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Phenotypic plasticity in the red alga *Porphyra abbottae*: Environmental factors influencing light harvesting ability

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PHENOTYPIC PLASTICITY IN THE RED ALGA *PORPHYRA ABBOTTAE*: ENVIRONMENTAL FACTORS INFLUENCING LIGHT HARVESTING ABILITY

by

GABRIELA HANNACH

A dissertation submitted in partial fulfillment
of the requirements for the degree of

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(Chairperson of Supervisory Committee)

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Abstract

PHENOTYPIC PLASTICITY IN THE RED ALGA PORPHYRA ABBOTTAE:

ENVIRONMENTAL FACTORS INFLUENCING

LIGHT HARVESTING ABILITY

by Gabriela Hannach

Chairperson of Supervisory Committee: Professor J. Robert Waaland
Department of Botany

Gametophytes of the red intertidal macroalga Porphyra abbottae were grown in batch cultures in order to examine some of their plastic responses to key environmental factors. Conchospores from a conchocelis stock culture (originated from a single carpospore) were grown under various conditions of photon flux density (PFD) and nutrient supply (nitrogen, phosphorus enrichment and water motion). The research centered on how these factors affect morphology (shape, thickness) and light harvesting by young gametophytic blades, and how changes in the latter influence photosynthesis and growth.

Blades grown at low PFD and high water motion were narrower; under nutrient limitation they were thinner.

Whole thallus broadband absorptance (400-700 nm) was used as a measure of light harvesting ability. Absorptance increased at PFDs limiting for growth, indicating photoacclimation by light harvesting pigments, but this increase was not sufficient to compensate for low

ambient PFD. The resulting decrease in the amount of light harvested at subsaturating PFDs did not account entirely for the decrease in growth rates at these PFDs: quantum yields of growth in plants grown under a range of PFDs showed decreased light use efficiency at low PFD, suggesting increased metabolic maintenance costs at limiting PFD.

Nutrient limitation decreased the light harvesting ability, light-limited and light-saturated photosynthetic rates, and growth. Minor shifts in maximal quantum yields of photosynthesis indicated that differences in light-limited rates were primarily determined by light absorption. Quantum yields of growth were increased by nitrogen limitation.

Increased absorptance, brought about by low PFD and/or high nitrogen, was accompanied by enrichment of the accessory pigments phycoerythrin and phycocyanin relative to chlorophyll a, and of phycocyanin relative to phycoerythrin. Addition of accessory pigments to photosynthetic membranes may thus involve an increase in phycobilisome density along with a reduction in the phycoerythrin content of phycobilisomes.

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CHAPTER 1

INTRODUCTION

The distribution of marine macroalgae in temperate zones is limited to coastal areas where substrate is available and sunlight can support growth. In these areas, light and nutrients are commonly major physical factors affecting macroalgal growth. Adequate water motion may also be critical for some species. Whereas temperature largely governs a species geographical distribution, it has a lesser influence on local abundance and growth.

On a local scale, light, nutrients and water motion may vary both temporally and spatially. Superimposed on the seasonal cycle of irradiance and photoperiod is a depth gradient in irradiance and spectral quality that results from the absorption of light by the water and the particles suspended in it. Nutrients in the water column undergo marked seasonal cycles related to phytoplankton growth, decomposition, and in certain areas, upwelling events. Spatially, nutrients can vary on a microscale as a result of excretion and regeneration from microorganisms. Marine organisms that inhabit the intertidal zone are subject to the greatest environmental variation and heterogeneity.

Given that marine macroalgae encounter such variation in essential resources, they can be expected to have evolved a variety of

mechanisms enabling them to adapt ontogenetically, i.e., acclimate to prevailing conditions. These morphological and physiological responses of the phenotype to the environment are known as phenotypic plasticity. Plastic responses to environmental variation are under genetic control just as any other plant trait. Heterogenous environments are thought to favor the evolution of phenotypic plasticity (Schlichting 1989). This trait is particularly important in multicellular plants, whose sessile life-style requires them to face ambient conditions (Schlichting 1986, 1989). However, plastic responses may only be considered adaptive if the phenotypic change results in an improvement of growth, survival, or reproduction (Stearns 1989).

The morphological variation of macroalgal populations has traditionally been a subject of taxonomic and descriptive studies. Attempts to explore the range of form variation in individual species has led to an appreciation of the extreme phenotypic plasticity of many species. In fact, variation in thallus form and structure under different habitat, seasonal or geographical conditions has been a major source of difficulty in algal taxonomy (Mathieson et al. 1981). Since the studies by Littler and Littler (1980) it has become apparent that seaweed form and function are strongly interrelated. The functional significance of a particular form is suggested when unrelated plants exhibit a similar appearance in the same habitat (Norton et al. 1982). In the model proposed by Littler and Littler (1980) macroalgal species are assigned to functional groups according to thallus morphology (e.g., blades, tufts, crusts), irrespective of phylogenetic

affiliation. A major parameter that characterizes an alga's morphology is its surface area : volume ratio. It has been possible to show that species with similar thallus construction often share a suit of ecological and physiological characteristics as well, such as life history traits, susceptibility to grazing, productivity (Littler and Littler 1980, Hay 1981, Steneck and Watling 1982), photosynthetic performance (Arnold and Murray 1980) and nutrient uptake and storage capabilities (Rosenberg and Ramus 1984, Ramus and Venable 1987, Duke et al. 1989).

Less attention has been given to evaluating the adaptive value of intraspecific variations in form. Several studies have examined the morphological differentiation of populations in relation to their degree of exposure to waves or currents (Jordan and Vadas 1972, Cheshire and Hallam 1988, Armstrong 1989, Bhattacharya and Druehl 1989). The relative contribution of environmental and genetic components of variation has been investigated in sporophytes of kelp species (e.g., Chapman 1974). Hanisak et al. (1988) have applied the functional-form model of Littler and Littler (1980) to examination of the functional significance of genetically distinct morphotypes within a single species.

Macroalgal populations have also been shown to differ with respect to physiological characteristics. Physiological differences between populations may have a genetic basis. For example, Gerard (1988) was able to differentiate ecotypes of Laminaria saccharina on the basis of photosynthesis-related traits; Espinoza and Chapman

(1983) found genetic differentiation of growth rate and maximum uptake rate of nitrate between geographically separated populations of Laminaria longicruris. Controlled experiments have shown that many aspects of an alga's physiology can also adjust phenotypically to ambient conditions. For example, Davison (1987) showed that Laminaria saccharina can adjust its photosynthetic physiology to ambient temperatures, and Laminaria groenlandica was shown to acclimate to ambient nitrate levels by altering rates of uptake (Druehl et al. 1989).

The best studied aspect of physiological acclimation in algae is the acclimation to light by the photosynthetic apparatus. The relationship between photosynthetic performance and light environment has been well studied in both land and marine plants (Boardman 1977, Bjorkman 1981). Acclimation to PFD involves changes that affect both light-limited and light-saturated rates of photosynthesis. The photosynthetic capacity, i.e., the maximum light-saturated rate of photosynthesis, increases at higher growth PFDs and is highly correlated with levels of those enzymes that are involved in fixation and reduction of CO_2, mostly RuBP-carboxylase (Bjorkman 1981).

Acclimation to low PFD involves an increase in light absorption efficiency, achieved largely through an increase in the concentration of light harvesting pigments. The different pigments rarely increase in concentration at the same rate, so that substantial changes can occur in the ratios between them (Waaland et al. 1974, Ramus et al. 1976, Rosenberg and Ramus 1982a). Because light availability is

considered a major factor limiting ocean productivity, a significant part of the light acclimation research has focused on marine phytoplankton. Although micro- and macroalgae share many physiological characteristics, unlike macroalgae, marine phytoplankton do not remain fixed in space with respect to a light field and may thus have acquired mechanisms to allow them to respond to variations in PFD in a shorter time than is necessary for a sessile plant. In some microalgae changes in intracellular pools of photosynthetic pigments are often observable within a few hours after transfer to a new light regime (Falkowski 1980). Such rapid and reversible responses suggest that the pigment metabolism of microalgae is tightly regulated, and that there is a relatively rapid turnover of pigment-proteins. There is some evidence that pigment adjustment times in macroalgae are on the order of several days (Lapointe and Tenore 1981, Henley and Ramus 1989b).

Growth PFD is not the only factor that influences the pigment content of marine plants. Pigments may increase during the growth and maturation of a seaweed thallus (Dring 1982). Nutrient availability, especially nitrogen, may also affect pigment content (Chapman et al. 1978, Rosenberg and Ramus 1982a, Shivji 1985, Amano and Noda 1987) and thus light harvesting efficiency. Any change in pigment concentration is likely to influence the photosynthesis-irradiance relationship. Although nitrogen is the element most frequently limiting the growth of seaweeds (Hanisak 1983), very little is known of how nitrogen availability affects the photosynthesis of macroalgae.

The major accessory pigments of red algae are the

phycobiliproteins. They absorb most of the energy that drives photosynthesis and exist in supramolecular complexes called phycobilisomes, located on the stroma side of the photosynthetic lamellae. They comprise the protein bound phycobilins phycoerythrin, phycocyanin, and allophycocyanin, with absorption peaks at 540-565 nm, 610-640 nm, and 650 nm, respectively. Together with their colorless linker polypeptides they are arranged in stacks that ensure efficient energy transfer to chlorophyll a (Gantt 1981). The phycobiliproteins of cyanobacteria can comprise up to 60% of the total soluble protein; thus a large part of the cell's resources is committed to their production.

The dissertation research presented here examines some of the plastic responses of Porphyra abbottae, a red macroalga, to key physical factors. Special emphasis is given to those phenotypic changes that affect the light harvesting ability. Chapter 2 explores the range of morphological variation for a thallus of simple construction, and the possible implications for the acquisition of light and nutrients. Chapter 3 deals with the range of variation in light absorption, pigment content and pigment composition in relation to PFD, nutrients and water motion. The significance of changes in pigmentation for photosynthesis and growth are examined in Chapters 4 and 5. Chapter 4 deals primarily with the effect of macronutrients on light-limited and light-saturated rates of photosynthesis. In Chapter 5 I examine light and nutrient induced pigment changes in relation to growth and the efficiency of light utilization. Since these studies

were all done in batch cultures with limited monitoring of nutrient concentrations, a model was developed in order to estimate time courses of nitrogen disappearance from the growth medium; those results are presented in Appendix I.

Porphyra abbottae is one of 17 species in the genus reported for the Pacific Northwest (Conway et al. 1975, Garbary et al. 1980). The gametophytic phase is a common spring-summer annual that inhabits the mid-intertidal of moderately exposed rocky shores. It is a broadly ovate and monostromatic blade. The margins of juvenile thalli are smooth, but they become increasingly ruffled in older, larger thalli.

Species of Porphyra are good representatives of the functional form group composed of thin, sheetlike thalli, i.e., with a high surface area: volume ratio. Seaweeds with this type of thallus morphology are characteristically fast growing and short lived, and often susceptible to grazing. Intertidal species of Porphyra presumably have largely escaped from grazing pressure by evolving a resistance to long emersion times.

CHAPTER 2

GROWTH AND MORPHOLOGY OF YOUNG GAMETOPHYTES OF *PORPHYRA ABBOTTAE*: EFFECTS OF ENVIRONMENTAL FACTORS IN CULTURE.

Studies on the environmental conditions that affect algal growth have largely been devoted to adult thalli. Growth optima and tolerance ranges may, however, vary greatly between developmental stages (Hanisak 1979, Fain and Murray 1982), such that growth to maturity may depend largely on survival of the microscopic stage (Chapman 1986).

Researchers have increasingly recognized the importance of studying the effects of combinations of factors on algal growth and metabolism (Durbin 1974, Santelices 1978, Hanisak 1979, Lobban et al. 1985). Temperature, light, nutrients and water motion are major physical factors most commonly associated with plant growth in the marine environment (Lobban et al. 1985). All are factors which could potentially interact so that the effect of any one factor may depend on the status of others. For example, light and nitrogen have been shown to act interactively on growth (Hoffmann and Santelices 1982, Lapointe 1981, Lapointe and Tenore 1981, Shivji 1985) and nitrogen uptake (Lapointe and Tenore 1981) of seaweeds and on their chemical (Lapointe 1981, Lapointe and Tenore 1981, Shivji 1985) and pigment (Lapointe 1981) composition. The interactive effect of water motion and nutrients may also be important. Water movement is believed to

facilitate nutrient uptake by decreasing boundary layer thickness and thus reducing gradients of nutrient concentrations adjacent to the thallus (Smith and Walker 1980, Wheeler 1980, Wheeler and Neushul 1981). The effect of water motion on growth may thus depend largely on nutrient availability in the medium (Matsumoto 1959, Conover 1968, Gavis 1976, Santelices 1978, Lapointe and Ryther 1979, Parker 1981, Fujita and Goldman 1985, Adey and Goertemiller 1987).

Environmental factors may also affect macroalgal thallus morphology, thus accounting for at least part of the morphological variation observed in natural populations (Chapman 1974, Johnstone 1978, Mathieson et al. 1981). The high degree of phenotypic plasticity in morphological features considered characteristic of many algal species points to a possibly important role of thallus geometry in the acquisition of resources for growth (Littler and Littler 1980, Norton et al. 1982, Raven et al. 1982, Rosenberg and Ramus 1984).

The present study was undertaken to learn how temperature, light, nutrients and water motion affect the early growth stages of the blade phase of Porphyra abbottae Krishnamurthy. In this chapter I examine the effects of these physical parameters on growth rate and blade morphology; in the next chapter I describe how they affect light absorption and pigment composition.

Porphyra abbottae is one of 17 species in the genus reported for the Pacific Northwest (Conway et al. 1975, Garbary et al. 1980). It is common in rocky intertidal communities in northwest Washington and has potential for mariculture. The gametophyte is a broadly ovate

monostromatic blade with margins that become ruffled as it grows. In British Columbia (Canada) and Washington (USA) it occurs as a spring-summer annual, inhabiting the mid-intertidal of moderately exposed rocky shores (Conway et al. 1975).

MATERIALS AND METHODS

Gametophytes of Porphyra abbottae Krishnamurthy (strain 1626, obtained from a single carpospore of a blade collected north of Rialto Beach, Washington, 47°58'N 124°39'W, on 5/30/83) were cultured from conchospores and subjected to various conditions of temperature, light, nutrients and water motion in two consecutive multifactor experiments. Spore release was induced by transferring laboratory cultured conchosporangial tufts from 10°C, 8L:16D h to 8°C, 16L:8D h, all at 25-50 µmol photons m^{-2} s^{-1} PAR (Lambda cosine collector). Conchospores were obtained within 3-4 weeks and seeded onto sterile microscope slides in a single tray to ensure homogenous settlement and germination of spores. The slides were then attached with silicone high vacuum grease to 80x100 mm Pyrex (#3280) culture dishes and incubated in 100 ml of enriched seawater medium at a 16L:8D h photoperiod under the appropriate sets of conditions described below. The medium was changed every 5-7 days. Nutrient analyses were not performed, but in a similar study (Chapter 4, Appendix 1) I found that, at comparable density, in 5 days 30-day old plants caused total depletion of nutrients in f/20 (see below) and partial depletion in f/2.

In the first experiment, the combined effects of temperature,

light and nutrients were studied in a 3x3x3 factorial design without replication (one culture flask per treatment combination). The temperatures, photon flux densities (PFD) and nutrient concentrations used were: 8, 10 and 12°C; 17.5, 70 and 140 µmol photons m^{-2} s^{-1} (Lambda cosine collector), provided by cool white fluorescent lamps (16L:8D h); and f/20, f/4 and f/2, prepared by filter sterilization (0.22 µm) of 5%, 25% and 50% solutions, respectively, of "f" enrichment medium in seawater (Guillard and Rythe r 1962). Stirring was provided by maintaining the cultures on rotary shakers at 100 rpm. Blade sizes (surface area) were determined at 14, 24 and 34 days after spore germination; the blades were subsequently preserved by mounting on microscope slides in a 50% "Karo" corn syrup solution.

In the second experiment, the combined effects of water motion and nutrient concentration were studied in a 4x4 factorial design with replication (2 culture flasks per treatment combination). Four different levels of water motion were provided by rotary shakers (at 0, 50, 100 and 150 rpm). The four nutrient concentrations were f/20, f/4, f/2 and full strength f medium. Cultures were kept at 12°C and at growth saturating PFD (70-80 µmol m^{-2} s^{-1}). Blade sizes were determined at 22, 32 and 45 days after spore germination. The blades were subsequently frozen and stored at -15°C.

Growth rates were assessed by measuring blade surface area at 10 to 13 day intervals. With the aid of a trinocular inverted microscope, images of the live, attached blades were projected onto a screen and traced on acetate sheets. I did not control for the effect of the high

PFD (3000 - 5000 μmol m^{-2} s^{-1}) that the plants were exposed to during surface area measurements, but damage from a short exposure (ca. 1 min) to high PFD seems unlikely given that low light acclimated P. perforata, a related species occurring in a similar habitat, is not photoinhibited when exposed to high PFD (2000 μmol m^{-2} s^{-1}) for less than 20 min (Herbert and Waaland 1988). Twenty blades per treatment combination were chosen at random on each sampling date, and from the tracings their area, length and width were determined with a digitizer (Numonics Digibit) attached to a microcomputer (MS-DOS). To estimate the relationship between fresh weight and surface area the 45-day old blades from the Water Motion x Nutrients experiment were carefully blotted and weighed with a 0.1 mg precision (range: 0.5 - 33 mg). Blade surface area was found to be an adequate predictor of fresh biomass (linear regression: y (mm^2) = 16.4x (mg), r^2=0.98) and therefore could be used as a reliable measure of growth in P. abbottae. Only a slight improvement in the linear fit (r^2 = 0.99) is obtained when blade fresh weight is regressed against blade volume instead of blade area. From the former a mean specific weight of 1.07 g/cm^3 was calculated for 45-day old blades in the Water Motion x Nutrients experiment. Preserved or frozen blades were later handsectioned for thickness measurements with an ocular micrometer. "Karo" mounts from the first experiment were immersed overnight in seawater prior to sectioning. A comparison of blade thickness determined from "Karo" mounted or frozen blade pieces showed no significant difference between the two methods (paired t test, n=10, p> 0.36). Except for those

thalli that were too small to section (low nutrient or low light cultures), 10 blades (Temperature x Light x Nutrients experiment) or 20 blades (Water Motion x Nutrients experiment) per culture flask were sectioned.

The main and combined effects of temperature, light, nutrients and water motion on blade size were tested by the appropriate analyses of variance (ANOVA, Neter et al. 1985). Since each culture flask represents one experimental unit, within flask variability cannot be used to estimate the total random variation and ANOVAs were therefore performed on the means for each flask to account for flask to flask variability. Differences between the levels of each factor were further analyzed by the Least Significant Difference (LSD) and Tukey methods for pairwise comparisons of main effect means and by contrasts (Steel and Torrie 1980).

RESULTS

Growth

The rate of increase in blade area was found to vary as growth proceeded. Early growth was exponential until a blade surface area of approximately 3 mm^2 was reached (Fig. 2.1). Thereafter, the specific growth rate (given by the slopes of the growth curves in Figs. 2.1 and 2.2) decreased with increasing plant size. However, the decline in growth rate noted for some of the treatments in the first experiment (Temperature x Light x Water Motion, Fig. 2.1) was small enough that growth could still be described adequately by an exponential function

(with few exceptions $r^2 \geq 0.8$, Table 2.1). Estimates of specific growth rates from this experiment are thus included here for comparison with other studies. Since growth of plants in the water motion and nutrients combinations did not occur at a constant rate during the period of the study (Fig. 2.2), no single specific growth rate describes their overall growth rate. Plant size (as blade surface area) at a given time is therefore a more appropriate estimate of cumulative growth and is used here to evaluate how temperature, light, nutrients and water motion affected growth rate.

Table 2.2 summarizes the results of the ANOVA test for 34 day old plants in the Temperature x Light x Nutrients experiment. The nature of the design requires that main effects and second order interactions be tested against the third order interaction mean square, resulting in highly conservative significance tests. There were significant, independent effects of PFD and nutrients on blade size (Table 2.2), resulting in significantly smaller blades at 17.5 than at 70 (but not at 140) µmol photons $m^{-2}s^{-1}$ ($p<0.05$) and in f/20 than in f/4 or f/2 ($p<0.01$, LSD and Tukey tests, Fig. 2.3). Further analysis indicates that PFD had a significant effect at 12°C only ($p<0.05$), and that the effect of nutrient concentration was significant at all levels of temperature and light ($p<0.05$). Even though the highest growth rates were obtained at 10 and 12°C, overall there was no significant effect of temperature on growth (Table 2.2). The general ANOVA failed to demonstrate the existence of second order interactions between temperature, light and nutrients (Table 2.2), possibly due to the low

power of this test (lack of replication). The results obtained at 10 and 12°C, however, suggest that the effect of nutrient concentration depends on PFD.

Water motion and nutrients had a highly significant interactive effect on blade size (Table 2.3), indicating that the effect of water motion on growth depends on the nutrient concentration. Irrespective of water motion, plants grown in f/4, f/2 and f were significantly larger than in f/20 (LSD and Tukey tests, $p<0.01$), although growth became nutrient saturated at 50% f medium (Fig. 2.4). While further addition of nutrients did not enhance growth, increasing the water motion did. Irrespective of nutrient concentration, blades grown at 100 and 150 rpm were significantly larger than at either 0 or 50 rpm (LSD and Tukey tests, $p<0.01$), suggesting that an important qualitative difference in the flow regime experienced by these juveniles may take place when the water motion is increased from 50 to 100 rpm. In addition, water motion was most effective in stimulating growth in the low nutrient cultures. At 45 days, an increase from 50 to 100 rpm resulted in a 4-fold increase in the blade area of f/2 plants (from 40 $\pm$ 1.4 mm^2 [SD, n=2] to 160 $\pm$ 35.8 mm^2), compared to a 25-fold increase in the blade area of f/20 plants (from 0.346 mm^2 [no replication]) to 8.6 $\pm$ 0.80 mm^2).

Blade Morphology

Blade length/width ratios were found to depend on the PFD, nutrient concentration and water motion (Figs. 2.5 and 2.6). At 17.5

μmol photons m^{-2} s^{-1} blades grew ca. 2.5 times more in length than in width, but only ca. 1.9 times at 70 μmol photons m^{-2} s^{-1} (Fig. 2.5). Whereas increasing water motion clearly resulted in more elongate blades (Fig. 2.6), the effect of nutrient enrichment on the length/width ratio was dependent on the level of water motion. At 0 and 50 rpm the ratio was significantly greater in f/2 than in full strength f medium, while at 100 and 150 rpm the ratios in the two media were not significantly different.

Blade thickness (Table 2.4) varied between 53 and 88 um and was most affected by water motion and nutrient concentration. While increasing water motion resulted in decreasing blade thickness, the opposite resulted from increasing nutrient concentrations. The largest differences in thickness were produced between the f/2 and f media and between 0 and 50 rpm. Temperature and light did not have a consistent effect on blade thickness (Table 2.4).

DISCUSSION

Light regime, nutrient enrichment and water motion significantly affected the growth rate and morphology of young Porphyra abbottae gametophytes.

Optimal PFD for growth was found to be 70 μmol m^{-2} s^{-1} with the higher PFD (140 μmol m^{-2} s^{-1}) being inhibitory. Although these values are very low compared to the levels of irradiance that adult plants would normally experience in their natural environment, they may well reflect the irradiance conditions of emerging sporelings. A few

studies have shown that sporelings of benthic algae have a relatively low light requirement compared to mature plants (Luning 1981, Fain and Murray 1982, Friedlander and Dawes 1984). Light requirements of adult P. abbottae are not known, but growth studies of the intertidal species P. sanjuanensis (Herbert, unpublished data) indicate near maximum growth of adult blades at 250 μmol photons m^{-2} s^{-1}. Net photosynthesis of juvenile P. abbottae is light-saturated at ca. 250 μmol photons m^{-2} s^{-1} (Chapter 4). Since none of the temperatures employed in this study were stressful, our finding that the inhibitory effect of PFD was more pronounced at the higher temperatures cannot at present be explained by an effect of temperature on photoinhibition of photosynthesis (Powles 1984). Also, whole thallus absorption spectra of these blades (Chapter 3) show no evidence of photodestruction of photosynthetic pigments in plants grown at 140 μmol m^{-2} s^{-1}.

