. Ecological studies of

economic red algae. I. Photosynthesis and respiration of *Chondrus crispus* Stackhouse and *Gigartina stellata* (Stackh.) Batters. *J. Exp. Mar. Biol. Ecol.* 7:197–206.

Matta, J. L. & Chapman, D. J. 1991. Photosynthetic responses and daily carbon balance of *Colpomenia peregrina*: seasonal variations and differences between intertidal and subtidal populations. *Mar. Biol. (Berl.)* 108:303–13.

McKersie, B. D., Chen, Y., deBeus, M., Bowley, S. R., Bowler, C., Inzé, D., D'Halluin, K. & Botterman, J. 1993. Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*Medicago sativa* L.). *Plant Physiol.* 103:1155–63.

Mishra, N. P., Mishra, R. K. & Singhal, G. S. 1993. Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant Physiol.* 102:903–10.

Mishra, R. K. & Singhal, G. S. 1992. Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. *Plant Physiol.* 98:1–6.

———— 1993. Photosynthetic activity and peroxidation of thylakoid lipids during photoinhibiton and high temperature treatment of isolated wheat chloroplasts. *J. Plant Physiol.* 141:286–92.

Moebus, K., Johnson, K. M., Sieburth, J. M. 1974. Rehydration of desiccated intertidal brown algae: release of dissolved organic carbon and water uptake. *Mar. Biol. (Berl.)* 26:127–34.

Munda, I. K. 1987. Preliminary investigation on the ascorbic acid content in some Adriatic seaweeds. *Hydrobiologia* 151/152:477–81.

Murthy, M. S. & Sharma, C.L.N.S. 1989. Peroxidase activity in *Ulva lactuca* under desiccation. *Bot. Mar.* 32:511–3.

Murthy, M. S., Rao, A. S. & Reddy, E. R. 1986. Dynamics of nitrate reductase activity in two intertidal algae under desiccation. *Bot. Mar.* 24:471–4.

Murthy, M. S., Rao, Y. N. & Faldu, P. J. 1988. Invertase and total amylase activities in *Ulva lactuca* from different tidal levels, under desiccation. *Bot. Mar.* 31:53–6.

Muslin, E. H. & Homann, P. H. 1992. Light as a hazard for the desiccation-resistant "resurrection" fern *Polypodium polypodioides* L. *Plant Cell Environ.* 15:81–9.

Norton, T. A. 1986. The zonation of seaweeds on rocky shores. *In* Moore, P. G. & Seed, R. [Eds.] *The Ecology of Rocky Coasts.* Columbia University Press, New York, pp. 7–21.

Nultsch, W., Pfau, J. & Materna-Weide, M. 1987. Fluence and wavelength dependence of photoinhibition in the brown alga *Dictyota dichotoma. Mar. Ecol. Prog. Ser.* 41:93–7.

Oates, B. R. 1985. Photosynthesis and amelioration of desiccation in the intertidal saccate alga *Colpomenia peregrina. Mar. Biol. (Berl.)* 89:109–19.

———— 1986. Components of photosynthesis in the intertidal saccate alga *Halosaccion americanum* (Rhodophyta, Palmariales). *J. Phycol.* 22:217–23.

———— 1988. Water relations of the intertidal saccate alga *Colpomenia peregrina* (Phaeophyta, Scytosiphonales). *Bot. Mar.* 31:57–63.

Oates, B. R. & Murray, S. N. 1983. Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds *Hesperophycus harveyanus* and *Pelvetia fastigiata* F. *gracilis. J. Phycol.* 19:371–80.

Öquist, G. & Fork, D. C. 1982. Effects of desiccation on the excitation energy distribution from phycoerythrin to the two photosystems in the red alga *Porphyra perforata. Physiol. Plant.* 56:56–62.

Parker, J. 1960. Seasonal changes in cold-hardiness of *Fucus vesiculosus. Biol. Bull. Mar. Biol. Lab. Woods Hole* 119:474–8.

Pearson, G. A. & Davison, I. R. 1993. Freezing rate and duration determine the physiological response of intertidal fucoids to freezing. *Mar. Biol. (Berl.)* 115:353–63.

———— 1994. Freezing stress and osmotic dehydration in *Fucus distichus* (Phaeophyta): evidence for physiological similarity. *J Phycol.* 30:257–67.

Quadir, A., Harrison, P. J. & DeWreede, R. E. 1979. The effects

of emergence and submergence on the photosynthesis and respiration of marine macrophytes. *Phycologia* 18:83–8.

Reiskind, J. B., Beer, S. & Bowes, G. 1989. Photosynthesis, photorespiration and ecophysiological interaction in marine macroalgae. *Aquatic Bot.* 34:131–52.

Rugg, D. A. & Norton, T. A. 1987. *Pelvetia canaliculata*, a high-shore species that shuns the sea. *In* Crawford, R.M.M. [Ed] *Plant Life in Aquatic and Amphibious Habitats.* Blackwell, Oxford, pp. 347–58.

Schonbeck, M. W. & Norton, T. A. 1978. Factors controlling the upper limits of fucoid algae on the shore. *J. Exp. Mar. Biol. Ecol.* 31:303–13.