Both water movement and high nutrient concentration seem essential for rapid growth of Porphyra abbottae gametophytes. At 12°C and 70 μmol photons m^{-2} s^{-1} growth rates remain high as long as nutrients are available at concentrations equal to or higher than those present in 25% f medium (i.e., an enrichment of 0.44 mM NO_3^-, 13 μM $PO_4^{\equiv}$, with added trace metals and vitamins) and adequate water motion is provided. The finding that water motion enhanced growth even at saturating nutrient concentrations most likely reflects the importance of water motion in facilitating carbon uptake (Wheeler 1980). Thus these experiments suggest that under favorable conditions of light and nutrients, carbon may soon become a limiting resource in batch cultures of this species.

It is often implied that the specific growth rate (the increase in plant tissue per unit plant tissue per day) of macroalgae remains constant through time (e.g., Chapman et al. 1978, Friedlander and Dawes 1984, Hurtado-Ponce and Umezaki 1987, Shivji 1985). However, thallus size, shape and possibly metabolism, change during development and may affect growth rate (Droop 1983). Here I show that the growth rate of P. abbottae slowed as the plants increased in size. In a 4 week study Bird et al. (1979) found that growth rates of Gracilaria tikvahiae remained exponential for 2.5 weeks in culture and decreased thereafter, whereas slower growing species (Fucus serratus and Chondrus crispus) grew at a constant rate throughout the study. Since nutrient concentrations were not monitored during the present study, the possibility exists that growth rates declined due to increasing nutrient limitation as plants grew larger. Alternatively, changes in growth rate may take place if different developmental stages differ in their ability to absorb or utilize light and nutrients for growth.

The morphological parameters examined here show that blade shape and thickness do respond to environmental factors early in development. Morphological plasticity could enhance light absorption, nutrient uptake and responses to water motion in different environments. I found that low PFD stimulated growth in length, a response that may allow emerging blades to reach a favorable light environment. The pattern observed for blade length/width in relation to the applied nutrient concentrations and water motion levels suggests that faster growing plants also tend to be more elongate. Two cultivars of

Porphyra yezoensis and P. tenera that have been selected for their vigorous growth also had an increased length/width ratio (Miura 1979). That low PFD produced slow growing but very elongate blades in P. abbottae suggests that blade shape is not determined by growth rate alone. In kelps, increasing exposure to waves and currents is known to produce blades that are either narrower or split, most likely an adaptation that reduces drag (Lobban et al. 1985). Although water motion levels in this study presumably were not stressful, our data also show a progressive narrowing of the blades as water motion is increased in the cultures.

Blade thickness is a direct measure of the surface area/volume ratio of the thallus. Because thinner blades have a higher proportion of their tissue in direct contact with the external medium, that increase in absorptive area may contribute to a greater efficiency of nutrient utilization at low ambient nutrient levels. On the other hand, since thinner blades of equal mass expose more surface area to the flow, they should have greater drag. The thinner blades obtained in the low nutrient cultures suggest an adaptive mechanism to enable adjustment of the surface area/volume ratio according to nutrient availability. Although water motion presumably facilitated nutrient uptake, it resulted in thinner blades. This may be merely a consequence of the higher growth rates of thalli at high water motion, since I have observed that, while cell size may vary, cell wall thickness seems to be the major factor contributing to increases in blade thickness. Thus fast growing thalli may invest relatively little

energy in increasing cell volume and addition of cell wall material. Assuming that the low water motion thalli were nutrient-limited, their greater thickness may correspond to increased allocation of cell wall polysacharides (Neish et al. 1977).

In terrestrial plants irradiance can affect leaf thickness, mesophyll area and specific weight (Björkman 1981, Fetcher et al. 1983, Oberbauer and Strain 1986). I did not find any relationship between light level and blade thickness in P. abbottae, but there is evidence that P. abbottae acclimates to the light environment by adjusting the amount of its photosynthetic pigments (Chapter 3).

Fig. 2.1. Blade area at 14, 24 and 34 day intervals of P. abbottae grown under various combinations of temperature (8, 10, 12°C), nutrient concentration (f/20, f/4, f/2) and PFD (■ = 17.5; ● = 70; ▲ = 140 μmol $m^{-2}s^{-1}$). Symbols represent the means (n=20) of $\log_e$ transformed values of blade area (original units are mm^2). Specific growth rates (day^{-1}) can be estimated from the slopes of the lines.

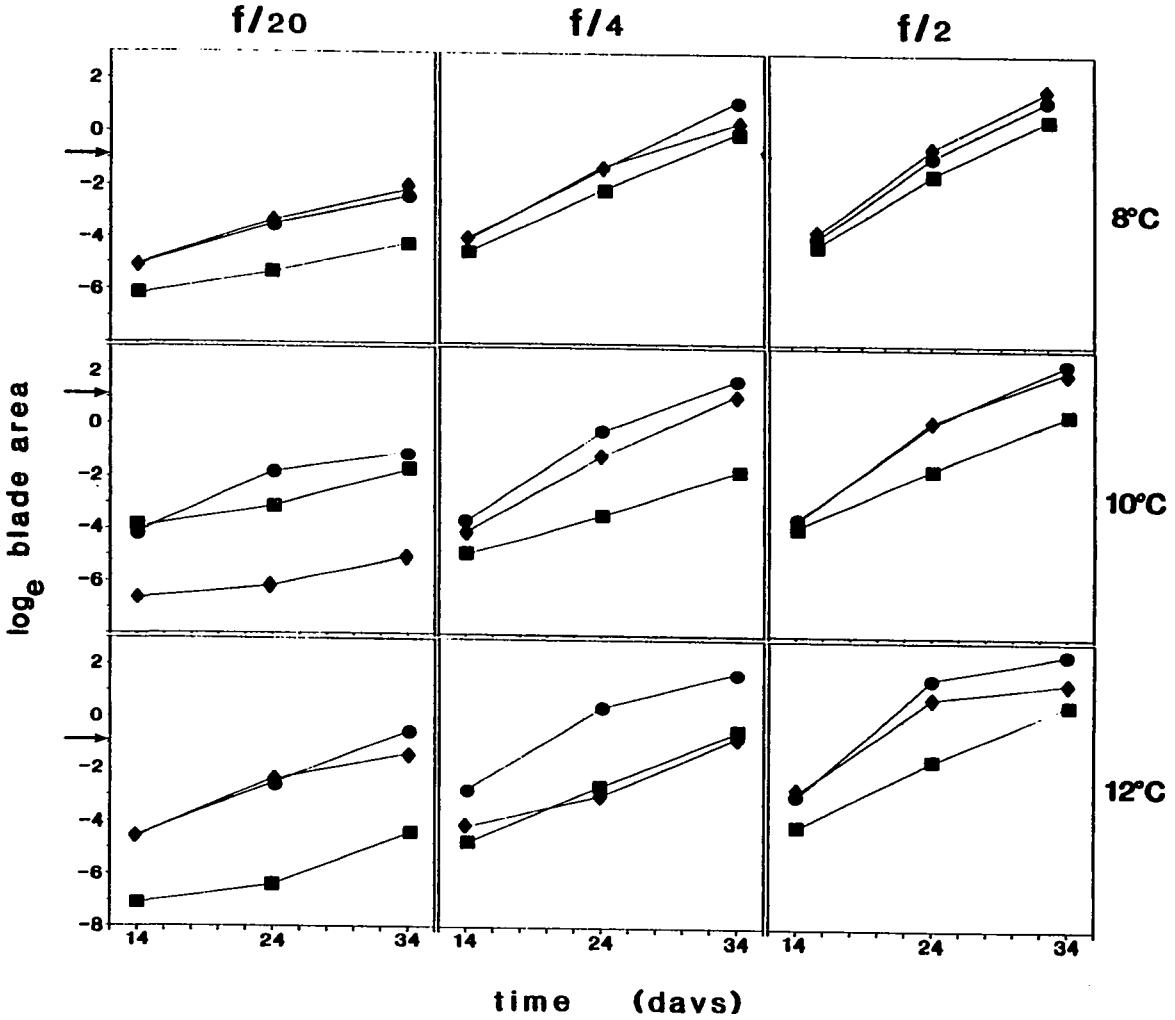

f/20
f/4
f/2
8°C
10°C
12°C
log$_e$ blade area
2
0
-2
-4
-6
-8
14
24
34
time (days)

Fig. 2.2. Blade area at 22, 32 and 45 day intervals of P. abbottae grown under various combinations of nutrient concentration (f/20, f/4, f/2, f) and water motion (■ = 0; ● = 50; ◆ = 100; ▲ = 150 rpm). Symbols represent the means (n=20) of $\log_e$ transformed values of blade area (original units are mm^2).

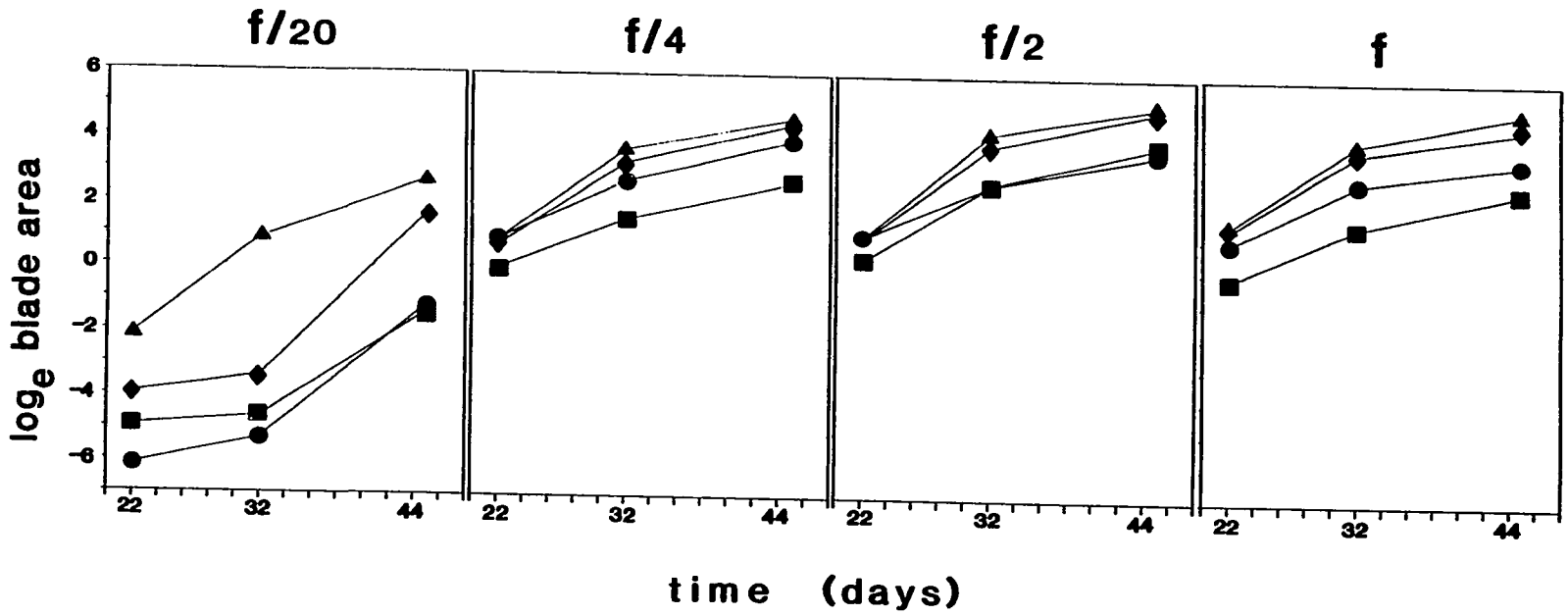
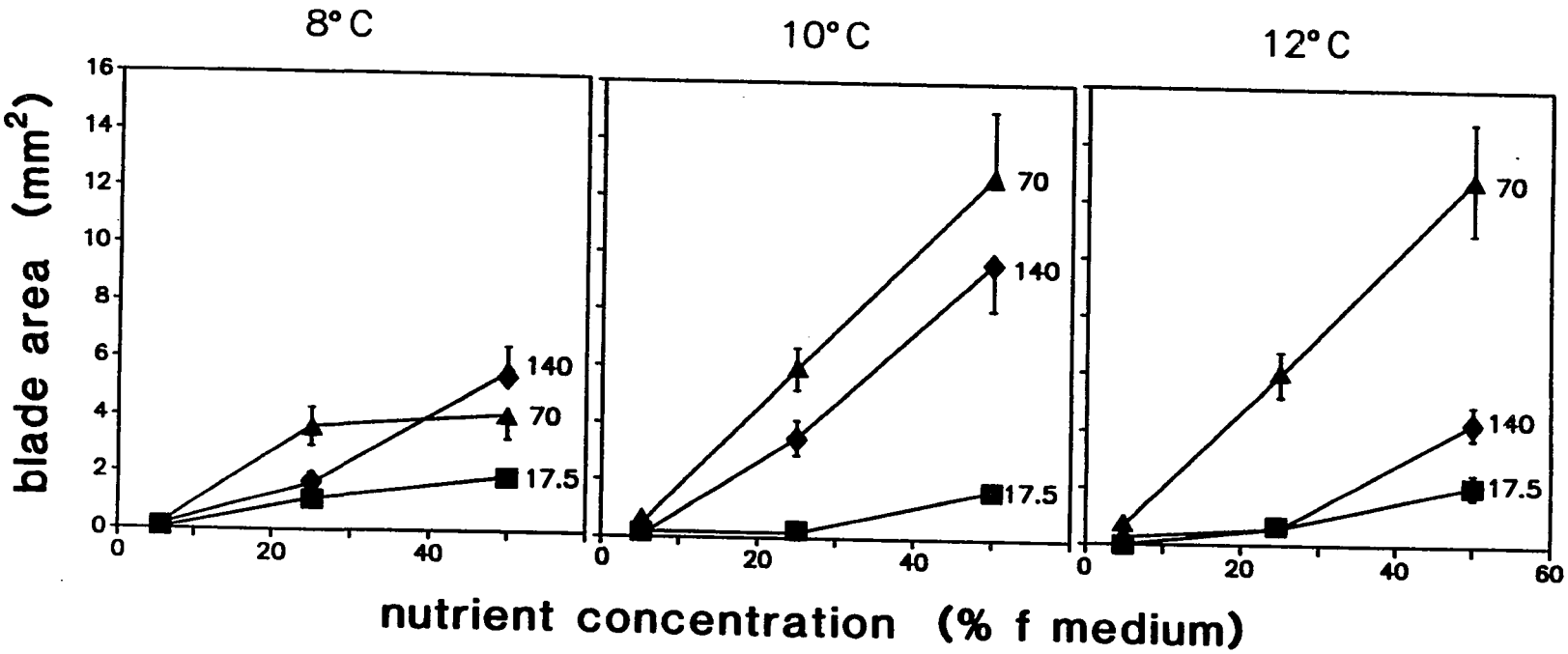

f/20
f/4
f/2
f
log$_e$ blade area
6
4
2
0
-2
-4
-6
22
32
44
time (days)

Fig. 2.3. Mean blade area (± 95% confidence intervals, n=20) of *P. abbottae* at 34 days for various combinations of temperature, nutrient concentration (as % f medium) and PFD (17.5, 70 and 140 μmol m^{-2} s^{-1}).


8° C
10° C
12° C
blade area (mm²)
nutrient concentration (% f medium)
140
70
17.5
0
2
4
6
8
10
12
14
16
0
20
40
60

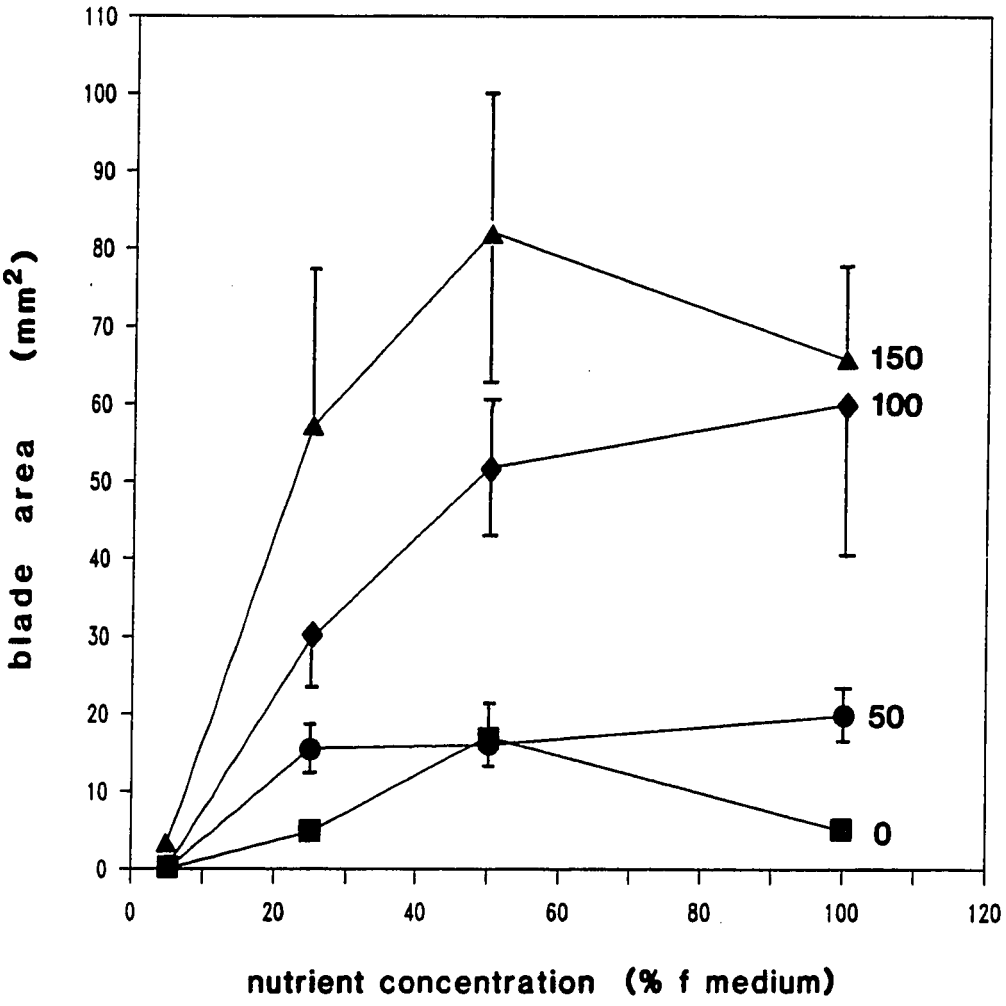


Fig. 2.4. Mean blade area (± 95% confidence intervals, n=20) of *P. abbottae* at 32 days for the various combinations of nutrient concentration (as % f medium) and water motion (0, 50, 100 and 150 rpm).

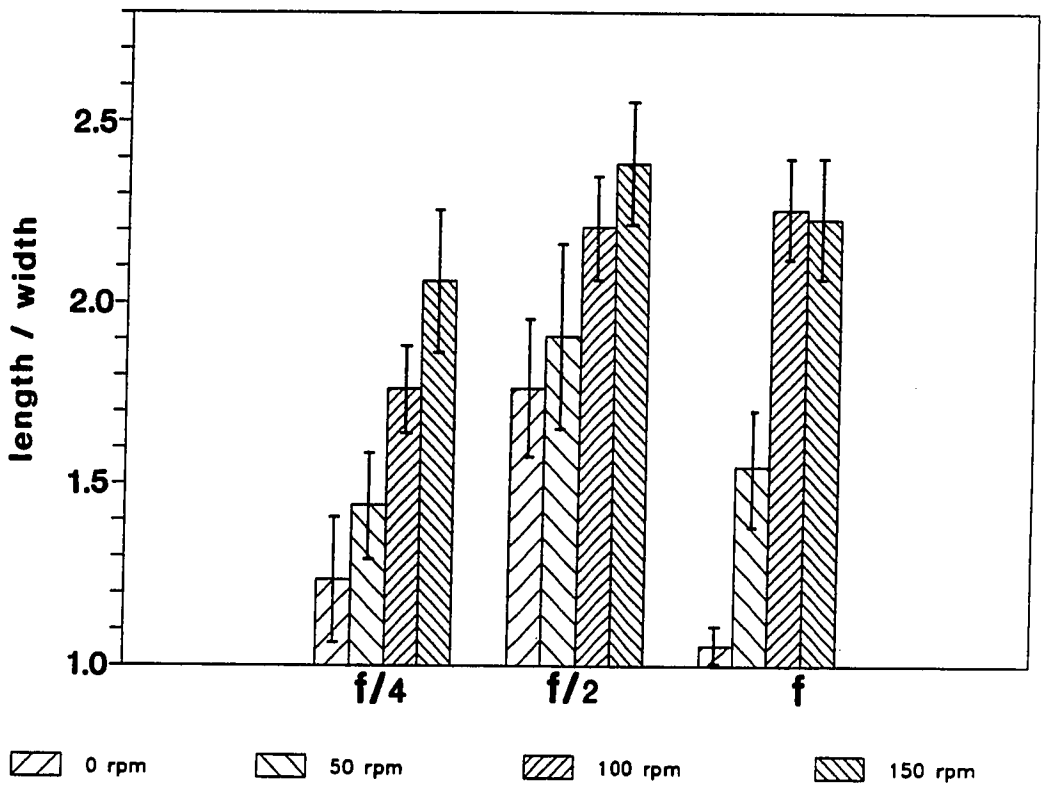


Fig. 2.5. Mean length/width ratios (± 95% confidence intervals) of P. abbottae blades grown under two nutrient concentrations and three PFDs (n = 283; surface area range = 0.37-2.7 mm^2).

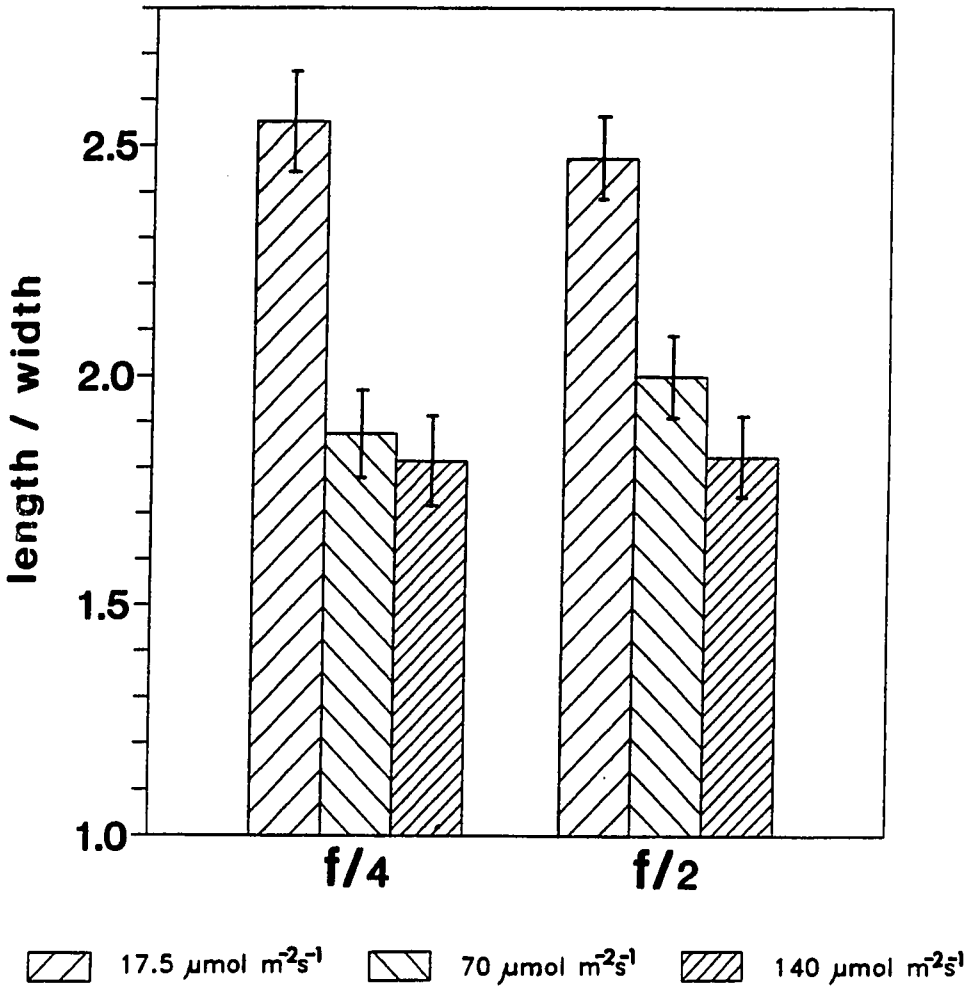


Fig. 2.6. Mean length/width ratios (± 95% confidence intervals) of *P. abbottae* blades grown under three nutrient concentrations and four water motion levels (n = 274; surface area range = 20-150 mm^2).

Table 2.1. Specific growth rates (day^{-1}) of Porphyra abbottae juvenile blades at various combinations of temperature, photon flux density (PFD) and nutrient concentration. Specific growth rates were determined from the slopes of linear regressions of the $\log_e$ (blade area) values versus time (n = 59 or 60). Determination coefficients (r^2) shown in parentheses.

Temperature (°C)	PFD ($\mu mol\ m^{-2}s^{-1}$)	Nutrient Concentration		
		f/20	f/4	f/2
		Specific Growth Rate (day^{-1})		
8	17.5	0.100 (0.65)	0.227 (0.96)	0.240 (0.95)
	70	0.137 (0.84)	0.260 (0.96)	0.262 (0.95)
	140	0.149 (0.87)	0.220 (0.95)	0.271 (0.97)
10	17.5	0.112 (0.82)	0.161 (0.89)	0.217 (0.97)
	70	0.155 (0.81)	0.270 (0.96)	0.301 (0.95)
	140	0.078 (0.58)	0.263 (0.96)	0.289 (0.95)
12	17.5	0.126 (0.78)	0.216 (0.97)	0.239 (0.95)
	70	0.200 (0.89)	0.226 (0.91)	0.274 (0.85)
	140	0.158 (0.80)	0.178 (0.79)	0.213 (0.81)

Table 2.2. ANOVA table for blade area at combinations of temperature, light (PFD), and nutrient concentration.[a]

Source of variation	df	Sum of squares[b]	Mean square	F
Temperature[c]	2	0.331	0.166	0.10
Light[d]	2	17.559	8.780	5.12 *
Nutrients[e]	2	73.687	36.843	21.49 ***
Temp. x Light	4	2.811	0.703	0.41
Temp. x Nutrients	4	1.934	0.298	0.17
Light x Nutrients	4	1.210	0.302	0.18
Temp. x Light x Nutrients	8	13.714	1.714	
Total	26	110.51		

* $p<0.05$
*** $p<0.001$
[a] 3x3x3-factorial design without replication
[b] The analysis is based on the $\log_e$ transformed means for each experimental unit (culture flask) at 34 days.
[c] 8, 10, 12°C
[d] 17.5, 70, 140 µmol photons $m^{-2}s^{-1}$
[e] f/20, f/4, f/2

Table 2.3. ANOVA table for blade area at various combinations of water motion and nutrient concentration.[a]

Source of variation	df	Sum of squares[b]	Mean square	F
Water Motion[c]	3	68.52	22.84	29.68 ***
Nutrients[d]	3	415.06	138.35	179.80 ***
Water Motion x Nutrients	9	37.91	4.21	5.47 **
$Error_1$	16	12.31	0.77	
Time	2	225.39	112.69	370.10 ***
Water Motion x Time	6	8.45	1.41	4.62 **
Nutrients x Time	6	18.87	3.14	10.33 ***
Water Motion x Nutrients x Time	18	4.69	0.26	0.86
$Error_2$	31[e]	9.44	0.30	
Total	94	800.63		

** $p<0.01$
*** $p<0.001$
[a] 4x4x3-factorial design with replication (n=2) and repeated measures on one factor (Time) (Neter et al. 1985).
[b] The analysis is based on the $\log_e$ transformed means for each experimental unit (culture flask) at 22, 32 and 45 days.
[c] 0, 50, 100, 150 rpm
[d] f/20, f/4, f/2, f
[e] One replicate flask missing.

Table 2.4. Mean blade thickness (μm) ± 95% confidence intervals[a] at different levels of temperature, photon flux density (PFD), water motion and nutrient concentration.

Temperature (°C)	PFD (μmol $m^{-2}s^{-1}$)	Water Motion (rpm)	Nutrient Concentration f/4	f/2	f
			Mean blade thickness (μm)		
8	70	100	53 ± 2.9	66 ± 5.7	
	140		57 ± 3.1	64 ± 6.8	
10	70		62 ± 5.1	58 ± 3.8	
	140		57 ± 4.2	66 ± 5.1	
12	70		66 ± 3.5	71 ± 4.6	
	140		63 ±12.2	64 ± 3.6	
	70	0	70 ± 6.7	56 ± 2.3	88 ±10.1
		50	61 ± 2.2	60 ± 2.8	67 ± 5.6
		100[b]	58 ± 2.9	57 ± 2.7	67 ± 2.6
		150	55 ± 3.7	54 ± 1.7	65 ± 2.2

[a]Confidence intervals (t test) of the means for all the blades measured within each treatment combination.
[b]Duplicate data from the Water Motion x Nutrients experiment.

CHAPTER 3

SPECTRAL LIGHT ABSORPTION BY INTACT BLADES OF *PORPHYRA ABBOTTAE*: EFFECTS OF ENVIRONMENTAL FACTORS IN CULTURE

Many algae acclimate to their light environment by changing their light harvesting pigments. In nearly all seaweeds examined so far, total pigment content as well as the ratio of accessory pigments to chlorophyll *a* increases at low levels of irradiance, such as those encountered at depth, in shaded habitats or during winter (e.g. Waaland et al. 1974, Ramus et al. 1976, Rhee and Briggs 1977, Rosenberg and Ramus 1982). This phenotypic response has been generally interpreted as an adaptation that enhances light absorption at photosynthesis limiting irradiances (Ramus 1981).

A plant's nutrient status will also affect its pigment content and thus might explain observed seasonal variations in pigment levels (Lapointe and Ryther 1979, Rosenberg and Ramus 1982, Wheeler et al. 1984). Though light limitation and nutrient abundance are both effective in raising pigment levels, there is some evidence that ratios of accessory pigments to chlorophyll *a* may be insensitive to ambient nutrient concentrations (Lapointe and Ryther 1979, Ramus 1983, Shivji 1985). A shift in the phycoerythrin:chlorophyll *a* ratio under nitrogen limitation has been interpreted as an indication that phycobiliproteins may serve as a nitrogen sink in red algae (Lapointe 1981). All this

would suggest that different physiological mechanisms are responsible for the control of pigment content by light or nutrients. Other factors are likely to determine plant pigmentation as well, provided they have an influence on the rate at which ambient light and nutrients are utilized. Of particular interest is the potential effect of water motion, given its importance in facilitating uptake processes mediated by diffusion (Smith and Walker 1980, Wheeler 1980). The effects of factors other than light and nutrients on the pigment content of macroalgae remain largely unexplored.

Pigmentation changes in response to light and nutrient availability have traditionally been evaluated through quantification of the extracted pigments. For ecological purposes, however, spectral light absorption by intact blades is a more meaningful way of evaluating the light trapping capability of a plant subjected to a particular set of environmental conditions. Because morphology and cellular architecture affect the propagation and internal distribution of light (Ramus 1978, Vogelmann and Björn 1986), whole thallus light absorption cannot readily be inferred from pigment amounts. Most but not all of light absorption by plant cells is due to the presence of photosynthetic pigments. Whole thallus absorptance spectra combined with photosynthetic action spectra are therefore necessary for an understanding of how efficiently marine plants can use ambient light.

The purpose of this study was to relate major environmental parameters (temperature, irradiance, nutrients, and water motion) to the spectral light absorption characteristics of laboratory cultured

gametophytes of Porphyra abbottae Krishnamurthy. The monostromatic thalli of this species are particularly well suited for measurements of whole thallus light absorption, because relatively small changes in pigment content will affect their absorptance. Several authors have compared absorptance spectra of macroalgal species (Haxo and Blinks 1950, Fork 1963, Kageyama et al. 1977, Ramus 1978, Lüning and Dring 1985), yet very few studies have examined the phenotypic changes in whole thallus spectral absorptance in terms of environmental parameters (Rhee and Briggs 1977, Ramus 1978). This study evaluates the significance of phenotypic changes in response to growth conditions in this alga of potential commercial value.

MATERIALS AND METHODS

Porphyra abbottae Krishnamurthy (University of Washington culture collection, strain 1626) gametophytes were cultured from conchospores at combinations of a) temperature (8, 10, 12° C), photon flux density (PFD; 17.5, 70, 140 µmol m^{-2} s^{-1}) and nutrient concentration (f/4, f/2 medium), all at 100 rpm (experiment 1), or b) water motion (0, 50, 100, 150 rpm) and nutrient concentration (f/4, f/2, f medium), all at 70 µmol m^{-2} s^{-1} (experiment 2), in two consecutive multifactor experiments. Experimental conditions were identical to those described in Chapter 2. Illumination was provided by cool white fluorescent lamps. Culture media were prepared by appropriate dilutions of Guillard and Ryther's (1962) "f" enrichment and were changed every 5-7 days; water motion was provided by rotary shakers. At the end of the

growing period (34-45 days) samples were taken to determine blade thickness and whole thallus absorption spectra.

Whole thallus absorption spectra were obtained by scanning from 400-750 nm with an Aminco DW-2 double beam spectrophotometer equipped with a beam scrambler and a condensing lens. Spectra were recorded for 4-6 individuals from one culture dish per treatment combination. The fresh blade (2-400 mm^2 surface area) was held between a piece of microscope slide and a cover slip attached to a holder that was positioned next to the photomultiplier to minimize light extinction resulting from scattering. Small holes (1.2 or 1.7 mm diam) drilled through the holder at the sites of beam incidence permitted measurements of very small individuals. Bleached blades (ca. 5 h at 5 cm from a 500 Watt focused projector lamp, room temperature; n=5) were used as scattering controls. Since in the absence of photosynthetic pigments light extinction in the 500-750 nm range was wavelength independent (Fig. 3.1), whole thallus peak absorbances of the phycobiliproteins and chlorophyll a were obtained by subtraction of 750 nm optical density values. In order to calculate broadband light absorption, the optical density scans were digitized (Numonics Digibit) and then interpolated numerically using a cubic spline algorithm to provide data at 2 nm intervals. This method was also used to reconstruct numerically a light scatter spectrum from the bleached blades and a PFD spectrum (measured with an ISCO spectroradiometer, model SR) of the light source used in the culture facility (Fig. 3.1). Absorptance (α), the fraction of the incident light that is absorbed

by the tissue, was calculated as $a = 1 - 10^{-A}$, where A = absorbance. Broadband absorptance (the fraction of incident photons absorbed in the 400-700 nm range) was obtained by numerical integration of the (absorptance-scatterance) x (light source) spectrum, where spectral PFD values of the light source are expressed as fractions of the 400-700 nm integrated curve.

Pigments were extracted from blades grown in experiment 2 only. Duplicate samples of 5 individuals (4-78 mg fresh wt.) per culture dish (2 dishes per treatment combination) were extracted on ice by grinding the tissue with a glass homogenizer in the presence of 10 mM potassium phosphate buffer (pH 7.0). Cell breakage was facilitated by repeatedly freezing and thawing the suspension (Merrill 1985). The homogenate was centrifuged for 45 min at 40,000 x g (4^{o} C), and the absorbances of the aqueous extract at 563 and 618 nm were used to determine phycobiliprotein concentrations assuming extinction coefficients ($E^{1\%}_{1cm}$) of 81.5 and 64.5 for phycoerythrin (PE) and phycocyanin (PC), respectively (O'Carra and O'hEocha 1976). PE concentrations were corrected for overlapping with PC using a coefficient (K_{563}) of 0.33, obtained by interpolation of values given by Merrill (1985) for Porphyra yezoensis. A second aqueous extraction was found to yield ca. 8% of the total extractable phycobiliproteins, and total content was thus estimated accordingly. The pellets were then extracted in 90% acetone, centrifuged at 20,000 x g for 20 min, and the concentration of chl a was calculated from the equations of Jeffrey and Humphrey (1975).

Effects of environmental parameters on broadband absorptance

values and on PE/chl *a* and PE/PC absorptance ratios were tested separately for each experiment by factorial analyses of variance (ANOVA). Since replicate absorption spectra were all derived from a single dish in each treatment combination ("pseudoreplication", Hurlbert 1984), the third order interaction was assumed non-significant in experiment 1 (temperature, PFD, nutrients) and that interaction term was used as the error term for the *F* test. In experiment 2 (water motion, nutrients), however, the replicate observations were included in the ANOVA in order to evaluate the second order interaction. The results from this analysis are therefore interpreted with caution. Differences between levels of one factor were further analyzed by contrasts among means (Steel and Torrie 1980). To determine the degree of association (Spearman's rank correlation) between growth and absorptance, growth rates (d^{-1}) were calculated from surface area measurements taken 10-13 d prior to and at termination of the experiments (data from Chapter 2).

RESULTS

Light level had a significant effect ($p<0.05$) on whole thallus broadband absorptance (Fig. 3.1). Absorptances were highest in the low light (17.5 µmol m^{-2} s^{-1}) treatments (Fig. 3.2) irrespective of temperature or nutrient level. In f/4, blades grown at 70 µmol m^{-2} s^{-1} had overall lower absorptance values than those grown at 17.5 or 140 µmol m^{-2} s^{-1}, whereas in f/2 absorptance decreased with increasing PFD.

Water motion, nutrients, and their interaction significantly

affected broadband absorptance ($p<0.001$). Absorptances generally increased with increasing nutrient concentration and decreasing water motion (Fig. 3.3). The interactive effect of these two factors is evident from the sharp decline in absorptances due to increased water motion in the f/4 cultures (all differences between water motions significant at $p<0.001$) compared to a correspondingly slow decline in the f/2 and f medium cultures (differences between water motions mostly not significant at $p<0.05$).

Patterns of variation in peak absorptances of Chl *a*, PE and PC (Tables 3.1, 3.2) were similar to those shown for broadband absorptance, as indicated by highly significant correlations between peak and broadband absorptance values (Table 3.3).

The PE/Chl *a* ratio ranged from 0.86-1.04 in experiment 1 (Table 3.1) and was affected both by PFD ($p=0.01$) and by an interaction of light with nutrients ($p<0.05$) but not significantly by nutrients alone. Since the PE/PC ratio (Table 3.1) did not vary significantly with either temperature, light or nutrients (grand mean = 1.50 ± 0.084, $n=17$), these results indicate that the two phycobilins change more with the light environment than does Chl *a*. Overall, PE/Chl *a* was lower at 70 than at 17.5 or 140 $\mu mol\ m^{-2}\ s^{-1}$ ($p<0.01$); for blades grown in f/4 this ratio was also significantly higher at 140 $\mu mol\ m^{-2}\ s^{-1}$ than at any other PFD ($p<0.05$).

Thalli from the water motion and nutrient combinations (experiment 2) had PE/Chl *a* and PE/PC ratios that ranged from 0.68-0.97 and from 1.23-1.51, respectively (Table 3.2). Water motion, f medium

concentration, and their interaction significantly affected these absorptance ratios ($p \leq 0.001$). PE/Chl *a* decreased with increasing water motion in all three nutrient concentrations, the decrease being largest in f/4 (all differences between water motions significant at $p<0.001$ in f/4). Only at 100 and 150 rpm did nutrients significantly increase the PE/Chl *a* ratio. The PE/PC ratio (Table 3.2) generally decreased with increasing nutrient concentration but showed no clear trend with respect to water motion, except perhaps in the low nutrient cultures.

Growth rate was significantly correlated with absorptance ratios but not with peak or broadband absorptance values (Table 3.3). Further analysis indicated that growth rate per incident photon (growth rate/PFD) was correlated with PE ($p<0.05$) but not with broadband absorptance (data not shown).

The pooled whole thallus absorptance values from experiment 2 are plotted as a function of blade thickness in Figure 3.4. Increased blade thickness did not result in a proportional increase in the per unit area absorption of light by Chl *a*, PE or PC.

Pigment content (mg g fr wt^{-1}, Table 3.4) varied with water motion and nutrient concentration in a manner that differed somewhat from the pattern observed for whole thallus absorptance. In the f/4 and f/2 cultures Chl *a*, PE and PC content generally decreased from 50 to 150 rpm, but in f medium only PE decreased at the higher water motion. Increasing the nutrient concentration resulted in higher pigment content at 100 and 150 rpm only. At low water motion (50 rpm) nutrients had no significant effect on pigment content, and in still

water (0 rpm) pigment content was highest in f/2 and lowest in f medium.

The relationship between pigment content and whole thallus absorptance was non-linear (Fig. 3.5), suggesting self-shading at high pigment densities.

DISCUSSION

Spectral light absorption by Porphyra abbottae was affected by a variety of environmental factors, reflecting their effect on pigment content, pigment composition and blade thickness. The present study indicates that in batch culture quantitative changes in absorptance occur in response to PFD, nutrients and water motion, and that these changes cannot be explained by differences in growth rate alone.

Previous work (Chapter 2) showed that growth of P. abbottae juveniles was light saturated at 70 μmol m^{-2} s^{-1}, light inhibited at 140 umol m^{-2} s^{-1} (10 and 12° C) and nutrient saturated in f/2 medium. Results from the present study (Fig. 3.2) suggest that absorptance in P. abbottae decreases with increasing PFD only as long as growth is not nutrient limited; absorptances of blades grown in f/4 were generally lowest at the intermediate, growth saturating PFD, indicating an inverse relationship between absorptance and growth rate under nutrient limitation. Whether this is merely a consequence of differences in nutrient levels arising between these cultures due to dissimilarity in the growth rates of blades grown under different PFDs cannot be determined from the data. The range of temperatures employed is representative of the habitat of this species and, although it was

broad enough to affect growth (Chapter 2), it had no apparent effect on thallus absorptance.

Although water motion and nutrient concentration both stimulated growth (Chapter 2), they had opposite effects on pigmentation (Fig. 3.3). Water motion reduced thallus pigmentation and thus did not make up for low ambient nutrient levels by increasing the rate of supply. This was most notable in the low nutrient cultures (f/4), where increasing water motion resulted in a drastic reduction of thallus absorptance, concomitant with a corresponding enhancement of growth (probably associated with increased carbon supply; Chapter 2). Cousens (1982), in contrast, found that the pigmentation of Ascophyllum nodosum increased along a gradient of wave exposures, with growth being highest at intermediate exposures. As with the effects of PFD and nutrients mentioned above, the effects of water motion and nutrients on thallus absorptance are confounded by their influence on growth rate. Thus, there are two possible explanations for a reduction in thallus absorptance with water motion. Synthesis of pigment-protein complexes in rapidly growing plants may not proceed at a rate comparable to that of slower growing ones, irrespective of ambient nutrient levels. Alternatively, significant fluctuations in the nitrogen content of the culture medium may take place, even when it is replaced frequently, if, as a result of nitrogen sequestering (Thomas and Harrison 1985), very rapid uptake is followed by a drop in the ambient nitrogen to levels below uptake saturation. Since in these experiments whole attached plants were grown from spores in closed vessels, between treatment

differences in the nutrient supply per unit plant material, at any one level of nutrients, could not be avoided. Monitoring nutrient levels in this type of culture may give an indication of rates of consumption, yet would be of limited predictive value unless combined with information on the uptake kinetics of these plants. It was therefore not attempted in this study.

The gametophytic phase of P. abbottae is a mid-intertidal spring-summer inhabitant of moderately exposed rocky shores in the Pacific Northwest (Conway et al. 1975). Since no further information on the natural conditions experienced by juveniles exists, I cannot assess how well the experimental conditions employed here match the natural environment.

The absorptance ratios show that the proportion of phycobiliproteins tends to increase with increasing thallus absorptance. Thus, low PFD, low water motion and high nutrient concentration all result in a greater proportion of the accessory pigments. Although the PE/PC ratio did not remain constant in response to water motion and nutrients (Table 3.2), variations did not follow a consistent pattern. The ratio decreased with increasing nutrient concentration in this experiment but did not in experiment 1 (Table 3.1). Conversely, water motion increased the ratio in the low nutrient culture only. The observed shifts in PE/PC and PE/Chl a indicate that changes in absorptance by pigments occur in the order PC>PE>Chl a. This inequality is coincident with the costs of synthesis and nitrogen contents of the three chromophores (Raven 1984). Since in addition the

phycobilins are less efficient photon absorbers than chlorophyll (Raven 1984) nutrient dependent changes in pigment composition may reflect the nitrogen cost of the light harvesting apparatus. The PE/PC ratio in some macroalgae may increase in low light (Waaland et al. 1974, Ramus 1983, Ramus and van der Meer 1983), but it also has been found to remain constant in others (Merrill 1985). I found no evidence that P. abbottae acclimates to low light by adjusting the ratio of its phycobilin pigments.

Changes in the proportion of phycobilin pigments with respect to chlorophyll a in response to ambient nutrients have been interpreted as evidence that the phycobiliproteins may serve as a site for nitrogen storage in some red algae (Lapointe 1981). P. abbottae also may allocate nitrogen preferentially to phycobiliproteins. The absence of a significant correlation between growth rate and thallus absorptance (Table 3.3) provides further evidence that photosynthetic pigments are synthesized in excess of immediate needs when a factor other than nitrogen supply (e.g. PFD, temperature, carbon supply) is limiting.

In the previous chapter it was shown that blade thickness of P. abbottae was affected by nutrients and water motion. Here I address the question of the influence of phenotypic changes in blade thickness for light absorption. Ramus (1978) and Lüning and Dring (1985) compared the absorptance spectra of species with thin and thick thalli and concluded that thicker thalli have flatter absorptance spectra, thereby approaching optical blackness. The pooled data from experiment 2 (nutrients, water motion) show a non-linear increase in the per unit

area light absorption with blade thickness, indicating a reduction in biomass-specific light absorption by the thicker blades. The rates of increase in the absorptance peaks of PE, PC and Chl *a* as a function of blade thickness did not differ appreciably from each other. These observations agree with predictions that absorptance by photosynthetic pigments decreases as the light path gets longer, due to attenuation within the thallus (Ramus 1978, Björkman 1981). Thicker *P. abbottae* thalli frequently had thicker cell walls (pers. observ.) and thus a greater proportion of non-absorbing material that may contribute to light scattering and reflectance.

The relationship between whole thallus absorptance and pigment content also shows the decrease in absorption efficiency by photosynthetic pigments as their concentration in the thallus increases (Fig. 3.5). While absorptance in a homogenous medium decreases exponentially with pathlength and pigment concentration, the heterogenous distribution of pigments within cells ("package effect") can further reduce the efficiency of light capture. The light gathering ability of highly pigmented blades may, however, be enhanced in natural light fields where light is diffuse and blade orientation is not fixed.

Pigment content in the water motion and nutrients combinations did not conform closely to the absorptance values obtained from blades in the same treatments. Although only the center part of the blade was used for measurements of absorbance spectra, extractions were performed on whole blades. Thus, variations in the proportion of non-pigmented

tissue such as cell wall or rhizoids are likely to affect the biomass specific pigment content and could account for the apparent incongruencies observed between whole thallus absorptance and pigment content.

Fig. 3.1. Whole thallus absorptance (PE, PC and Chl *a* peaks at 566, 624 and 680 nm, respectively) and scatterance spectra of *Porphyra abbottae*. Area under spectral PFD curve of the fluorescent light source is 1 μmol photons m^{-2} s^{-1}.