———— 1979a. Drought-hardening in the upper shore seaweeds *Fucus spiralis* and *Pelvetia canaliculata. J. Ecol.* 67:687–96.

———— 1979b. An investigation of drought avoidance in intertidal fucoid algae. *Bot. Mar.* 22:133–44.

———— 1980a. The effects on intertidal fucoid algae of exposure to air under various conditions. *Bot. Mar.* 23:141–7.

———— 1980b. Factors controlling the lower limits of fucoid algae on the shore. *J. Exp. Mar. Biol. Ecol.* 43:131–50.

Schöner, S. & Krause, G. H. 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta (Berl.)* 180:383–9.

Seel, W. E., Hendry, G. A. F. & Lee, J. A. 1992a. The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. *J. Exp. Bot.* 43:1023–30.

———— 1992b. Effect of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. *J. Exp. Bot.* 43:1031–7.

Smith, C. M. 1984. Drying enhances tolerance to high temperature stress for an intertidal alga, *Porphyra perforata* J. Ag. *Am. Zool.* 24:29A.

Smith, C. M. & Berry, J. A. 1986. Recovery of photosynthesis after exposure of intertidal algae to osmotic and temperature stresses: comparative studies of species with different distributional limits. *Oecologia* 70:6–12.

Smith, C. M., Satoh, K. & Fork, D. C. 1986. The effect of osmotic tissue deydration and air drying on morphology and energy transfer in two species of *Porphyra. Plant Physiol.* 80:843–7.

Surrif, M. B. & Raven, J. A. 1989. Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia* 78:97–105.

Thomas, T. E. & Turpin, D. H. 1980. Desiccation enhanced nutrient uptake rates in the intertidal alga *Fucus distichus. Bot. Mar.* 23:479–81.

Thomas, T. E., Harrison, P. J. & Taylor, E. B. 1987a. Nitrogen uptake and growth of the germlings and mature thalli of *Fucus distichus. Mar. Biol. (Berl.)* 84:267–74.

Thomas, T. E., Turpin, D. H. & Harrison, P. J. 1987b. Desiccation enhanced nitrogen uptake rates in intertidal seaweeds. *Mar. Biol. (Berl.)* 94:293–8.

Thomas, T. E., Harrison, P. J. & Turpin, D. H. 1987c. Adaptation of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. *Mar. Biol. (Berl.)* 93:569–80.

Todd, C. D. & Lewis, J. R. 1984. Effect of low air temperature on *Laminaria digitata* in south-western Scotland. *Mar. Ecol. Prog. Ser.* 16:199–201.

Tsang, E. W. T., Bowlet, C., Hérouart, D., Van Camp, W., Villarroel, R., Generello, C., Van Montagu, M. & Inzé, D. 1991. Differential regulation of superoxide dismutases in plants exposed to environmental stress. *Plant Cell* 3:783–92.

Tschiersch, H. & Ohmann, E. 1993. Photoinhibition in *Euglena gracilis*: involvement of reactive oxygen species. *Planta (Berl.)* 191:316–23.

Underwood, A. J. 1986. Physical factors and biological interactions: the necessity and nature of ecological experiments. *In* Moore, P. G. & Seed, R. [Eds] *The Ecology of Rocky Coasts.* Columbia University Press, New York, pp. 372–90.

Underwood, A. J. & Fairweather, P. G. 1989. Supply-side ecology and benthic marine assemblages. *Trends Ecol. Evol.* 4:16–20.

Vadas, R. L., Johnson, S. & Norton, T. A. 1992. Recruitment and mortality of early post-settlement stages of benthic algae. *Br. Phycol. J.* 27:331–51.

Vayda, M. E. & Yuan, M-L. 1994. The heat shock response of an antarctic alga is evident at 5° C. *Plant Mol. Biol.* 24:229–33.

Vierling, E. 1991. The roles of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:570–620.

Weich, R. G. & Granéli, E. 1989. Extracellular alkaline phosphatase activity in *Ulva lactuca. J. Exp. Mar. Biol. Ecol.* 129: 33–44.

Wheeler, P. A. & Björnsäter, B. R. 1992. Seasonal fluctuations in tissue nitrogen, phosphorous and N:P for five macroalgal species common to the Pacific Northwest coast. *J. Phycol.* 28: 1–6.

Wiltens, J. Schreiber, U. & Vidaver, W. 1978. Chlorophyll fluorescence induction: an indicator of photosynthetic activity in marine algae undergoing desiccation. *Can. J. Bot.* 56:2787–94.

Wing, S. R. & Patterson, M. R. 1993. Effects of wave-induced lightflecks in the intertidal zone and photosynthesis in the macroalgae *Postelsia palmaeformis* and *Hedophyllum sessile* (Phaeophyceae). *Mar. Biol. (Berl.)* 116:519–25.

Wing, S. R., Leichter, J. J. & Denny, M. W. 1993. A dynamic model for wave-induced light fluctuations in a kelp forest. *Limnol. Oceanogr.* 38:396–407.

Wise, R. W. & Naylor, A. W. 1987. Chilling-enhanced photooxidation. *Plant Physiol.* 83:278–82.

Zaneveld, J. S. 1969. Factors contolling the limitation of benthic marine algal zonation. *Am. Zoo.* 9:267–91.