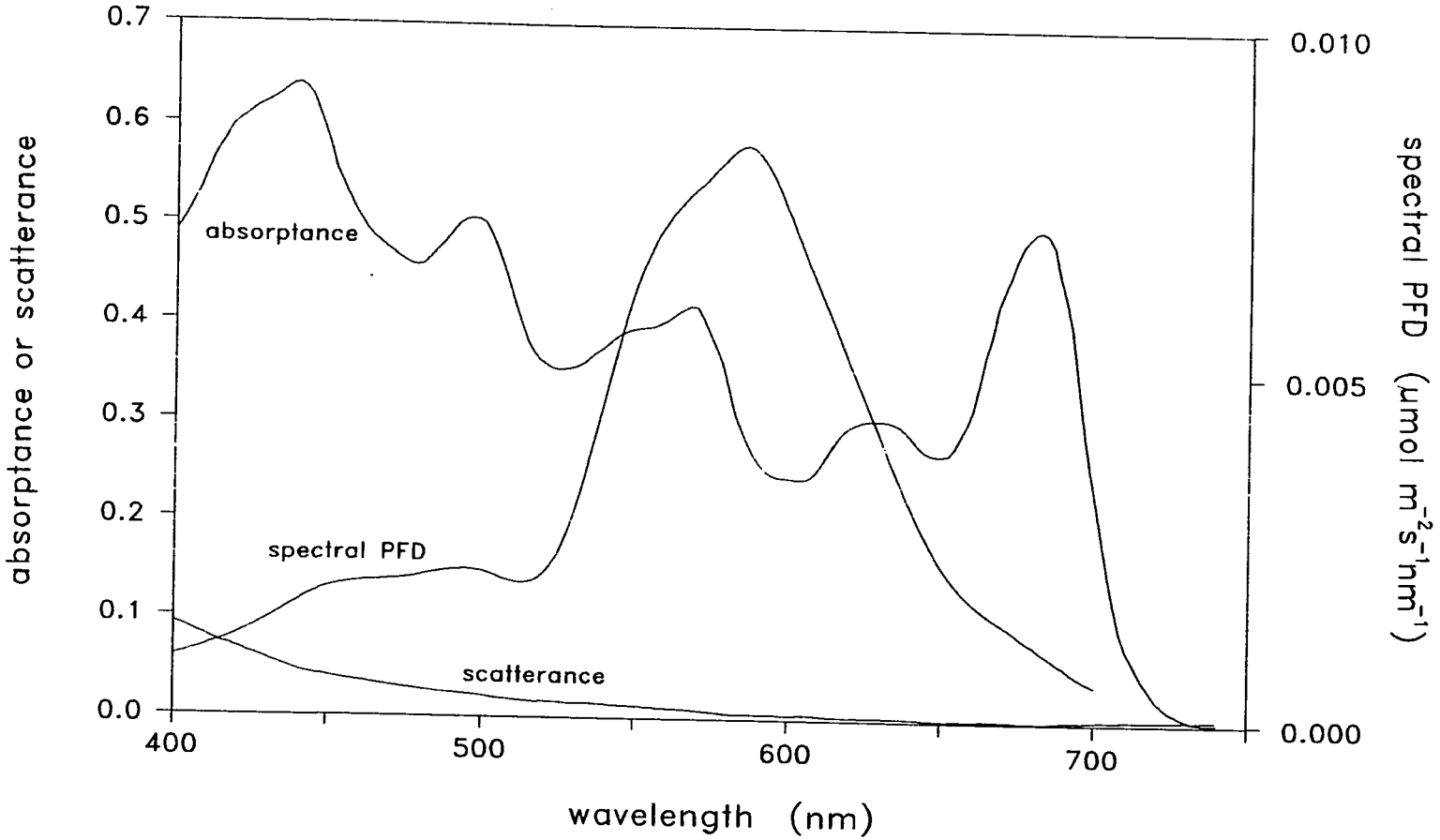

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
absorptance or scatterance
absorptance
spectral PFD
scatterance
400
500
600
700
wavelength (nm)
0.010
0.005
0.000
spectral PFD (μmol m⁻² s⁻¹ nm⁻¹)

Fig. 3.2. Broadband absorptance of *P. abbottae* juvenile blades grown at various combinations of nutrients (f/4 or f/2 medium), temperature, and PFD (17.5, 70 or 140 µmol m^{-2} s^{-1}). Data for f/4, 10° C, 17.5 µmol m^{-2} s^{-1} not available. Means ± 95% confidence limits of four individuals per treatment combination.

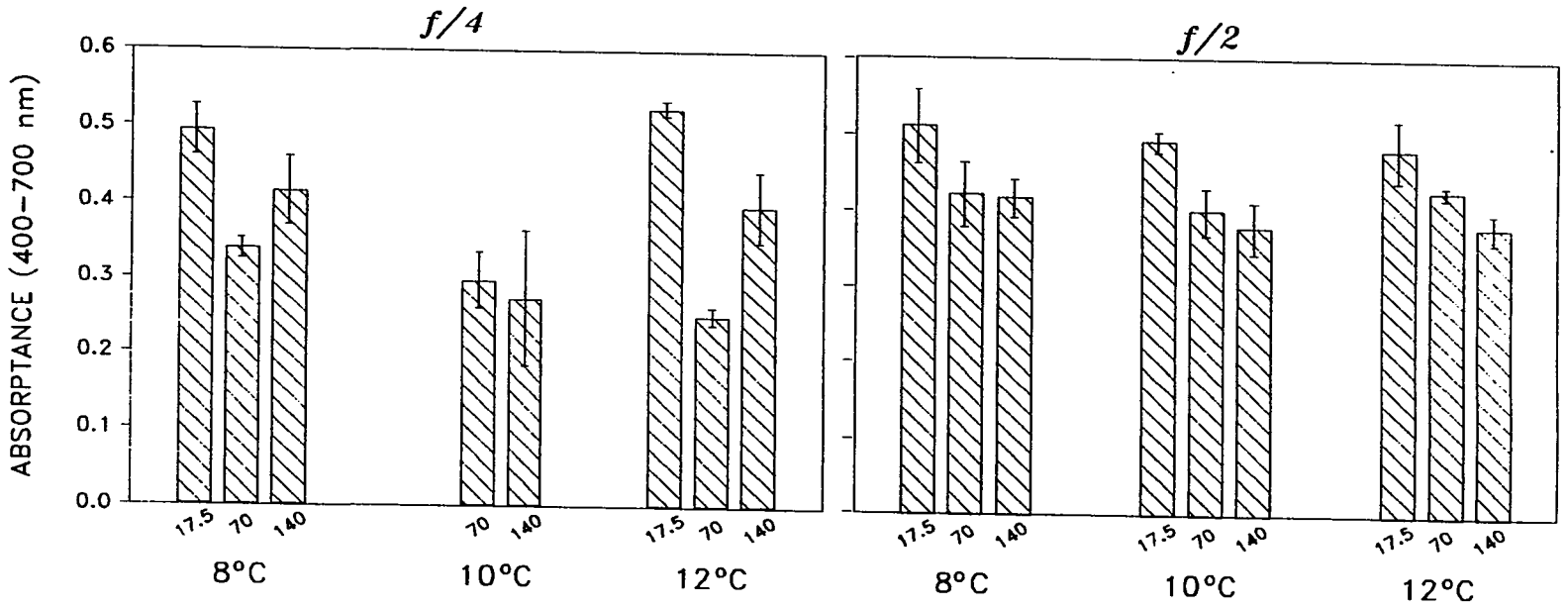

f/4
f/2
ABSORPTANCE (400–700 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
17.5
70
140
8°C
10°C
12°C

Fig. 3.3. Broadband absorptance of P. abbottae juvenile blades grown at various combinations of nutrients (f/4, f/2 or f medium) and water motion (0, 50, 100 or 150 rpm). Means ± 95% confidence limits of four individuals per treatment combination.

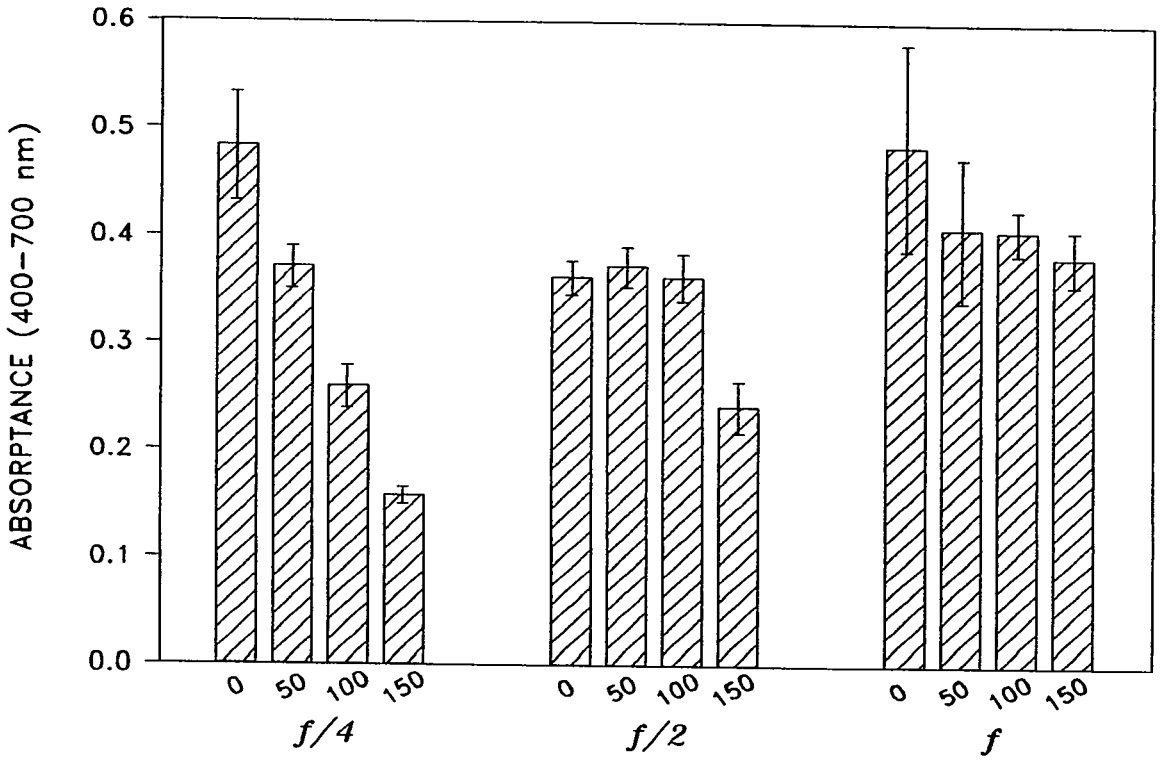
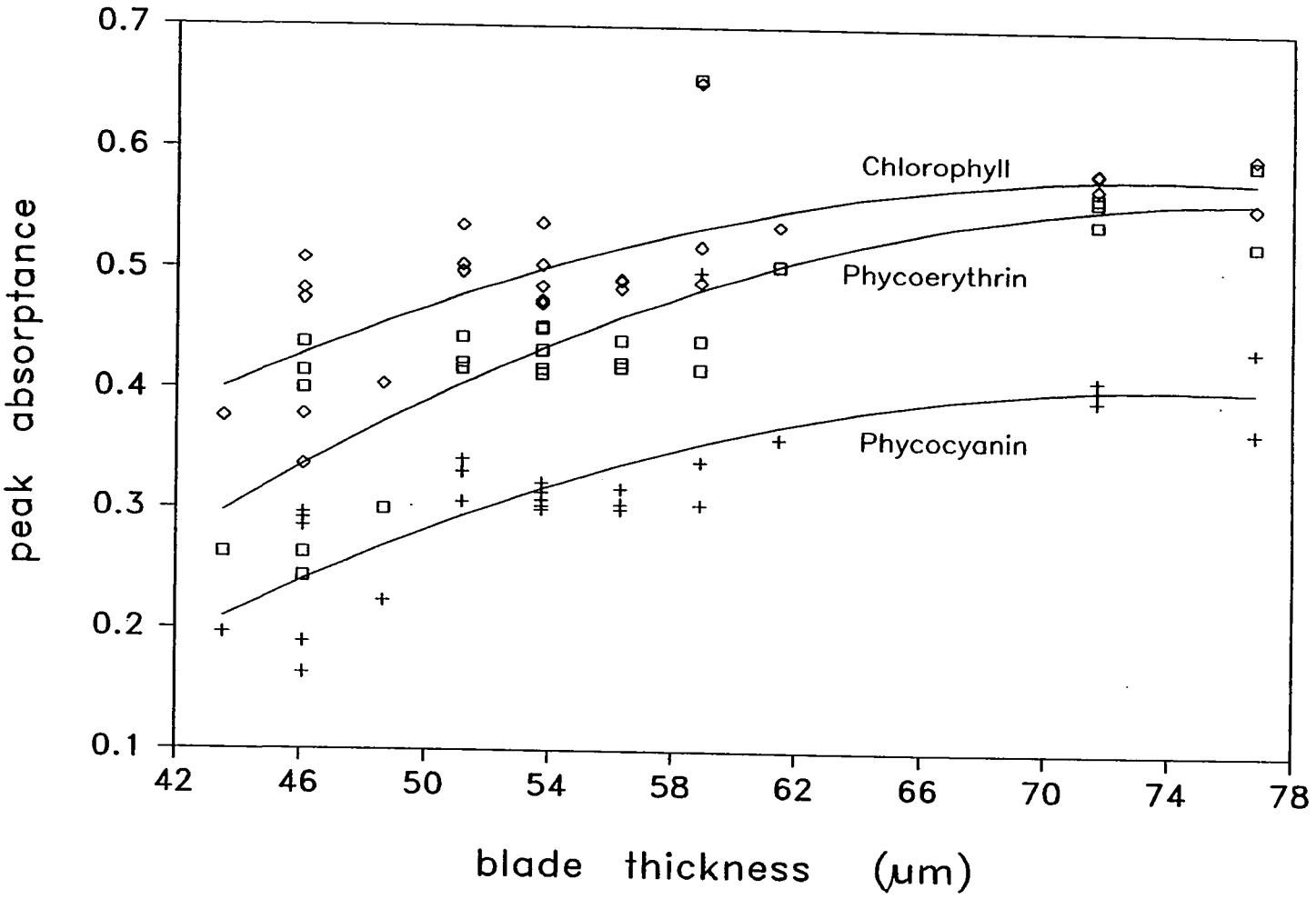

ABSORPTANCE (400–700 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
f/4
f/2
f

Fig. 3.4. Peak absorptances of Chl a (◇), PE (□) and PC (+) as a function of blade thickness. Curves were fitted by polynomial regression.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
peak absorptance
Chlorophyll
Phycoerythrin
Phycocyanin
42
46
50
54
58
62
66
70
74
78
blade thickness (μm)

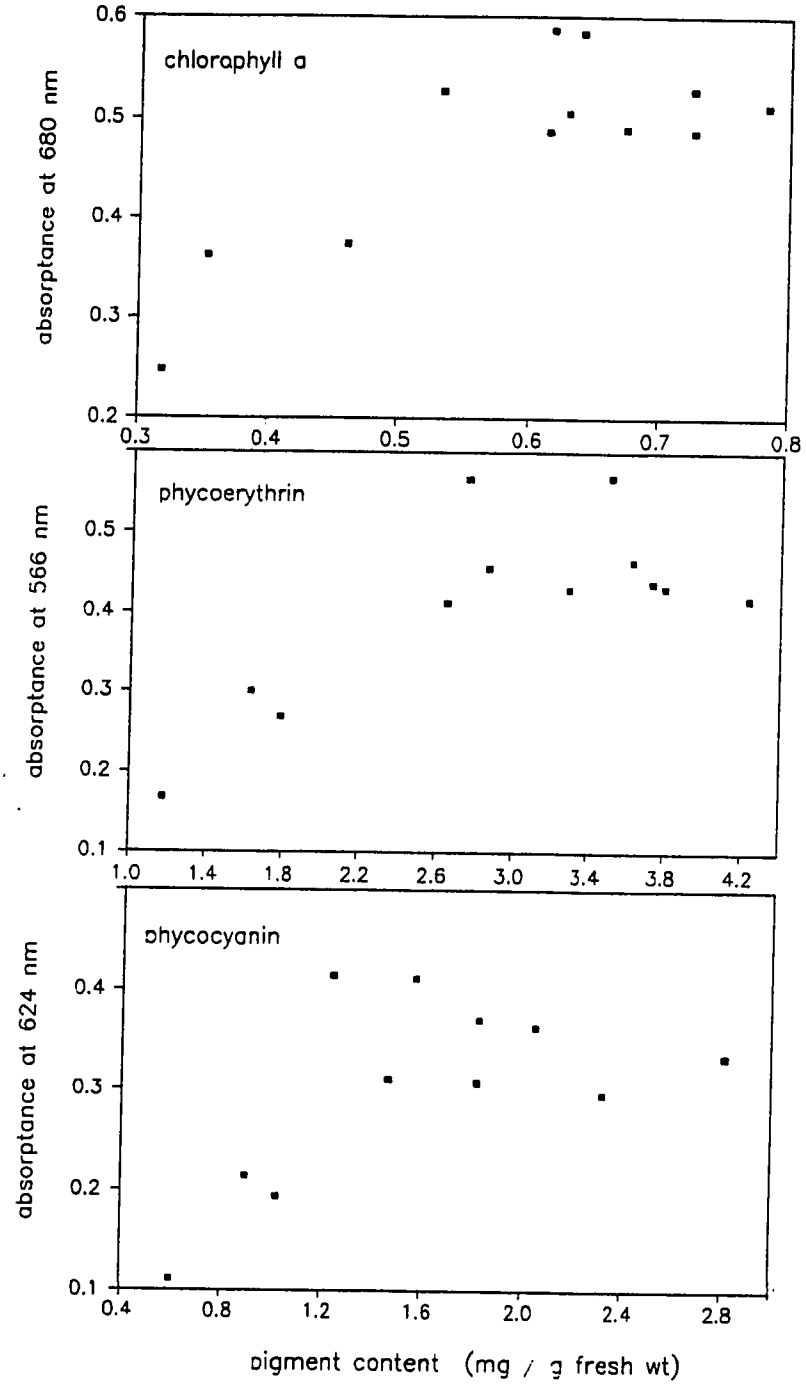


Fig. 3.5. Peak absorptances of Chl a, PE and PC as a function of pigment content pooled from all water motion and nutrients combinations (n=12). Data points are means from one replicate flask per treatment combination.

Table 3.1. Peak absorptances of Chl *a* (680 nm), PE (566 nm), and PC (624 nm), and absorptance ratios (566/680, 566/624) in intact blades of *P. abbottae* grown at various temperatures, PFDs and f medium concentrations. Means ± SD of 4 or 6 blades per treatment combination.

Medium	Temp. (°C)	PFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	680 nm	566 nm	624 nm	566/680	566/624
f/4	8	17.5	0.58 ±0.03	0.58 ±0.02	0.42 ±0.03	1.00	1.40
		70	0.44 ±0.01	0.43 ±0.01	0.28 ±0.02	0.97	1.54
		140	0.49 ±0.03	0.51 ±0.02	0.35 ±0.03	1.04	1.45
	10	70	0.41 ±0.02	0.38 ±0.03	0.23 ±0.03	0.91	1.65
		140	0.34 ±0.04	0.34 ±0.05	0.21 ±0.05	0.98	1.65
	12	17.5	0.63 ±0.00	0.64 ±0.01	0.44 ±0.01	1.02	1.43
		70	0.33 ±0.03	0.29 ±0.03	0.18 ±0.04	0.86	1.62
		140	0.45 ±0.03	0.47 ±0.03	0.33 ±0.04	1.04	1.44
f/2	8	17.5	0.62 ±0.04	0.61 ±0.04	0.43 ±0.03	1.00	1.45
		70	0.53 ±0.02	0.51 ±0.02	0.34 ±0.03	0.96	1.50
		140	0.52 ±0.02	0.50 ±0.01	0.32 ±0.02	0.97	1.56
	10	17.5	0.61 ±0.00	0.61 ±0.01	0.41 ±0.02	1.00	1.50
		70	0.51 ±0.02	0.49 ±0.02	0.32 ±0.02	0.95	1.51
		140	0.48 ±0.02	0.45 ±0.03	0.30 ±0.02	0.95	1.51
	12	17.5	0.58 ±0.03	0.60 ±0.03	0.40 ±0.03	1.03	1.50
		70	0.52 ±0.02	0.49 ±0.01	0.36 ±0.01	0.94	1.35
		140	0.46 ±0.02	0.46 ±0.01	0.30 ±0.01	0.99	1.51

Table 3.2. Peak absorptances of Chl *a* (680 nm), PE (566 nm), and PC (624 nm), and absorptance ratios (566/680, 566/624) in intact blades of *P. abbottae* grown at various f medium concentrations and water motions. Means ± SD of 4 blades per treatment combination.

Medium	Water motion (rpm)	680nm	566 nm	624 nm	566/680	566/624
f/4	0	0.58 ±0.02	0.57 ±0.03	0.41 ±0.03	0.97	1.38
	50	0.49 ±0.01	0.44 ±0.01	0.31 ±0.01	0.89	1.42
	100	0.36 ±0.02	0.30 ±0.01	0.21 ±0.01	0.82	1.40
	150	0.25 ±0.02	0.17 ±0.01	0.11 ±0.01	0.68	1.51
f/2	0	0.49 ±0.02	0.42 ±0.02	0.30 ±0.01	0.86	1.41
	50	0.51 ±0.02	0.43 ±0.02	0.31 ±0.01	0.85	1.40
	100	0.49 ±0.03	0.41 ±0.02	0.31 ±0.02	0.85	1.33
	150	0.37 ±0.03	0.27 ±0.02	0.19 ±0.03	0.71	1.39
f	0	0.59 ±0.05	0.57 ±0.07	0.41 ±0.06	0.96	1.37
	50	0.53 ±0.04	0.46 ±0.05	0.36 ±0.04	0.88	1.27
	100	0.53 ±0.02	0.45 ±0.02	0.37 ±0.01	0.86	1.23
	150	0.51 ±0.02	0.43 ±0.01	0.33 ±0.01	0.84	1.29

Table 3.3. Rank correlation coefficients among whole thallus peak absorptances, broadband absorptance, absorptance ratios, and growth rate. ** $p<0.01$, *** $p<0.001$.

	Peak absorptance					Broadband absorptance
	Chl *a* (680 nm)	PE (566 nm)	PC (624 nm)	PE/Chl *a*	PE/PC	(400-700 nm)
Broadband absorptance (400-700 nm)	0.95***	0.98***	0.97***	0.67***	-0.28	
Growth rate	0.08	0.30	0.12	0.57**	0.55**	0.21

Table 3.4. Chl *a*, PE, and PC content (mg g fr w^{-1}, mean ± SD) for blades grown at the various nutrient concentrations and water motions. n = 2 dishes per treatment combination.

Medium	Water motion (rpm)	Chl *a*	PE	PC	PE/Chl *a*	PE/PC
f/4	0	0.65 ±0.01	3.85 ±0.34	1.84 ±0.27	5.90	2.09
	50	0.66 ±0.02	3.83 ±0.10	2.11 ±0.29	5.82	1.82
	100	0.55 ±0.19	2.72 ±1.09	1.50 ±0.59	4.96	1.81
	150	0.47 ±0.15	2.32 ±1.14	1.38 ±0.78	4.92	1.68
f/2	0	0.72 ±0.01	4.06 ±0.16	2.26 ±0.06	5.63	1.80
	50	0.72 ±0.09	3.45 ±0.18	2.03 ±0.21	4.81	1.70
	100	0.66 ±0.05	3.19 ±0.53	1.98 ±0.51	4.81	1.61
	150	0.61 ±0.15	2.68 ±0.89	1.68 ±0.66	4.40	1.60
f	0	0.57 ±0.04	2.75 ±0.01	1.33 ±0.09	4.80	2.07
	50	0.71 ±0.02	3.53 ±0.10	1.99 ±0.06	4.99	1.77
	100	0.62 ±0.09	3.33 ±0.46	2.14 ±0.31	5.36	1.56
	150	0.70 ±0.09	3.12 ±0.58	2.17 ±0.64	4.48	1.44

CHAPTER 4

GROWTH, LIGHT ABSORPTION AND PHOTOSYNTHESIS OF *PORPHYRA ABBOTTAE* GAMETOPHYTES IN RELATION TO THEIR NUTRIENT CONSUMPTION.

The marine environments commonly inhabited by marine macroalgae often undergo pronounced spatial and temporal variations in levels of nutrients. Nitrogen is considered the primary limiting nutrient for macroalgal growth in many coastal areas (e.g., Chapman and Craigie 1977), but in certain marine waters phosphorus may commonly be the limiting nutrient (Lapointe 1987). Studies on how the physiology of photosynthesis of marine plants has adapted to a variable nutrient supply are thus relevant to an understanding of the mechanisms that control their growth in these environments.

Increasing evidence suggests that the content of light-harvesting pigments is affected by nutrients (nitrogen in particular) in a number of phylogenetically and morphologically diverse macroalgae (e.g., Chapman et al. 1978, Rosenberg and Ramus 1982a, Shivji 1985, Amano and Noda 1987). In the red phycobilin-containing seaweeds, nutrient availability affects both the total content and the relative proportions of photosynthetic pigments (Rosenberg and Ramus 1982a, Lapointe and Duke 1984, Macler 1986). There is evidence that the biliprotein phycoerythrin may serve as a means of storing nitrogen in marine cyanobacteria (Wyman et al. 1985). While a similar role has

been proposed for some rhodophytan phycobiliproteins (Lapointe 1981, Bird et al. 1982, Lapointe and Duke 1984), no study has yet examined how nutrient-related pigment changes affect photosynthesis in these algae. Studies with microalgae have revealed many physiological responses to nitrogen limitation, including decreased rates of photosynthesis and lowered ribulose bisphosphate (RuBP) carboxylase activity in addition to pigment loss (Syrett 1981). These kinds of adjustments of the photosynthetic apparatus to the nitrogen environment also seem to operate in the macroalgal species that have so far been examined (Ramus 1983, Wheeler and Weidner 1983, Lapointe and Duke 1984, Macler 1986, Lapointe 1987).

Light and nutrient availability were shown to affect the content and proportions of photosynthetic pigments and hence whole thallus light absorption in gametophytes of the red alga Porphyra abbottae (Chapters 2 and 3). The purpose of the present study was to evaluate how variations in the nutrient supply affect the photosynthetic light response and quantum yield in this alga. Because water motion is likely to enhance nutrient uptake (Chapter 2), young attached P. abbottae gametophytes were grown in batch cultures subjected to two nutrient regimes and two water motion speeds. In addition, the response to a change in the nutrient concentration was examined in disks excised from adult blades. Growth rate and average daily nitrate and phosphate consumption of juveniles and disks were examined in relation to the photosynthesis-light response and to whole thallus spectral absorptance.

MATERIALS AND METHODS

Disk cultures: Disks excised from Porphyra abbottae Krishnamurthy blades (strain 1626, obtained from a single carpospore of a blade collected north of Rialto Beach, Washington, 47°58'N 124°39'W, on 5/30/83) were grown in normal seawater (ca. 30 µM NO_3^-, 2.7 µM $PO_4^{\equiv}$) enriched with f/2 (Guillard and Ryther 1962) or f/20, a medium containing 10% of the usual f/2 macronutrient enrichment. The resulting nitrogen and phosphorus concentrations were ca. 912 µM NO_3^-, 39 µM $PO_4^{\equiv}$ in f/2, and ca. 117 µM NO_3^-, 6.2 µM $PO_4^{\equiv}$ in f/20. Both media contained f/2 concentrations of vitamins and trace metals. Eight 10 to 12-mm diameter disks per dish were grown with aeration in one of two replicate deep Pyrex dishes with 200 ml of medium. After 26 to 32 days the nutrient regimes were reversed by transferring f/2-grown disks to f/20 and vice versa. In these and in juvenile cultures, cool-white fluorescent tubes provided a photon flux density (PFD) of 70 µmol m^{-2} s^{-1} (measured with a Lambda cosine collector) with a 16L:8D photoperiod (this PFD had been found to be saturating for the growth of juveniles, Chapter 2). The temperature was kept at 12±2 °C. Every 6-8 days the disks were blotted and weighed, the media sampled for NO_3^- determinations (Strickland and Parsons 1972), and then changed. Biomass was maintained at $\leq$ 3g fresh weight L^{-1}. Specific growth rates were determined from fresh weights, assuming exponential growth.

Juvenile cultures: Conchospores were seeded into 8 deep Pyrex dishes (see Chapter 2 for induction of conchospore formation and release). The sporelings were kept in f/2 and allowed to grow for 3-4

weeks, at which time their density was reduced to 2-4 individuals cm^{-2}. Two more thinnings brought the density down to ca. 1 individual cm^{-2} (80 per dish) at 40 d. Approximately 30 days after germination (mean blade area: 1.2-4.6 mm^2) the dishes were transferred to 1 of 4 experimental conditions (2 replicate dishes per treatment combination): media with low (f/20) or high (f/2) N and P content, and slow (50 rpm) or fast (150 rpm) water motion. Dishes contained 100 ml of media which were changed every 5 days. Water motion was provided by rotary shakers.

Samples of 10 blades per dish were generally taken at approximately 10 d intervals (occasionally at shorter or longer intervals, depending on growth rate) and stored frozen for surface area determinations (Chapter 2). Specific growth rates (μ, d^{-1}) were obtained by linear regression: $\log_e$ (Area) = $\mu \cdot t + c$, where Area=mean blade area of sample, t=time, and c=constant. Calculations were based on samples taken during exponential growth only, i.e., before the growth rate started to decline. Because of the disparity in growth rates among treatments, the number of sampling dates varied from 4 to 11.

NO_3^- and $PO_4^{\equiv}$ were determined with every medium change. Area-specific NO_3^- and $PO_4^{\equiv}$ consumption was calculated from the change in concentration of the medium and an estimate of the total blade area in the dish. To account for growth, total blade area was estimated from the geometric mean of the areas at the beginning and end of the measuring period. Daily rates of consumption are based on total

consumption over the age period used to determine growth rate.

Photosynthesis: Light response (photosynthesis vs. PFD or "P vs. I") curves of f/20- and f/2- grown disks were obtained 2-15 d before the nutrient regimes were reversed. Light-limited (50 μmol m^{-2} s^{-1}) and light-saturated (500 μmol m^{-2} s^{-1}) photosynthetic rates of disks were measured at 10 days following the change in nutrient regimes. Disks (10-13 mm diameter) or whole juveniles (10-19 mm long, 43-100 days old). were fastened to a plunger in a 4 ml cuvette filled with f/20 and illuminated by a 500 W tungsten CZA projector lamp with integral heat filter (Fig. 4.1). Light response curves (one curve for each sample, 3 samples per dish) were constructed from oxygen exchange rates determined with a polarographic electrode (Beckman model 39553) at 8 PFDs ranging from 10 to 2000 μmol m^{-2} s^{-1}. At each light level, measurements of linear rates of oxygen evolution in the light (10-20 min) were followed by measurements of oxygen consumption in the dark (generally 5-10 min), and gross photosynthesis estimated from the sum of the two. The tissue was thereafter stored at -40 °C for later determination of surface area and absorbance spectrum.

P vs. I values were fitted to the equation $P = P_s (1-e^{-a}) e^{-b}$, where $a = \alpha \times I/P_s$, $b = \beta \times I/P_s$, P = gross photosynthetic rate, I = PFD (Platt et al. 1980). In this exponential model, α is the initial slope of the light-limited phase, P_s is the photosynthetic rate at saturating PFD, and β is a parameter that evaluates the effect of photoinhibition ($\beta = 0$ for no inhibition). Curve-fitting was performed by non-linear regression (SYSTAT, Inc.).

Light Absorption: Whole thallus absorbance spectra (400-730 nm) were recorded with an Aminco DW-2 double beam spectrophotometer as described in Chapter 3. Absorbance spectra were used to calculate peak absorptances of phycoerythrin (PE, 566 nm), phcycocyanin (PC, 624 nm), and the spectrally weighted broadband absorptance (400-700 nm) by numerical integration (Chapter 3) for both the fluorescent growth lights and the tungsten projector lamp.

Quantum yield: The maximal photosynthetic quantum yield (ϕ_p) was calculated for each sample by linear regression of gross photosynthesis vs. PFD absorbed at 10, 25 and 50 μmol photons m^{-2} s^{-1}. Because the very slow rates of respiration recorded after exposure to low light levels resulted in an underestimation of gross photosynthesis at these light levels (Fig. 4.2), regression lines were not forced through the origin. There was no indication of curvature (i.e. a decline in the quantum yield) at 50 μmol photons m^{-2} s^{-1} and correlations were high (r^2=0.62-1.00).

Since in these experimental designs each replicate dish represents one experimental unit, the mean for a dish was considered as a single observation. The effect of nutrients and water motion on each variable was tested by a series of 2-way ANOVAs. Spearman's rank correlation coefficient (r_s) was used for multiple correlation analysis of the dependent variables. Analyses were performed with STATISTIX (NH Analytical Software).

RESULTS

Nutrient loading had a significant effect on growth rate, NO_3^- consumption, the photosynthetic light response, and absorptance by all major photosynthetic pigments (Table 4.1), but only growth rate was significantly affected by water motion. This resulted in strong positive correlations of absorptance (peaks and broadband) with NO_3^- consumption and photosynthesis (Table 4.2).

Growth Rate and Nutrient Consumption: Specific growth rates of juveniles (Table 4.3) were 49-91% higher in f/2 than in f/20. Water motion enhanced growth by 63% in f/2 as compared to only 29% in f/20; however, at this level of replication ANOVA results do not demonstrate significant interaction of nutrients with water motion on growth ($p=0.053$).

NO_3^- consumption rates of juveniles at the two water motion speeds were 2.0-3.7 times higher in f/2 (mean$\pm$SD = 5.0$\pm$1.3 nmoles mm^{-2} d^{-1}, n=4) than in f/20 (1.8$\pm$0.7 nmoles mm^{-2} d^{-1}). Because f/2 cultures always remained nutrient saturated (Appendix 1), NO_3^- consumption and growth rate were increased by water motion in these cultures (Fig. 4.3). In contrast, f/20 cultures were nitrogen limited for most of the time (Appendix 1) and became NO_3^- depleted betweeen media changes. In spite of increased NO_3^- limitation, plants at 150 rpm grew at a faster rate (27% higher) than those at 50 rpm (Fig. 4.3).

$PO_4^{\equiv}$ consumption of juveniles (grand mean$\pm$SD = 0.19$\pm$0.08 nmoles mm^{-2} d^{-1}, n=8) did not vary significantly among treatments (Table 4.1) and was weakly correlated to most of the other variables (Table 4.2),

suggesting that phosphorus was not a limiting nutrient in any of the cultures.

Growth rates of disks prior to nutrient addition or removal (Table 4.4) were not significantly different in f/20 than in f/2 ($p>0.2$). During this period, f/20 cultures depleted all available nitrate (mean uptake rate$\pm$SD = 32.8$\pm$21.4 µmol g fr.wt.$^{-1}$ d^{-1}) and f/2 cultures consumed 10-30% of the nitrate supply (uptake = 50.4$\pm$44.3 µmol g fr. wt.$^{-1}$ d^{-1}). Nutrient addition increased the growth rate at 5 days, whereas growth rate was not affected until 5-11 days following nutrient removal (Table 4.4).

Photosynthesis: The photosynthetic light response was greatly affected by nutrients (Fig. 4.4, Table 4.5). P_s was 3-4 times higher, and α 2-3 times higher in f/2- than in f/20-grown plants. P_s and α of f/20-grown juvenile blades were slightly higher in fast than in slow water motion, but the difference was not significant. Estimates of photosynthesis at 70 µmol photons m^{-2} s^{-1} thus indicate rates 2-3 times higher in f/2 than in f/20. When normalized to broadband absorptance (Fig. 4.4B), light-limited rates were similar for f/2 and f/20 plants, but differences between light-saturated rates remained high, indicating an effect of nutrients on dark reaction rates.

During the 10 days following nutrient addition to f/20-grown disks, photosynthetic rates (µmol O_2 m^{-2} s^{-1}) almost reached those of f/2-grown disks (Table 4.4). On the contrary, at 10 days the photosynthetic rates of disks transferred to f/20 were still more than twice those of f/20-grown disks.

Maximal quantum yields (ϕ_p, Table 4.6) ranged from 0.04 to 0.08 mol O_2 mol photons^{-1}. Nutrients significantly affected the ϕ_p of disks and that of juveniles at slow water motion. Because at high water motion ϕ_ps were very similar in f/20 and f/2, there was no significant overall correlation between ϕ_p and NO_3^- consumption of juveniles.

Light Absorption: Absorptance peaks of PE, PC and Chl *a* all significantly increased with nutrient availability (Table 4.1), thus causing an increase in broadband absorptance with NO_3^- consumption (Fig. 4.5). Increased nitrate consumption also caused a significant increase in the PC/Chl *a* absorptance ratio and a decrease in the PE/PC ratio (Fig. 4.5, Table 4.4). The PE/Chl *a* ratio was not significantly correlated with NO_3^- consumption or any other variable (Table 4.4) although it increased with NO_3^- consumption at slow water motion (Fig. 4.5)

Whole thallus absorptances (broadband and single wavelength) were also significantly correlated with growth rate and the photosynthetic light response (Table 4.2). Absorptances correlated most strongly with NO_3^- consumption (r_s = 0.95-0.98) and with P_s, and thus also with P(70) (the estimated photosynthetic rate at the growth irradiance).

Disks transferred from f/20 to f/2 doubled their broadband absorptance in 10 days, almost reaching levels of f/2-grown disks (Table 4.4). Absorptance of disks transferred to f/20 only declined to 65% of its original value in 10 days (Table 4.4). These disks reached absorptances similar to those of f/2 plants in 3-4 weeks (data not shown).

DISCUSSION

Nutrient loading had a broad effect on growth, photosynthetic response and light absorption of Porphyra abbottae gametophytes. In contrast to juvenile blades, the growth rates of blade disks in f/2 and f/20 differed by less than 2% d^{-1} in spite of large differences in their photosynthetic rates. That measured growth rates of disks in f/2 averaged only 10.8% d^{-1} as compared to 14% d^{-1} in juveniles suggests that either carbon limitation or tissue loss were responsible for the small difference in growth rate between the two nutrient regimes.

If water motion facilitates nutrient uptake it should also positively affect growth and N status. Wheeler (1982) has demonstrated that uptake of inorganic N by Macrocystis is greatly enhanced at increased current speeds. The nutrient-rich f/2 cultures of P. abbottae consumed almost 50% more NO_3^- at 150 rpm than at 50 rpm. Because f/20 cultures became NO_3^- depleted and concentrations were not monitored between media changes, it was not possible to detect differences in the uptake rates at the two water motions. As shown in Appendix 1, in batch cultures with a limited nutrient supply, such as the f/20 media used during this study, higher water motion leads to increased nutrient limitation, thus explaining the slight decrease in the overall consumption per unit plant area. Since N limitation causes a decrease in photosynthesis and absorptance (Appendix 1), the result of this temporal variation in the degree of N limitation is to increase the variability between measurements of these parameters. Because the method used to determine nutrient consumption rates may be subject to

sources of error that have not been accounted for (e.g., bacterial activity, evaporation, the error involved in determining changes at very high concentrations) values given here are only approximations to actual nutrient uptake rates.

As suggested in Chapter 2, the effect of water motion on growth is not limited to facilitating uptake of the macronutrients supplied with the enrichment. In f/2, the increase in growth due to high water motion exceeded the increase in NO_3^- consumption by 15%, and in f/20 high water motion resulted in a 27% increase of growth in spite of reduced NO_3^- consumption. Because there is no active transport system for inorganic carbon, its diffusion through the boundary layer is an important rate-limiting process for photosynthesis (Smith and Walker 1980, Wheeler 1980), and growth may become carbon limited in poorly stirred cultures (Hanisak 1979). Water motion probably facilitated carbon uptake in the present study, thus stimulating growth even under NO_3^- limiting conditions.

The photosynthetic response to nutrient loading reflected the importance of N status in determining both light-limited and light-saturated photosynthesis. In both juvenile and adult blades nutrient limitation caused a 3-fold reduction of the light harvesting and photosynthetic capacity. Effects of nitrogen supply on the photosynthetic capacity of plants have largely been ascribed to changes in the content or specific activity of RuBP carboxylase (Evans 1989). For only a few macroalgae has N availability been shown to affect the photosynthetic capacity (Ramus 1983, Lapointe and Duke 1984, Macler

1986, Lapointe 1987) and activities of carbon assimilation enzymes (Wheeler and Weidner 1983, Lapointe and Duke 1984).

A strong correlation between cell or tissue N and light-saturated photosynthesis has been shown for a number of terrestrial plants (Evans 1989) and a diatom (Osborne and Geider 1986). Thus because many plants can store N, tissue N may be a better indicator of the N directly available to the plant than ambient N. Particulate N was not measured in the present study, but P_s was positively correlated with N consumption. A close coupling between photosynthetic performance and ambient concentration can be expected to occur only in the absence of internal pools. Luxury N consumption is also common in seaweeds, but the nature of the storing coumpounds and the storage capacity vary (Hanisak 1983) and may largely be constrained by the degree of morphological complexity (Rosenberg and Ramus 1982b). Thin high surface-area/ volume forms such as Porphyra or Ulva species are characteristically fast-growing (Littler and Littler 1980), yet probably have a limited capacity to store N (Rosenberg and Ramus 1982b). Amino acids and proteins are an important and rapidly utilizable nitrogen pool in the thicker thallus of Gracilaria foliifera (Bird et al. 1982, Rosenberg and Ramus 1982b). By contrast, in Porphyra perforata starvation caused a significant decrease in the content of thallus NO_3^- and little or no decrease in amino acids or soluble proteins (Thomas and Harrison 1985). I have also found (unpublished results) that nutrient sufficient P. abbottae contains pools of soluble NO_3^- which are depleted during starvation.

The N content of seaweeds has been found to range from 0.05 to 6.5% (De Boer 1981), and that of Porphyra tenera varied between 1.9 and 6.5% of the dry weight throughout the year (Iwasaki and Matsudaira 1954). Calculations based on the NO_3^- consumed over 5-day periods by P. abbottae yield values of 1.2-2.6 % N in f/20 and 4-7% N in f/2 (assuming 0.061 mg mm^{-2} (Chapter 2) and a 1:10 dry:fresh weight ratio). The values for f/20 are close to the critical N concentrations (the internal concentration that just limits its maximal growth) established for Codium (1.9%) and Gracilaria (2%) by Hanisak (1983). This agrees with model predictions (Appendix 1) that f/20 cultures were N limited for most of the time and further suggests that internal N pools were probably depleted soon after each medium change, thus causing a decline in photosynthetic performance as the blades used their ambient supply (Appendix 1). Conversely, the N consumption of f/2 blades suggests the sustained presence of internal pools and thus N sufficient photosynthesis at all times.

Differences in light-limited photosynthesis and in α (initial slope of P vs. incident PFD) may be caused by differences in the efficiency of light capture (absorptance) and/or the fraction of the radiation absorbed that is converted into chemical energy, expressed as photosynthetic efficiency (ϕ_p, Radmer and Kok 1977). The lower α of f/20-grown plants are accounted for largely, but not completely, by their reduced photon absorption, indicating a small effect of N limitation on the efficiency of energy conversion.

While the photosynthetic quantum yield (ϕ_p) can remain invariant

under a range of environmental conditions (Senger and Fleischhaker 1978, Geider and Osborne 1986, Osborne and Geider 1986), it has been reported to decrease during nitrogen starvation in batch culture (Welschmeyer and Lorenzen 1981, Cleveland and Perry 1987). The large decrease observed in photon absorption of P. abbottae under N limitation, with preferential loss of absorptance by the accessory pigments, was accompanied by a much smaller reduction of the o. This suggests that the decrease in light-limited photosynthesis was caused primarily by a reduction in the content and/or efficiency of light-harvesting pigments, with only a small additional reduction in reaction center or electron transport activity. A more severe N defficiency (such as in the experiments of Welschmeyer and Lorenzen 1981 and Cleveland and Perry 1987) may be necessary to bring about a greater reduction in the efficiency of photosynthetic energy conversion. The maximal quantum yields obtained for Porphyra abbottae in nutrient sufficient disk cultures (0.077 mole O_2/mole photons) are close to those obtained for P. umbilicalis by Dring and Lüning (1985) in a similar light field (0.087 mole O_2/mole photons).

A comparison of P vs. I parameters indicates that light saturated rates were ca. 50% - 100% higher for adult than for juvenile blades but that their light-limited rates were similar or up to 60% higher. Overall, adult blades also had higher growth rates than the juveniles. However, because of the different stirring methods employed for the 2 kinds of cultures, it is not possible to directly compare their growth rates. In f/20 medium, adults had a comparably higher photosynthetic

rate at 70 μmol photons m^{-2} s^{-1}, suggesting that P(70) may be a good indicator of growth rate.

Light-limited and light-saturated rates responded more rapidly to nutrient addition than to nutrient limitation: at 10 d, rates of disks transferred to f/2 approached rates measured for f/2-grown plants, whereas rates of disks transferred to f/20 declined more gradually. Changes in absorptance also occurred faster in disks transferred to f/2 than in disks transferred to f/20. The gradual declines in photosynthesis and absorptance produced by limiting the nutrient supply may be linked to utilization of internal nitrogen reserves. Red macroalgae have been found to contain utilizable reserves of inorganic nitrogen (Rosenberg and Ramus 1982b, Thomas and Harrison 1985), amino acids and proteins (Bird et al. 1982, Rosenberg and Ramus 1982b). Phycobiliproteins have also been implicated in nitrogen storage (Lapointe 1981, Bird et al. 1982, Lapointe and Duke 1984). That absorptance of P. abbottae declined faster than the light-limited photosynthetic rate upon transfer to f/20 may reflect degradation of a surplus pool of pigment proteins.

Absorptances by all major pigments increased with nutrient supply, in agreement with earlier observations (Chapter 3). Trends in absorptance ratios, however, did not closely conform to those reported in Chapter 3. In the previous study (Chapter 3), PE/Chl a ratios (range: 0.71-0.97) were generally found to increase with nutrient supply and also to be a good predictor of broadband absorptance and growth rate. Evidence that the relative phycobiliprotein content may

vary seasonally (Rhee and Briggs 1977, Rosenberg and Ramus 1982a) and that phycobilin content falls to undetectable levels during N starvation (Macler 1986) also suggests that red algal phycobiliprotein pigments are more sensitive to nutrient limitation than Chl *a*. Mean PE/Chl *a* ratios measured here were higher (0.96-1.15) and independent of nutrient loading, and thus not significantly correlated with any of the variables examined. It is possible that the lower range of PE/Chl *a* absorptance obtained previously is indicative of a more severe N limitation (due to larger blades and/or higher density). The increase in the absorptance by PC relative to PE and Chl *a* with nutrient loading suggests that, within a suboptimum range of N availability, PC is preferentially allocated, followed by PE and Chl *a*.

Because the rate of nutrient supply (nitrogen in particular) influences the ability of *Porphyra abbottae* to absorb light and to photosynthesize, in addition to its effect on growth, there is a high degree of correlation among many of the variables used here as measures of physiological performance. This shows that nutrient availability affects a variety of physiological processes and that these are highly interdependent.

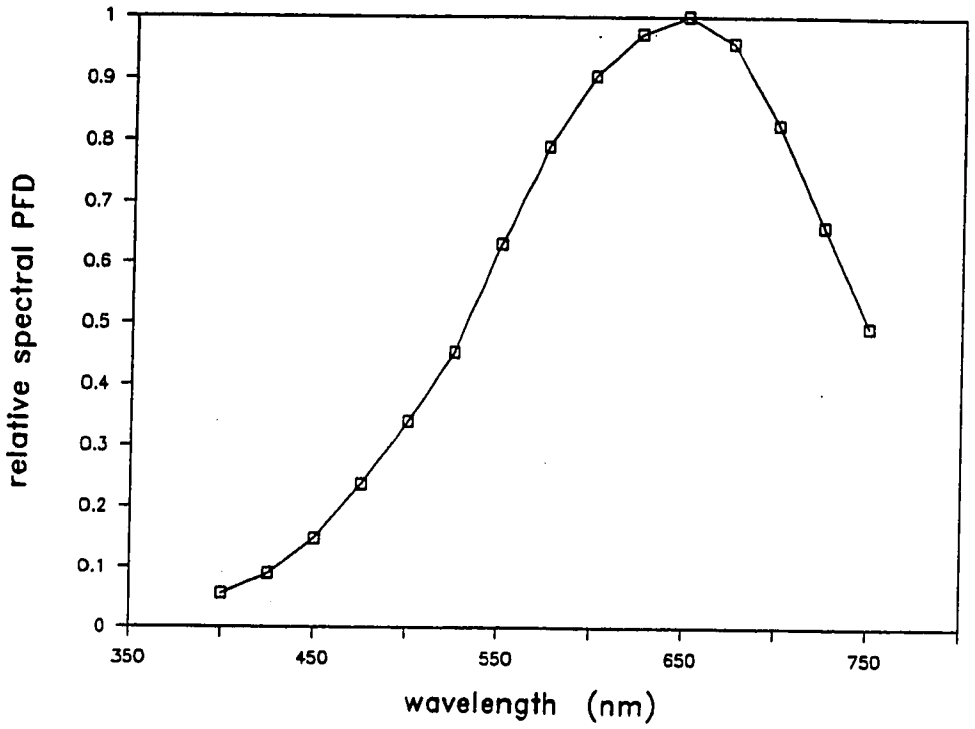


Fig. 4.1. Spectral quality of "CZA" tungsten light source plus heat filter used for measurements of photosynthesis, as recorded with an ISCO spectroradiometer.

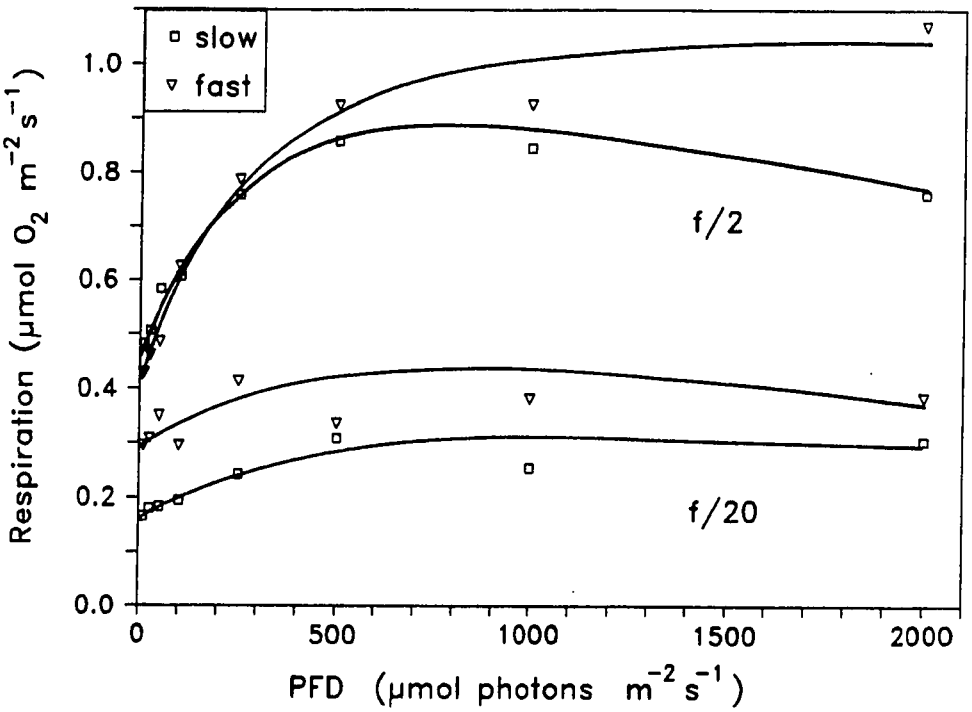


Fig. 4.2. Respiration rates of juvenile blades grown in f/2 or f/20 media, and at fast (150 rpm) or slow (50 rpm) water motion. Dark O_2 consumption was recorded after each measurement of photosynthesis at the PFDs shown. Symbols represent means for each light level (n=6). Curves were fitted by eye.

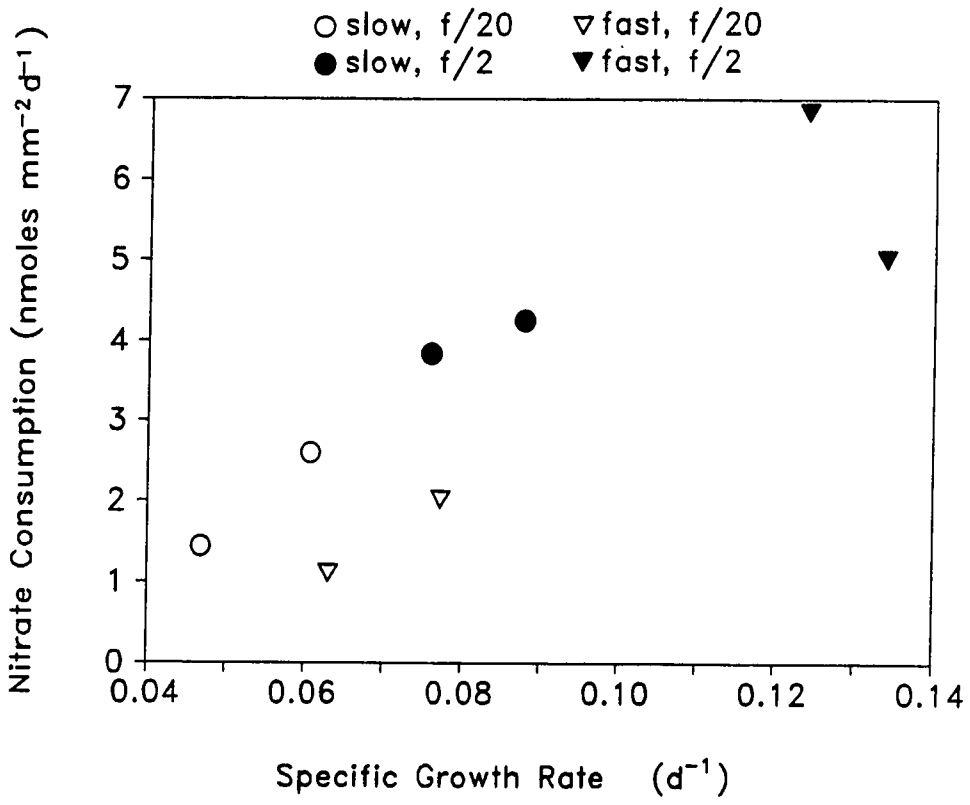


Fig. 4.3. Relationship between nitrate consumption and specific growth rate for juvenile blades grown at fast (150 rpm) or slow (50 rpm) water motion. Each value represents one dish.

Fig. 4.4. Photosynthetic light response curves for juvenile blades and disks grown in f/2 or f/20 media. Juveniles were kept at fast (150 rpm) or slow (50 rpm) water motion and disks were aerated. A) Gross photosynthesis vs. incident PFD. B) Gross photosynthesis (μmol O_2 m^{-2} s^{-1}) normalized to the spectrally weighted broadband absorptance vs. incident PFD. Symbols represent means for each light level (n=6). Curves are based on the exponential model given in Table 4.5. See Table 4.5 for parameter estimates and confidence intervals of curves in A.

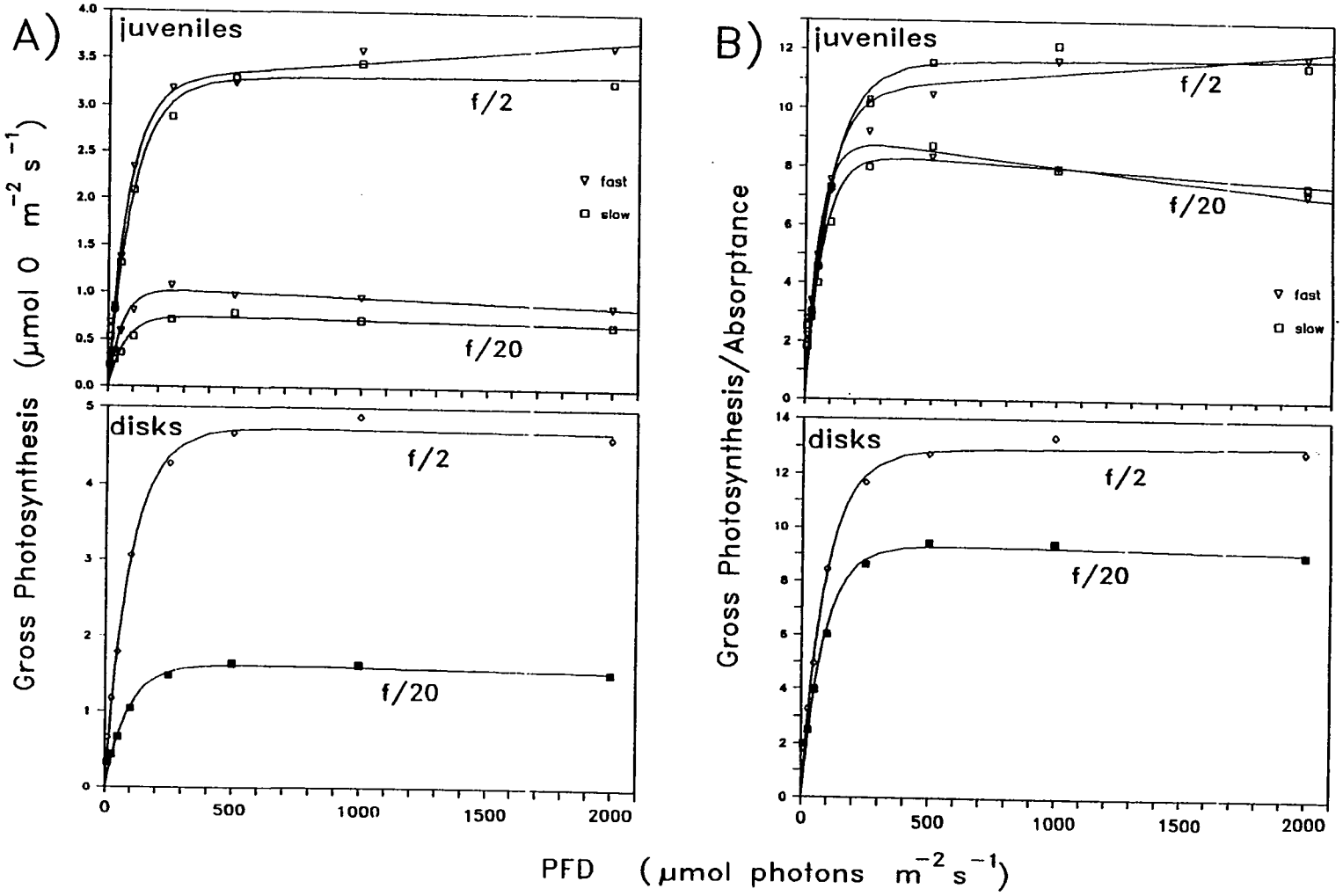

A)
juveniles
f/2
f/20
fast
slow
disks
f/2
f/20
Gross Photosynthesis (µmol O m^{-2} s^{-1})
B)
juveniles
f/2
f/20
fast
slow
disks
f/2
f/20
Gross Photosynthesis/Absorptance
PFD (µmol photons m^{-2} s^{-1})

Fig. 4.5. Whole thallus absorptance as a function of nitrate consumption for juvenile blades grown in f/20 or f/2 and at slow (50 rpm) or fast (150 rpm) water motion. PE/Chl a, PC/Chl a, PE/PC: Ratios of absorptance peaks; 400-700 nm: spectrally weighted broadband absorptance. Each value represents one dish.

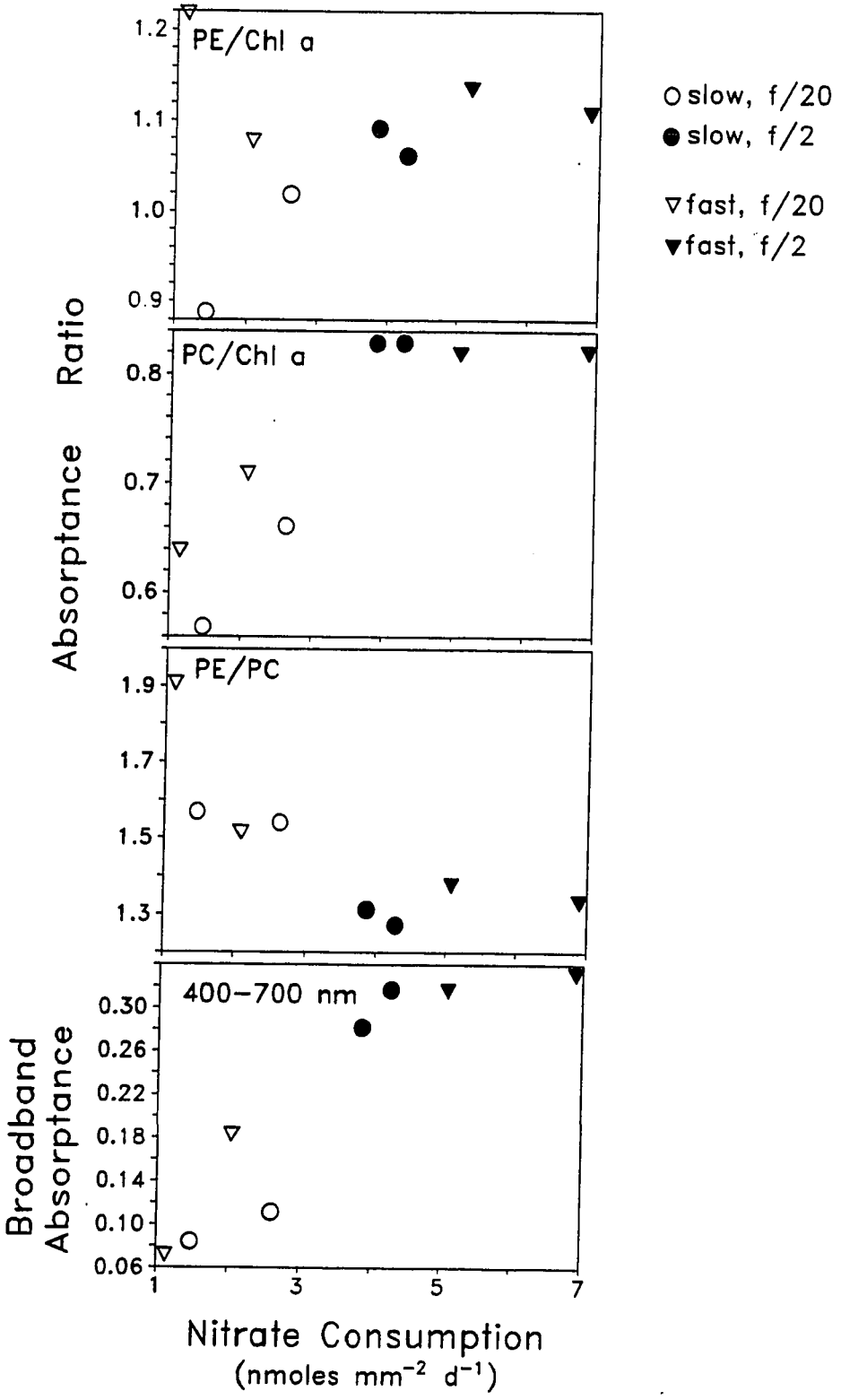

PE/Chl a
PC/Chl a
PE/PC
400–700 nm
Absorptance Ratio
Broadband Absorptance
Nitrate Consumption
(nmoles mm^{-2} d^{-1})
slow, f/20
slow, f/2
fast, f/20
fast, f/2

Table 4.1. Results of ANOVA significance tests for the effect of nutrients (f/20, f/2) and water motion (50 rpm, 150 rpm) on growth, nutrient consumption, photosynthetic response and spectral light absorption of juvenile blades. n = 2 replicate dishes per treatment combination. ns = not significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$

	Nutrients	Water Motion
μ[a]	***	**
N cons.	**	ns
P cons.	ns	ns
P_s	**	ns
	*	ns
P(70)	**	ns
A(400-700)	***	ns
A(PE)	***	ns
A(PC)	***	ns
A(Chl *a*)	***	ns
A(PE)/A(PC)	*	ns
A(PE)/A(Chl *a*)	ns	ns
A(PC)/A(Chl *a*)	***	ns

[a] μ = specific growth rate (d^{-1})
N cons. = nitrate consumption (nmoles mm^{-2} d^{-1})
P cons. = phosphate consumption (nmoles mm^{-2} d^{-1})
P_s and are parameters of the P *vs.* I curve (see Table 4.5)
P(70) = estimated gross photosynthesis (μmol O_2 m^{-2} s^{-1}) at 70 μmol photons m^{-2} s^{-1} (PFD for growth), calculated from parameters in Table 4.5.
A(400-700 nm) = spectrally weighted broadband absorptance
A(PE) = absorptance at 566 nm
A(PC) = absorptance at 624 nm
A(Chl *a*) = absorptance at 680 nm

Table 4.2. Rank correlation coefficients (r_s) among various variables, pooled for all treatments. Analysis based on the means for each replicate dish (n = 8). ns = not significant; * = p<0.05; ** = p<0.01; *** p<0.005. See Table 4.1 for abbreviations.

	μ	N cons.	P cons.	P_s	α	P(70)	A(400-700 nm)
μ	--						
N cons.	0.81*	--					
P cons.	ns	ns	--				
P_s	ns	0.86*	0.76*	--			
α	0.95***	0.83*	ns	ns	--		
P(70)	0.81*	0.90**	ns	0.93***	0.88*	--	
A(400-700 nm)	0.83*	0.95***	0.74*	0.95***	0.86*	0.98***	--
A(PE)	0.88*	0.98***	ns	0.90**	0.88*	0.93***	0.98***
A(PC)	0.83*	0.95***	0.74*	0.95***	0.86*	0.98***	1.00***
A(Chl a)	0.83*	0.95***	0.74*	0.95***	0.86*	0.98***	1.00***
A(PE)/A(PC)	ns	-0.79*	ns	-0.90**	ns	-0.93***	-0.88*
A(PE)/A(Chl a)	ns	ns	ns	ns	ns	ns	ns
A(PC)/A(Chl a)	ns	0.76*	ns	0.86*	0.76*	0.90**	0.86*

Table 4.3. Specific growth rates (μ) of juvenile blades grown in media with low (f/20) or high (f/2) nutrient concentration and in slow (50 rpm) or fast (150 rpm) water motion. μ was obtained by linear regression of $\log_e$(mean blade area) vs. time for the age period shown, where day 0 is the day of germination ($\pm$ 3d). n = 2 replicate dishes x 4-11 samples/dish in time.

Nutrients	Water Motion (rpm)	μ (SE) (d^{-1})	n	r^2	Age Period (d)
f/20	50	0.059 (0.007)	18	0.83	30-104
	150	0.075 (0.004)	22	0.94	30-104
f/2	50	0.088 (0.005)	14	0.96	30-82
	150	0.143 (0.012)	8	0.96	30-68

Table 4.4. Growth rate, absorptance and photosynthesis of disks grown in f/20 and transferred to f/2 medium or *vice versa*. μ = specific growth rate (% d^{-1}); abs = spectrally weighted absorptance; PS(50) = photosynthetic rate (umol O_2 m^{-2} s^{-1}) at 50 µmol photons m^{-2} s^{-1}; PS(500) = photosynthetic rate at 500 µmol photons m^{-2} s^{-1}. Means ± SD, n=6.

	Grown in f/20	After change to f/2	
μ	9.1 ± 1.9	0-5 days: 5-11 days:	12.4 + 1.8 14.0 ± 3.6
abs	0.17 ± 0.05	10 days:	0.33 ± 0.03
PS(50)	0.66 ± 0.27		1.68 ± 0.15
PS(500)	1.64 ± 0.98		3.60 ± 0.42

	Grown in f/2	After change to f/20	
μ	10.8 ± 3.0	0-5 days: 5-11 days:	10.9 ± 0.2 6.5 ± 1.8
abs	0.37 ± 0.06	10 days:	0.24 ± 0.02
PS(50)	1.79 ± 0.47		1.42 ± 0.15
PS(500)	4.66 ± 1.22		3.51 ± 0.54

Table 4.5. Parameter estimates (and their 95% confidence intervals) for photosynthesis - light response curves based on the model $P = P_s (1 - e^{-\alpha I/Ps}) e^{-\beta I/Ps}$. n = 2 replicate dishes x 3 blades/dish at 8 light levels.

Nutrients	Water Motion (rpm)	P_s (μmoles O_2 $m^{-2}s^{-1}$)	α (mol O_2 mol photons^{-1})	$\beta \times 10^3$
a) Juvenile blades				
f/20	50	0.76 (0.64 - 0.87)	0.011 (0.008 - 0.015)	0.036 (-0.064 - 0.139)
	150	1.05 (0.45 - 1.64)	0.019 (-0.004 - 0.041)	0.100 (-0.492 - 0.692)
f/2	50	3.28 (2.61 - 3.95)	0.033 (0.021 - 0.046)	-0.025 (-0.533 - 0.481)
	150	3.26 (2.55 - 3.97)	0.040 (0.023 - 0.057)	-0.197 (-0.708 - 0.314)
b) Disks				
f/20	air	1.64 (1.42 - 1.89)	0.018 (0.013 - 0.022)	0.044 (-0.150 - 0.239)
f/2	air	4.75 (4.28 - 5.22)	0.048 (0.040 - 0.057)	0.025 (-0.344 - 0.392)

Table 4.6. Maximum quantum yields of photosynthesis (ϕ_p) for a) whole juvenile blades and b) disks from adult blades grown in f/20 or f/2. Means $\pm$ SD, n=6; (a) difference significant at p=0.02; (b) difference significant at p=0.01.

Nutrients	Water Motion (rpm)	$\phi_p \pm$ SD (mol O_2 mol photons^{-1})
a) Juvenile blades		
f/20	50	0.037 $\pm$ 0.009 (a)
	150	0.056 $\pm$ 0.020
f/2	50	0.069 $\pm$ 0.027 (a)
	150	0.058 $\pm$ 0.015
b) Disks		
f/20	air	0.050 $\pm$ 0.016 (b)
f/2	air	0.077 $\pm$ 0.014 (b)

CHAPTER 5

EFFECTS OF LIGHT AND NITRATE ON THE QUANTUM YIELD OF GROWTH OF YOUNG *PORPHYRA ABBOTTAE* GAMETOPHYTES

Plants exhibit a variety of phenotypic responses to irradiance (Boardman 1977). In the morphologically simple algae such as unicells, filaments or non-differentiated blades, acclimation to the light environment is likely to be primarily by physiological and biochemical, rather than morphological adjustments, although in certain algae chromophore displacement may also play a role in light acclimation (Nultsch and Pfau 1974).

Regulation of the light harvesting ability by light-induced pigment changes is well established for a wide variety of unicellular (Richardson et al. 1983) and multicellular (Ramus 1981) algae and is generally regarded as a mechanism that leads to the optimization of photosynthesis and growth. While the photosynthetic response to photoacclimation has been well studied (Bjorkman 1981), relatively little is known of how the changes in pigment content and composition characteristically associated with photoacclimation influence growth, or to what extent these changes can compensate for a decrease in ambient irradiance. Because the light-harvesting efficiency of closely packed pigment molecules is greatly reduced (Kirk 1983), energetic constraints may impose a limit on low light acclimation. In the red

algae, the high N content of phycobiliproteins further increases the cost of maintaining high pigment levels (Raven 1984). Optimal use of ambient light levels thus presumably involves balancing the increase in the light harvesting capacity with the costs of synthesis and maintenance of the light harvesting pigments and associated membranes.

Henley and Ramus (1989) have examined the limits to photoacclimation in Ulva rotundata and have concluded that increases in pigmentation at low irradiance are not sufficient to maintain high growth rates, yet at saturating irradiance U. rotundata contains pigments in excess of those needed to sustain maximal growth. Other evidence suggests that pigment changes may not always be linked to an optimization of the light-harvesting ability. For example, in the siphonous green seaweed Codium fragile the increased pigment content at low irradiance cannot significantly enhance its already high absorptance (Ramus 1978); in the marine cyanobacterium Synechococcus non-functional phycobiliproteins are accumulated as a means of storing nitrogen (Wyman et al. 1985).

Whole-thallus absorption spectra of Porphyra abbottae gametophytes showed that in this species low-light acclimation involves an increase in total photon absorption and in the proportion of phycobiliproteins relative to chlorophyll a (Chapter 3). Ramus (1989) has stressed that in order to evaluate the ecological significance of photoacclimation in algae, studies are needed which relate light absorption to the efficiency with which light is utilized for growth. Therefore, in the present study the effect of long term

photoacclimation on plant growth is evaluated by means of the quantum yield of growth, expressed as the increase in blade area per photon absorbed. Because N limitation causes a reduction in photon absorption and photosynthetic capacity (Chapter 4), quantum yields of growth were determined from growth and light absorption measurements of plants acclimated to various irradiance and N environments.

MATERIALS AND METHODS

Porphyra abbottae Krishnamurthy (strain 1626) conchospores were seeded onto deep Pyrex culture dishes (Chapter 2). The sporelings were allowed to grow for 2-3 weeks in f/2 medium (Guillard and Ryther 1962) at ca. 70 µmol photons m^{-2} s^{-1}. The cultures were then thinned to ca. 30 individuals/dish and transferred to one of the light and nitrogen conditions described below. For the light gradient, cultures were grown at 8 or 9 photon fluence densities (PFDs) ranging from 9.5 to 154 µmol photons m^{-2} s^{-1} (Lambda cosine collector) in medium (150 ml) containing 5 or 100 µM nitrate. For the nitrogen gradient, additional cultures were grown in 2.5, 10, 25, and 50 µM $NaNO_3$ at 126 µmol photons m^{-2} s^{-1}. The media were prepared by adding f/2 enrichment with varying amounts of $NaNO_3$ to seawater that had been depleted of N by Ulva plants (3 g fresh weight L^{-1} for 3 d). The temperature was maintained at 10-11°C. Light was provided by cool-white fluorescent tubes at a 16L:8D photoperiod. The dishes were kept on rotary shakers (100 rpm).

Plants were acclimated to the experimental light and nitrogen conditions for 2-4 weeks prior to measurements of growth and light

absorption. The density in each dish was reduced to 16 individually-marked blades, and the surface areas of 10 blades were determined (Chapter 2) at the beginning and end of the growth period (10-18 d). Halfway through the growth period (5-9 d,) 5 blades were removed for measurements of whole thallus spectral absorbance (Chapter 3). The medium was changed daily during the growth period and the remaining NO_3^- concentration determined every other day.

Whole thallus absorbance spectra were used to calculate peak absorptances of phycoerythrin (PE, 566 nm), phycocyanin (PC, 624 nm), chlorophyll a (Chl a, 680 nm), and the spectrally weighted broadband absorptance (i.e., the fraction of the incident PFD in the 400-700 nm range absorbed by the blade) by the method described in Chapter 3.

Specific growth rates (d^{-1}) were calculated for each blade from surface area increases by assuming exponential growth.

The quantum yield of growth, i.e., the surface area increase per mol photons absorbed, was calculated for each dish as

$$\phi_g = 17.36 \frac{\mu}{a \cdot I}$$

where ϕ_g = quantum yield of growth (m^2 mol $photon^{-1}$), μ = specific growth rate on a surface area basis (d^{-1}), a = spectrally weighted broadband absorptance (dimensionless), I = incident PFD (μmol photons m^{-2} s^{-1}), and the constant 17.36 converts μmol s^{-1} to mol d^{-1} (assuming 16 h light d^{-1}).

RESULTS

Whole thallus broadband absorptance and the PE/Chl *a* and PC/Chl *a* peak absorptance ratios all decreased curvilinearly with increasing PFD (Fig. 5.1). Broadband absorptance (Fig. 5.1A) decreased more drastically in the low-N (5 μM NO_3^-) than in the high-N (100 μM NO_3^-) cultures, from 0.46±0.025 (mean±SD, n=5) at 9.5 μmol photons m^{-2} s^{-1} to 0.13±0.007 at 56 μmol photons m^{-2} s^{-1}. Broadband absorptance of high-N cultures only decreased from 0.50±0.015 at 9.5 μmol photons m^{-2} s^{-1} to 0.31±0.008 at the highest growth irradiance (126 μmol photons m^{-2} s^{-1}). PE/Chl *a* and PC/Chl *a* peak absorptance ratios (Fig. 5.1B,C) also decreased more sharply in low-N than in high-N cultures. These differences between low-N and high-N cultures were established at the lowest PFDs (10-30 μmol photons m^{-2} s^{-1}). Thereafter, the rate of decrease of broadband absorptance and absorptance ratios with PFD did not differ notably between low-N and high-N plants. The PE/PC absorptance ratio (Fig. 5.1D) of low-N cultures increased sharply between 9.5 and 31.5 μmol photons m^{-2} s^{-1} and then remained approximately constant at 1.60-1.65, whereas in high-N cultures PE/PC gradually increased with PFD.

Broadband absorptance and the PE/Chl *a* and PC/Chl *a* peak absorptance ratios all increased in a nearly linear manner with initial NO_3^- concentrations of 2.5-100 μM in cultures maintained at 126 μmol photons m^{-2} s^{-1} (Fig. 5.2). Broadband absorptance (Fig. 5.2A) increased from ca. 0.1 at 2.5-5.0 μM to almost 0.5 in f/2 (ca. 900 μM), and thus varied within the same range of values elicited by the PFD

gradient. In the 2.5-100 µM range, linear regression of absorptance over initial NO_3^- concentration (r^2=0.98) shows that absorptance was increased by 0.01 (1%) for every 5.6 µM increase in the initial concentration of NO_3^-, equivalent to an increase in consumption of 426 µmol NO_3^- m^{-2} d^{-1}. The PE/PC absorptance ratio (Fig. 5.2D) remained high (1.61-1.66) between 2.5 and 100 µM NO_3^-, but decreased to 1.51±0.04 in f/2 medium.

There was a close negative correlation (r^2=0.77) between the PE/PC ratio and the peak absorptance by PC for the data pooled from all light and N regimes (Fig. 5.3).

Growth vs. incident PFD curves (Fig. 5.4A) indicate maximal growth rates at 50-80 µmol photons m^{-2} s^{-1} and a decrease of growth rate at higher PFDs, both in low-N and high-N. Differences in growth rate between low-N and high-N cultures were largest at optimum PFDs. Since the value for the growth rate of the high-N culture at 77 µmol photons m^{-2} s^{-1} (0.157 d^{-1}, Fig. 5.4A) is an outlier, it is possible that the actual optimum PFD is > 56 µmol photons m^{-2} s^{-1}.

NO_3^- in the low-N (5 µM) cultures became depleted daily at all PFDs ≥ 20 µmol m^{-2} s^{-1}. NO_3^- consumption in these cultures varied between 1.1 and 1.8 mmol m^{-2} d^{-1} (Table 1) and averaged 1.7± 0.6 mmol m^{-2} d^{-1} at 77 µmol photons m^{-2} s^{-1}, the PFD of maximum growth. In the high-N (100 µM) cultures, NO_3^- was depleted daily at PFDs ≥ 41 µmol photons m^{-2} s^{-1}; the remaining NO_3^- concentration never exceeded 0.8 µM. NO_3^- consumption of high-N cultures varied between 4.2 and 9.9 mmol m^{-2} d^{-1} (Table 1). The highest growth rate of high-N cultures (0.240 d^{-1})

was obtained at 56 μmol photons m^{-2} s^{-1}. At this PFD the NO_3^- consumption averaged 8.4±4.4 mmol m^{-2} d^{-1}, ca. 98% of the daily supply.

At 126 μmol photons m^{-2} s^{-1} growth rates varied from 0.131 to 0.198 d^{-1} with increasing N concentration (Fig. 5.5A). Growth was N-saturated at 50 μM and declined at higher concentrations. NO_3^- consumption of the 50 μM culture averaged 3.3±1.6 mmol m^{-2} d^{-1} (Table 1), corresponding to 95% of its daily supply. There was a close positive linear correlation between NO_3^- consumption and initial NO_3^- concentration in the 2.5-100 μM range (r^2=0.98).

The quantum yield of growth (ϕ_g) increased sharply up to a PFD of 30-40 μmol photons m^{-2} s^{-1} and then gradually declined at higher PFDs (Fig. 5.4B). The highest ϕ_g (0.43 m^2 mol photons^{-1}) was obtained in low N (5μM) at 40 μmol photons m^{-2} s^{-1}. This irradiance is slightly subsaturating for growth (Fig. 5.4A). At 40-100 μmol photons m^{-2} s^{-1}, ϕ_gs of low-N blades were more than twice as high as the ϕ_gs of high-N blades. At 126 μmol photons m^{-2} s^{-1} ϕ_g decreased from 0.15 to 0.05 m^2 mol photons^{-1} with increasing N concentration (Fig. 5.5B).

DISCUSSION

Light Absorption

The response of *Porphyra abbottae* juvenile gametophytes to growth irradiance indicated photoacclimation by light harvesting pigments at irradiances subsaturating for growth. Light limitation or N addition caused an increase in absorption by PE, PC and Chl *a*, thus causing an increase in the spectrally weighted broadband absorptance, i.e., the

proportion of the incident radiation absorbed by the blades.

N availability affected the relationship between absorptance and growth PFD, in agreement with studies showing interaction of light and nutrients on seaweed growth and chemical composition (Duke et al. 1986, Lapointe 1981, Lapointe and Tenore 1981, Rosenberg and Ramus 1982a, Shivji 1985). PFD dependent changes in absorptance were more pronounced in low-N than in high-N cultures. In low N, absorptance decreased more than 3 times with increasing PFD; 33% more of the incident light could thus be harvested at the lowest PFD. In high N, absorptance decreased by about 30% with increasing PFD; the higher absorptance of blades grown at the lowest PFD would thus allow only for an additional 15% of the incident radiation to be harvested. Thus light harvesting pigments increased more dramatically at low PFD when N was limiting, possibly because light becomes more limiting to growth than N uptake and assimilation. The mechanisms by which light controls pigment synthesis are complex and not well understood (Anderson 1986). Falkowski (1980) has proposed a model for the regulation of chlorophyll synthesis by photosynthetically produced ATP which involves diversion of N from amino acids to chlorophyll precursors. According to this model, N could limit the synthesis of chlorophyll at low irradiance. As pointed out by Henley and Ramus (1989), since phycobilins and chlorophyll are both tetrapyrroles their synthesis may be regulated in a similar manner.

The highest absorptances, 0.46-0.50, were those of blades grown at the lowest PFD (9.5 μmol m^{-2} s^{-1}) in low or high N, and those of

light-saturated blades grown in f/2. Because 9.5 μmol photons m^{-2} s^{-1} is not much higher than the compensation PFD (see below), it can be concluded that these absorptance values are near the upper limit possible for P. abbottae blades. Although absorptance is not directly proportional to pigment content, except at very low concentrations (Chapter 3), differences in absorptance can be used to infer changes in pigment content. At high concentrations the effect of pigment content on absorptance is greatly reduced (Fig. 3.5). Thus differences in absorptance at low PFD reflect relatively larger differences in pigment content than at high PFD. In addition to the lowered absorption efficiency that results from the packaging of pigment molecules, a limit to the size of the light harvesting antenna is probably set by the loss in efficiency of energy transfer within a large light harvesting apparatus (Richardson et al. 1983). For P. abbottae a limit to the light harvesting capacity seems to be approached when it reaches an absorptance of 50%. Whether pigment content increases beyond this limit can not be determined from the present results.

Ramus (1983) has suggested that increases in pigment content at low irradiance may reflect nitrogen economy in addition to photoacclimation. This implies that differences in pigment content would not arise solely by N reallocation, but also by an increase in total tissue N at limiting PFD. The increase in absorptance of N sufficient, low-light acclimated blades was accompanied by a decrease in the daily N consumption (per unit surface area). Although cell N was not measured, these data suggest that light limitation caused a

decrease in the N content, and thus preferential allocation to pigment proteins at the expense of other nitrogen-containing compounds. In many terrestrial plants, growth at low PFD is associated with a reduction in the N content per unit leaf area, and a greater proportion of the N is partitioned into the thylakoids at the expense of other proteins (Evans 1989). Results of studies with microalgae are diverse, showing either an increase (Falkowski 1980) or a decrease (Laws and Bannister 1980) in cell N with low light acclimation. Total protein per cell has been found to remain constant in spite of large variations in light harvesting pigments (Beardall and Morris 1976, Claustre and Gostan 1987), thus indicating that PFD can affect patterns of N allocation in microalgae as well. Little information exists on how irradiance affects N allocation in macroalgae. Total N content has been found to increase at low PFD in Gracilaria and juvenile sporophytes of Macrocystis pyrifera (Lapointe et al. 1984, Shivji 1985), suggesting that these species accumulate N when they are light-limited.

The high absorptance values obtained for P. abbottae blades grown in low N and at the lowest PFD (Fig. 5.1A) suggest that a large proportion of the N is invested in light harvesting pigments under simultaneous light and N stress. Shivji (1985) reported a similar pattern for juvenile Macrocystis pyrifera. In contrast, the data of Fredriksen and Rueness (1989) show that in Gelidium latifolium a constant proportion of the N was devoted to light harvesting pigments under N limitation. How PFD affects patterns of N allocation thus

seems to vary among species and may be related to the storage capacities of thalli with different morphologies (Rosenberg and Ramus 1982b).

Absorptance Ratios

Absorptance ratios indicate that PFD induced changes in absorptance by light harvesting pigments occurred in the order PC>PE>Chl a. Relative proportions of PC, PE and Chl a also depended on N availability. Phycobiliprotein to chlorophyll a ratios increased with decreasing PFD and increasing N supply as expected (Chapter 3).

The relative proportions of the two phycobiliproteins was affected more by light than by N. In low N the proportion of PC relative to PE decreased sharply with increasing PFD but then remained constant, whereas in high N the decrease was gradual. Under light saturation, N did not alter the relative proportions of PE and PC, except at the very high N concentration present in f/2. The combined data presented in Fig. 5.3 suggest that the relative proportions of PE and PC depend on the total phycobiliprotein content, irrespective of whether pigment changes were elicited by altering light or N supplies. Thus the increased phycobiliprotein content caused by low light or high N is accompanied by a decrease in the relative contribution of PE.

Changes in phycobiliprotein content or composition may reflect changes in number and/or size of the phycobilisomes. Variations in the PE/PC ratio with growth irradiance are of interest because they are most likely an expression of phycobilisome structure. Waaland et al.

(1974) determined that in Griffithsia pacifica reduced light caused a marked increase in the number of phycobilisomes per unit area of thylakoid membrane along with a small increase in the PE/PC ratio. However, Merrill (1985) did not detect any variations in the PE/PC ratios of G. pacifica grown at different PFDs. A marine cyanobacterium has been shown to undergo changes in phycobilisome size at irradiances which saturated growth and changes in phycobilisome number at irradiances which limited growth (Kana and Glibert 1987). In some chromatically adapting cyanobacteria changes in the PE/PC ratio have been correlated with changes in the length of the rods composed of PE and PC (Tandeau de Marsac 1983). Based on current models of red algal phycobilisome structure (Gantt 1981, Larkum and Barrett 1983, Merrill 1985), shifts in PE/PC ratios can best be explained by changes in the number of phycoerythrin hexamers comprising the outer portion of the phycobilisome. Such changes may be dependent on the linker polypeptides responsible for phycobilisome assembly. The existence in wild P. umbilicalis of two kinds of phycobilisome, one with a reduced content of linker polypeptides and PE, has been proposed as an adaptation that may allow for rapid binding or dissociation of the phycoerythrobiliproteins in response to changes in environmental factors (Algarra et al. 1990). The decrease in the PE/PC ratio accompanying low light acclimation in P. abbottae could thus be achieved by an increase in the number of phycobilisomes in combination with a decrease in the average PE content of the phycobilisomes. If this assumption is correct, then the PC content would vary in direct

proportion to the number of phycobilisomes. A similar conclusion has been reached by Kana and Glibert (1987) regarding PFD related pigment changes in a strain of Synechococcus. However, they observed an increase in the PE/PC ratio with decreasing PFD, whereas the opposite was found for P. abbottae. The relationship between PE/PC and PC shown in Fig. 5.3 may thus represent a gradual impoverishment in PE as more phycobilisomes are added to a given area of thylakoid membranes.

PE/PC absorptance ratios in this study varied between 1.2 and 1.6. Based on results from a previous study (Chapter 3) these values would correspond to approximate molar ratios of 1.4 and 2.2, respectively. PE/PC molar ratios obtained by Merrill (1985) for P. yezoensis also increased (although only slightly) with irradiance, from 1.2 at 5 μmol photons m^{-2} s^{-1} to 1.5 at 45 μmol photons m^{-2} s^{-1}. This contrasts with results obtained from PE rich species where PE/PC decreases at higher PFD (e.g. Waaland et al. 1974, Wyman et al. 1985, Kana and Glibert 1987). This difference may thus be related to the much higher PC content of Porphyra species.

Growth

Growth of P. abbottae was saturated at ca. 70 μmol photons m^{-2} s^{-1} and was somewhat inhibited at higher PFDs, in agreement with earlier observations (Chapter 2). Extrapolation of the growth curves gives a compensation PFD of approximately 5 μmol photons m^{-2} s^{-1}. That the decline of growth at PFDs > 70-80 μmol photons m^{-2} s^{-1} was not caused by N limitation is indicated by the fact that plants grown at

126 µmol photons m^{-2} s^{-1} were N-saturated at initial concentrations > 50 µM (Fig. 5.5A).

Light saturation of growth occurred at approximately the same PFD for low-N and high-N cultures, in spite of large differences in their light harvesting capacities: at 70 µmol photons m^{-2} s^{-1}, low-N and high-N blades absorbed 9 and 25 µmol photons m^{-2} s^{-1}, respectively. According to Dring (1982), the irradiance required to saturate growth should be decreased in low nutrient concentrations, since the supply of nutrients becomes limiting for growth before the rate of the dark reaction has become limiting for photosynthesis. This interpretation fails to recognize the importance of pigment loss with nutrient limitation. The present data demonstrate that it is more meaningful to examine growth saturation in terms of absorbed, rather than incident PFD.

Quantum Yield of Growth

Quantum yields of growth (ϕ_g) were highest at subsaturating PFD (30-40 µmol m^{-2} s^{-1}) and decreased sharply at lower PFDs. This sharp drop in ϕ_g at low PFD results from the large increase in absorptance along with a decrease in growth rate. At the compensation PFD for growth, ϕ_g should be zero. At saturating PFDs, ϕ_g declines because these cultures are no longer light-limited. The slower growth rates of N-limited plants were greatly offset by their low absorptance. Consequently, their ϕ_gs were higher than those of N-sufficient plants.

Henley and Ramus (1989) reported a very similar pattern for ϕ_g in

Ulva rotundata grown under natural, fluctuating conditions of irradiance. However, the values for quantum yield of growth obtained for P. abbottae are about one order of magnitude higher than those of U. rotundata. This discrepancy can probably be explained largely by the effects of shading in their culture tanks, as well as by the high PFD requirements of the plants used for their study and the somewhat higher absorptance of the distromatic thalli of Ulva. ϕ_gs obtained for the diatom Phaoedactylum tricornutum by Geider et al. (1985) also indicate that the maximum quantum yield of growth occurs at subsaturating PFD.

Because different processes may be involved in the effects of N on growth and absorptance, it is not easy to predict whether the PFD at which ϕ_g is maximal should be affected by the N regime. The maximal ϕ_g of high-N cultures occurred at 32 µmol photons m^{-2} s^{-1} (Fig. 5.4B). Due to accidental loss no growth data were available for low-N cultures at this PFD. However, based on the low-N growth curve (Fig. 5.4A), ϕ_g at 32 µmol photons m^{-2} s^{-1} is estimated as 0.32 m^2 mol $photons^{-1}$, and thus lower than the value at 41 µmol photons m^{-2} s^{-1} (0.43 m^2 mol $photons^{-1}$). This suggests that N limitation caused an upward shift in the PFD required for maximal light use efficiency.

The relationship between ϕ_g and growth PFD contrasts with the photosynthetic response to PFD of blades grown at 70 µmol photons m^{-2} s^{-1} (Chapter 4). In the latter, as is typical for photosynthesis vs. PFD curves of plants acclimated to a given PFD, the quantum yield of photosynthesis is maximal at PFDs approaching 0 µmol m^{-2} s^{-1}. These

different patterns in the response to PFD are explained by the increased absorptance of low light acclimated blades which causes a drop in the quantum yield of growth.

The units used here for ϕ_g (m^2 mol photons^{-1}) are consistent with using blade surface area for measurements of growth, but units of mol C mol photons^{-1} have a greater physiological meaning (Ramus 1989). Thus carbon-based ϕ_gs will be estimated by assuming a carbon content of 27% of the dry weight (Atkinson and Smith 1983), a dry/fresh weight ratio of 0.1, and 61 g fresh weight m^{-2} (Chapter 2). Maximal ϕ_gs thus calculated are 0.059 and 0.034 mol C mol photons^{-1} for low-N and high-N cultures, respectively. Similar calculations give values of 0.040 and 0.016 mol C mol photons^{-1} for the quantum yields of growth at 70 µmol photons m^{-2} s^{-1} (estimated from Fig. 5.4B). These values are somewhat lower than those obtained for Ulva rotundata by Henley and Ramus (1989), who derived values in the range of 0.045-0.085 mol C mol photons^{-1}. The maximal ϕ_g of Phaeodactylum tricornutum (Geider et al. 1985) was 0.08 mol C mol photons^{-1}, and thus also higher than that of P.abbottae.

Because maximal quantum yields of photosynthesis (ϕ_p) should be independent of growth PFD (Boardman 1977, Geider and Osborne 1986), it is possible to compare maximal ϕ_ps obtained for plants grown at 70 µmol photons m^{-2} s^{-1} (Chapter 4) with light-limited ϕ_gs obtained here in order to evaluate the relation of gross to net production. Maximal ϕ_ps reported in Chapter 4 are based on rates of O_2 evolution at 10-50 µmol photons m^{-2} s^{-1} and can thus be compared to maximal ϕ_gs by means of a

photosynthetic quotient (mol O_2/mol CO_2). For a photosynthetic quotient of 1.0 maximal ϕ_gs would be 0.059 and 0.034 mol O_2 mol photons^{-1} for low-N and high-N cultures, respectively. Maximal photosynthetic quantum yields (Table 4.6) of juveniles grown at 150 rpm and in f/20 or f/2 media were 0.056 and 0.058 mol O_2 mol photons^{-1}, respectively. Absorptances of these plants were comparable to those of low-N and high-N cultures of the present study. A comparison between maximal ϕ_ps and maximal ϕ_gs indicates an apparent 100% net growth efficiency for N-limited cultures. This conflictive result can probably be attributed to an overestimation of ϕ_p at the PFD where ϕ_g is maximal, and/or differences in the growth conditions of plants used for measurements of ϕ_p and ϕ_g. In spite of this inconsistency, the results suggest that the difference between photosynthetic and growth quantum yields is much larger for N-sufficient than for N-limited plants. This would imply that N limitation causes an increase in the efficiency with which absorbed light is used for growth. Differences between photosynthetic and growth quantum yields are caused by respiratory and excretory losses. In a study of three marine phytoplankters, Falkowski et al. (1985) found that excretory losses were negligible and that 47 to 86% of the fixed C is retained for growth, while the rest is respired. The comparison between photosynthetic and growth quantum yields of P. abbottae thus suggests that a much smaller proportion of the photosynthetically fixed C is lost to respiration by the N limited plants. Unfortunately, effects of nutrient limitation on rates of respiration in algae are still poorly documented (Burris 1980).

Fig. 5.1. Spectrally weighted broadband absorptance (A) and peak absorptance ratios (B-D) as a function of growth PFD. ● : 5 μM, △ : 100 μM initial NO_3^- concentration. Curves fitted by eye.

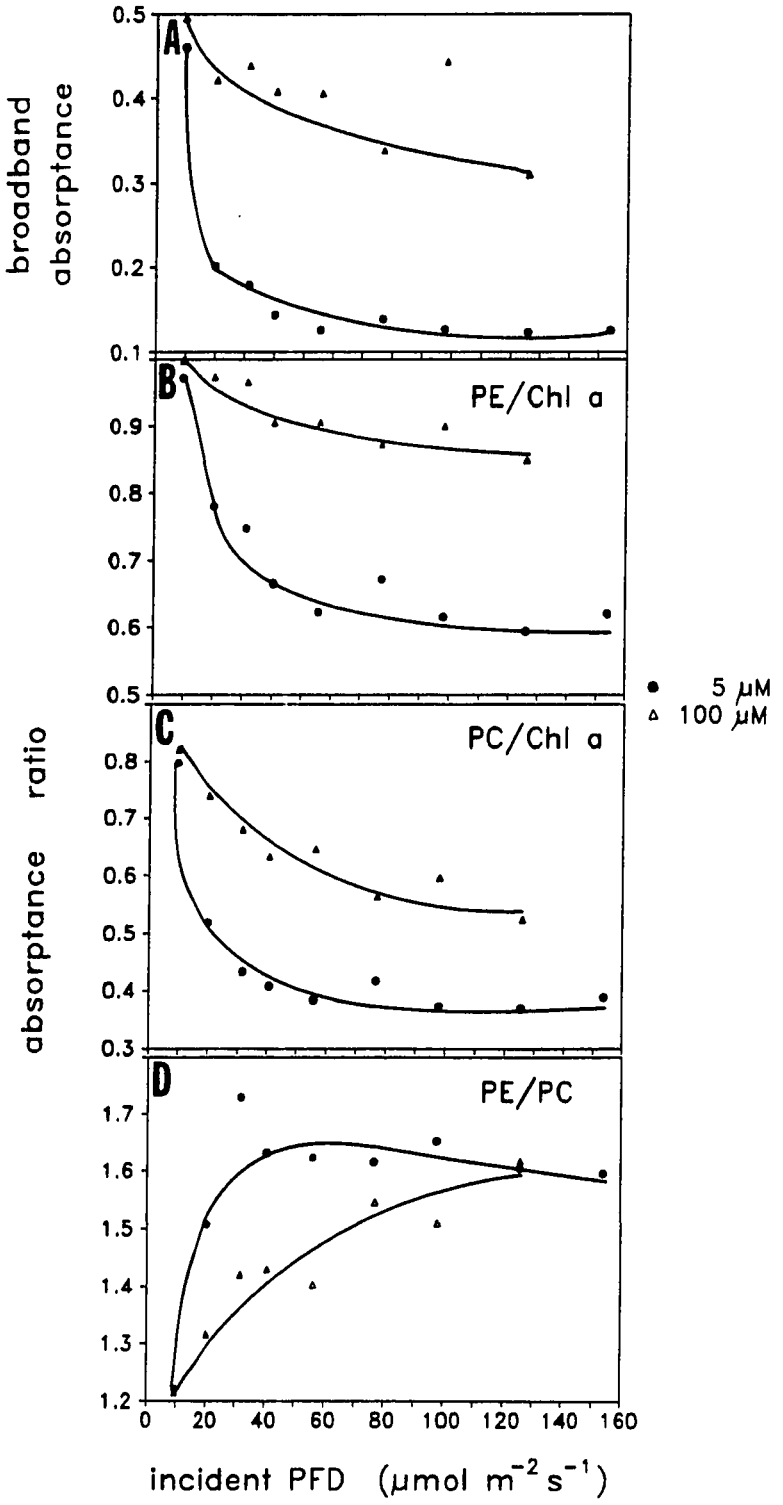
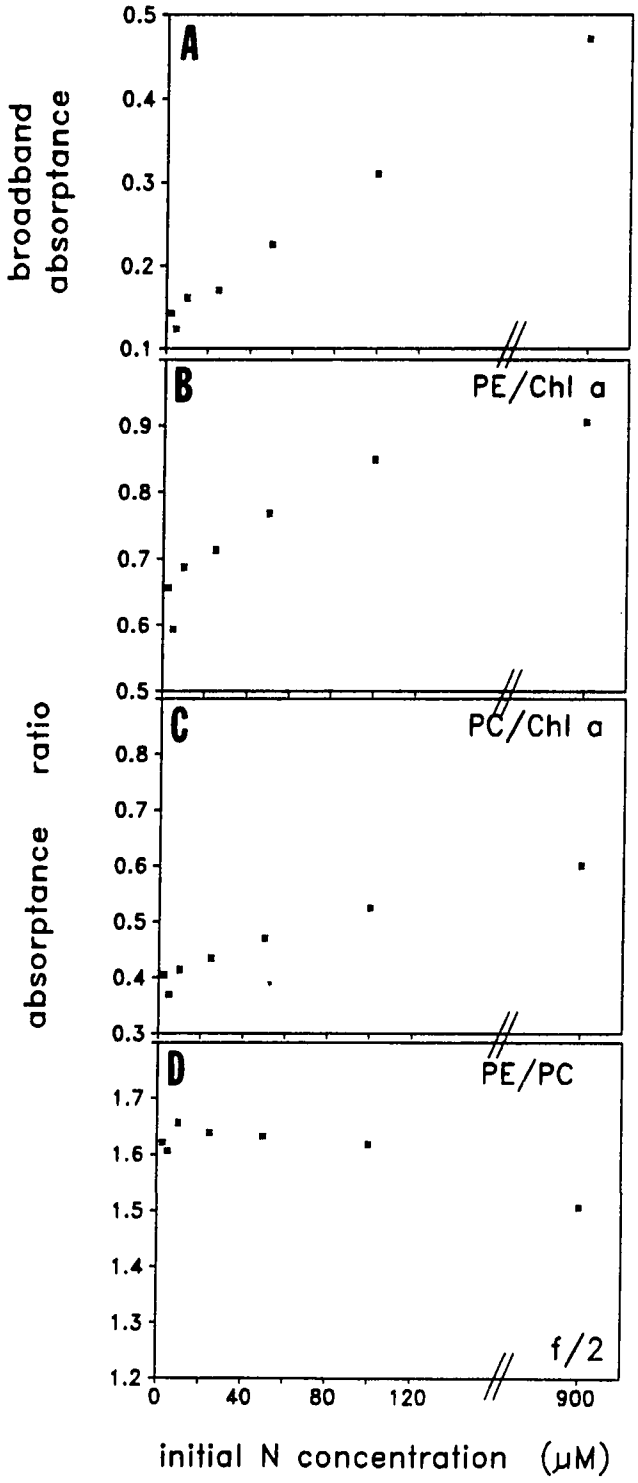

broadband absorptance
absorptance ratio
A
B
PE/Chl a
C
PC/Chl a
D
PE/PC
5 μM
100 μM
incident PFD (μmol m⁻² s⁻¹)

Fig. 5.2. Spectrally weighted broadband absorptance (A) and peak absorptance ratios (B-D) as a function of media NO_3^- concentration. Media were changed daily. PFD = 126 μmol photons m^{-2} s^{-1}.


broadband absorptance
absorptance ratio
A
B
PE/Chl a
C
PC/Chl a
D
PE/PC
f/2
0.5
0.4
0.3
0.2
0.1
0.9
0.8
0.7
0.6
0.5
0.8
0.7
0.6
0.5
0.4
0.3
1.7
1.6
1.5
1.4
1.3
1.2
0
40
80
120
900
initial N concentration (μM)

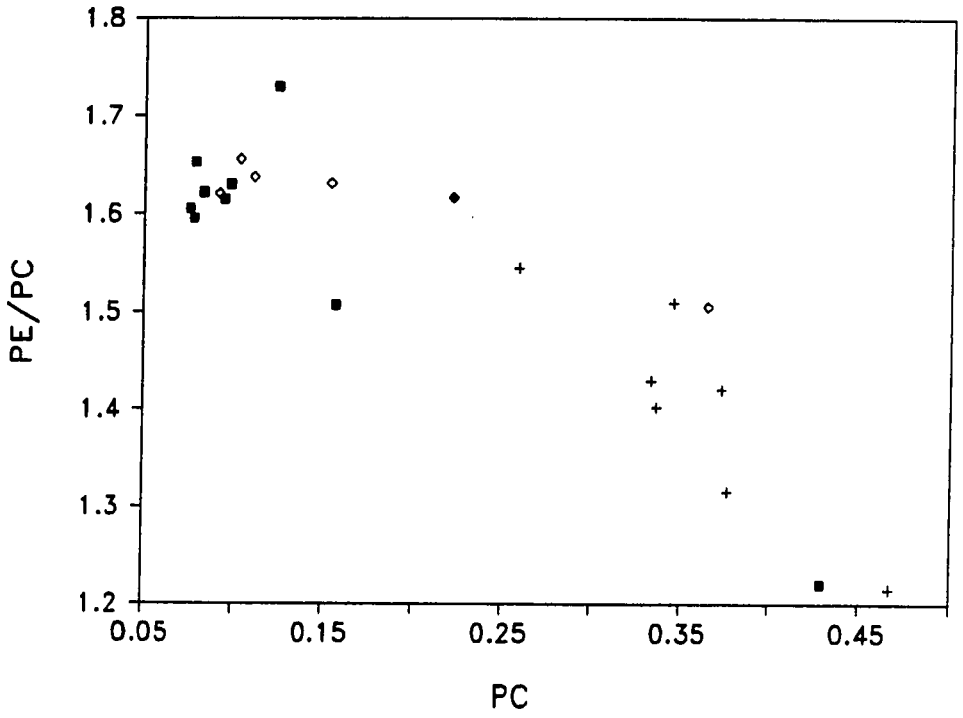


Fig. 5.3. The relationship between the PE/PC absorptance ratio and peak absorptance by PC. Data pooled from all PFD and NO_3^- regimes. ■ : 5 µM cultures, + : 100 µM cultures, ◇ : light-saturated (126 µmol photons m^{-2} s^{-1}) cultures.

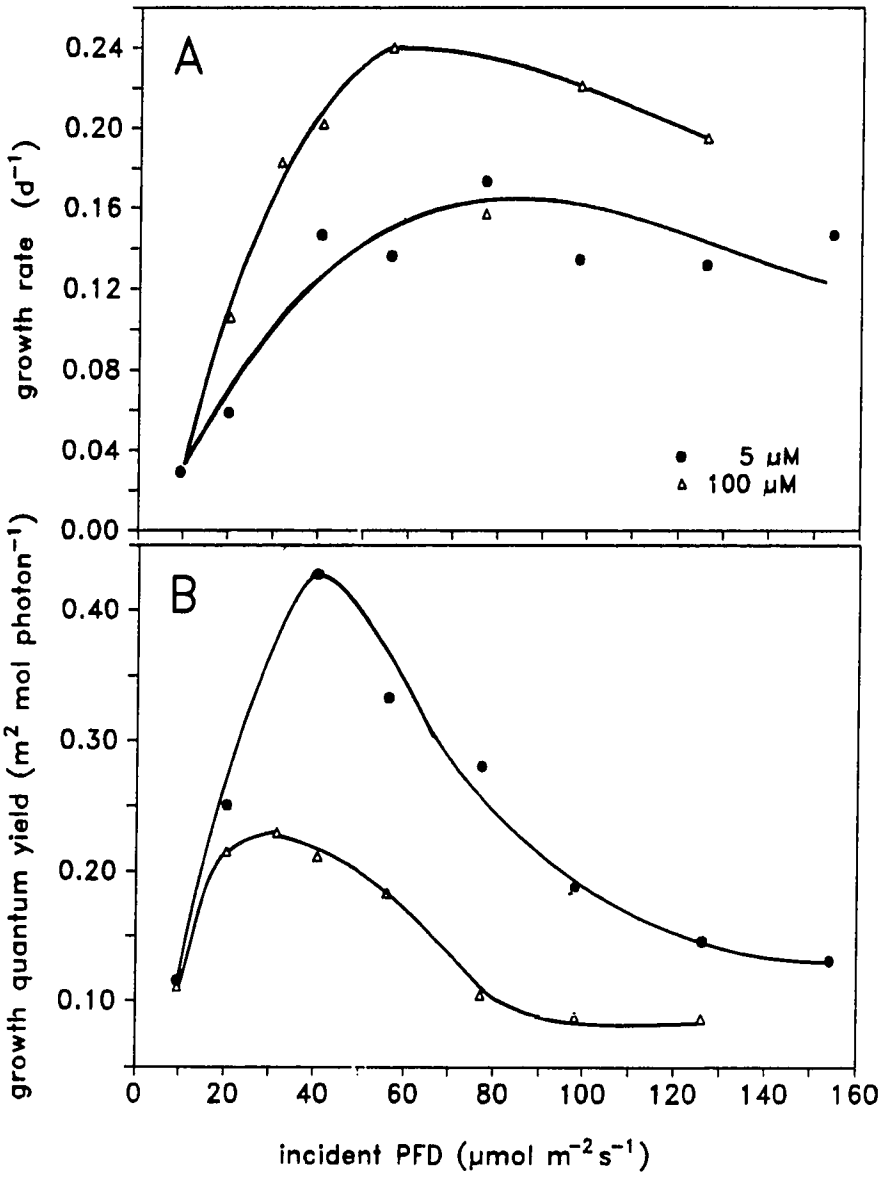


Fig. 5.4. Growth rate (A) and quantum yield of growth (B) as a function of growth PFD. ● : 5 µM, △ : 100 µM initial NO_3^- concentration.

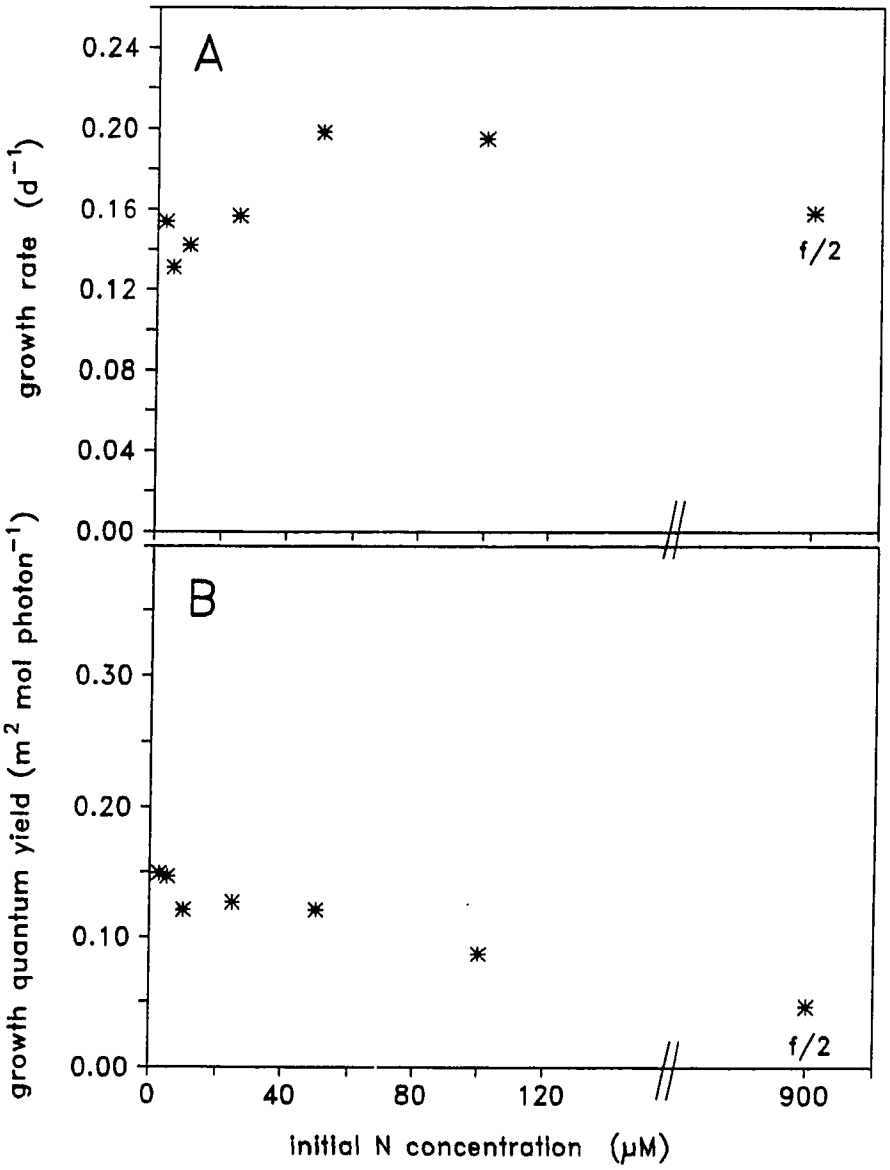


Fig. 5.5. Growth rate (A) and quantum yield of growth (B) as a function of media NO_3^- concentration. Media were changed daily. PFD = 126 μmol photons m^{-2} s^{-1}.

Table 5.1. NO_3^- consumption (mmol m^{-2} d^{-1}) of Porphyra abbottae juveniles grown at various PFDs (µmol photons m^{-2} s^{-1}) and media NO_3^- concentrations. Values are means (SD) of 5 or 6 measurements during a 10-day period. nd = no data.

PFD	NO_3^- concentration (uM)					
	2.5	5	10	25	50	100
9.5		nd				4.2 (5.8)
20		1.2 (0.8)				6.0 (0.8)
32		nd				9.6 (3.0)
41		1.4 (0.3)				8.8 (3.8)
56		1.2 (0.3)				8.4 (4.4)
77		1.7 (0.6)				9.1 (3.3)
98		1.8 (0.7)				9.9 (4.7)
126	0.4 (0.1)	1.1 (0.2)	1.3 (0.4)	1.6 (0.6)	3.3 (1.6)	7.8 (3.1)
154		1.4 (0.3)				nd

CHAPTER 6

SUMMARY AND CONCLUSIONS

The objective of these studies was to examine some of the plastic responses of Porphyra abbottae, a red macroalga with an intertidal gametophytic phase, to growth-limiting factors which are known to vary, often unpredictably, in the natural environment. The underlying assumption was that in a heterogenous environment there would be selection for phenotypic plasticity of those traits which are directly related to the acquisition of the variable resource. Marine plants, particularly macroalgae inhabiting the intertidal zone, encounter great variation in the supply of light and nutrients. Consequently the research focused on the plastic responses of P. abbottae gametophytes to light and nutrient availability during growth.

The emphasis was set on identifying long term (several weeks) morphological or physiological adjustments to specific environmental conditions. To this end, a conchocelis stock culture originated from a single carpospore was maintained to be used as a source of conchospores with a low degree of genetic variability. The attached sporelings were grown in vessels under controlled conditions for a period of 5 to 15 weeks, at which time the juveniles measured 1-4 cm in length. Although it is likely that many of the mechanisms involved in long term acclimation also operate for short term responses to

environmental change, temporal variation was not addressed directly.

Because Porphyra gametophytes have a very simple thallus construction, morphological plasticity is limited to variations in blade shape, ruffles, and thickness, and possibly changes in cell shape or structure. It is thus likely that morphological plasticity plays a minor role in their acclimation to the local environment. Nevertheless, shape and thickness of P. abbottae blades were shown to respond to environmental factors early in development. Changes in blade shape, described by the length/width ratio, and blade thickness were identified in response to variations in the supply of PFD, nutrients and water motion.

Low PFD stimulated growth in length, a response that may help emerging blades reach a more favorable light environment. Narrower blades were also obtained with increased water motion. Although the possibility exists that this trait is linked to growth rate or mechanical stress, it may also have an adaptive value if it reduces drag in the natural flow environment. Blades grown under nutrient limitation were found to be thinner, suggesting that P. abbottae can adjust its surface area/ volume ratio according to nutrient availabity.

A major part of the research was devoted to examining the effect of light and nutrient supply on the light harvesting ability of the young gametophytes. Light harvesting ability was determined through measurements of whole thallus broadband absorptance, i.e., the

proportion of the incident radiation absorbed in the 400-700 nm range. This approach has a greater ecological meaning than the more commonly used quantification of photosynthetic pigments following their extraction. However, the drawback of this method is that it may not be used directly to infer absolute pigment content or composition and thus yields limited information on the energetic implications of variations in the size and composition of the light harvesting antennae. Nevertheless, changes in the ratios of absorptance peaks can be used to identify shifts in the relative proportions and thus in the relative contributions of the individual pigments to the overall light harvesting capacity. Shifts in absorptance ratios also yield some information on the mechanisms involved in light acclimation.

Absorptance increased at PFDs limiting for growth, indicating photoacclimation by light harvesting pigments. In a PFD gradient, maximum broadband absorptance was ca. 0.5 at a PFD not much higher than compensation. It is concluded that P. abbottae can increase its light harvesting capacity to gather up to 50% of the incident radiation. Under nitrogen sufficiency, absorptances stayed above 0.3 at all PFDs. Because growth of P. abbottae is light-saturated at 70 μmol photons m^{-2} s^{-1}, an increase in absorptance from 0.35-0.40 at light saturation to 0.50 at 10 μmol photons m^{-2} s^{-1} implies that the increase in light harvesting ability cannot compensate for the decrease in available light: the total amount of light harvested at 10 μmol photons m^{-2} s^{-1} would be only one fifth of the amount harvested at light saturation. Presumably there are energetic constraints on increasing pigment

content further, related to the decrease in the efficiency of light absorption by closely packed pigment molecules. It was shown that peak absorptances correspond with pigment contents at low pigment concentrations only, as would be predicted from self-shading and packaging effects. However, no data were obtained regarding the total pigment content of blades approaching maximum absorptance, and thus the question whether at very low light levels P. abbottae increases its pigment content even when this will not further enhance light harvesting remains unanswered. This question would be worth pursuing in the light of evidence that pigment enrichment may serve the dual purpose of increasing the light harvesting ability as well as a means of storing nitrogen.

The decrease in the amount of light harvested at subsaturating PFDs does not account entirely for the decrease in growth rates at low PFD. In addition to the limitation set by the amount of light absorbed to drive photosynthesis, at low PFD there is a decrease in the efficiency with which the light absorbed is used for growth. It can thus be concluded that light-limited growth rate is not governed exclusively by the light harvesting ability, but other processes, probably related to metabolic maintenance costs, are limiting as well.

The influence of PFD on pigmentation cannot be easily separated from the effects of nitrogen availability. Nitrogen is a major component of the light harvesting apparatus and thus greatly influenced pigment content and composition. Since nitrogen is often likely to

limit the growth of macroalgae, interactive effects of light and nitrogen on pigment content are relevant to the light harvesting and photosynthetic abilities of these plants.

Because all experiments were done in batch culture, the plants did not receive a steady supply of nutrients. It was therefore not possible to determine what concentrations saturate the uptake or growth of Porphyra abbottae under natural conditions. The approach was to compare cultures growing simultaneously and that differed with respect to their total nutrient supply, without attempting to quantify the nitrogen status of the plants. For this reason comparisons between experiments regarding the effect of nitrogen are restricted to an overall assessment of the effects of nitrogen limitation on pigmentation, photosynthesis and growth.

Nutrient limitation resulted in a decrease of absorptance, light-limited and light-saturated photosynthesis, and growth. Light conversion efficiency, i.e., the quantum yield of photosynthesis, was only slightly affected by nitrogen limitation, an indication that light-limited rates of photosynthesis were determined primarily by the light harvesting ability. However, quantum yields of growth were increased by nitrogen limitation, regardless of acclimation PFD. Differences in the quantum yield of growth of N-sufficient (highly pigmented) and N-limited (poorly pigmented) plants may arise either from 1) differences in their quantum yields of photosynthesis, or 2) differences in maintenance metabolism (respiration). Based on evidence from P vs. I curves that photosynthetic quantum yield was not greatly

affected by nitrogen limitation, the second alternative seems to be the more likely explanation.

Conflicting results were obtained concerning the correlation between growth rate and absorptance. Those from the experiments reported in Chapter 3 indicate a lack of association between peak absorptances (PE, PC, Chl *a*) or broadband absorptance and growth rate, whereas the opposite conclusion was reached for the results presented in Chapter 4. This discrepancy is explained by the opposing effects that light and nutrients have on thallus absorptance. Whereas the increased absorptance that results from nitrogen sufficiency will be associated with a higher growth rate, the increased absorptance that results from light limitation will be associated with a lower growth rate because light will continue to be limiting.

Variations in the absorptance ratios indicated that the phycobiliprotein content changes more readily than Chl *a*. Increases in broadband absorptance are accompanied by an enrichment in PE and PC relative to Chl *a*, with a relatively greater increase in the proportion of PC. This pattern seems to hold regardless of whether the response is to light limitation or nitrogen enrichment. It is proposed that the observed variations in the content and relative proportions of PE and PC may reflect that the addition of accessory pigments to the thylakoids involves an increase in phycobilisome density along with a reduction of the average PE content of the phycobilisomes.

Water motion was supplied by rotary shakers and the effects of different speeds on growth and pigmentation were assessed in order to test the hypothesis that enhancement of nutrient uptake resulting from increased water motion should be reflected in the pigmentation of the thalli. Although the type of water motion supplied is far from mimicking the complexity of the natural flow environment, it is adequate for addressing questions relating to the diffusion of solutes across the boundary layer.

Experiments in which both nutrient supply and water motion were varied demonstrated that effects of water motion on pigmentation are confounded by its effect on growth rate and/or the rate of nutrient depletion from the medium. That water motion enhanced growth even under nitrogen limitation is taken as indirect evidence that facilitation of carbon uptake is at least partly responsible for the stimulation of growth by water motion. In Chapter 4 the degree of enhancement was shown to be subject to nitrogen availability. The apparent conflict of these results with those presented in Chapter 2 nevertheless may be explained by the greater effectiveness of water motion at low nutrient concentrations as long as these do not become depleted. Effects of water motion on nitrogen consumption and absorptance were also subject to nitrogen availability, and small in comparison to the response obtained by changing nitrogen concentrations. Although very preliminary, these results support the prediction that adequate water motion may be essential to meet the

nutrient requirements of macroalgae in certain areas or during the times of year when nutrient levels in seawater are low.

A common theme of this research has been the complexity that arises from the interaction of environmental factors on traits related to photosynthesis and growth. Of particular interest is the wide range of interactive effects that are associated with the nutrient status of the plants. In order to find out to what extent nutrients are limiting for the survival, growth and reproduction of macroalgae, and what adaptations allow for efficient exploitation of this resource, more information needs to be gathered on the nutrient environment experienced by macroalgae in conjunction with studies of their nutrient uptake and assimilation capabilities.

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APPENDIX 1

A MODEL FOR THE TEMPORAL CHANGE IN THE NITROGEN CONCENTRATION OF A MACROALGAL BATCH CULTURE.

The results reported in Chapters 2-4 are based on batch cultures, with medium changes at 5 to 7-day intervals. Since nitrogen in the medium was not monitored daily, its rate of depletion is not known. However, in order to evaluate whether a measured physiological response has been altered by nitrogen stress it is necessary to know whether and for how long the plants have been exposed to limiting N concentrations.

Here I present a model that predicts the temporal change in the concentration of a nutrient in a batch culture as a function of initial concentration, initial amount of plant material, growth rate, and the kinetic parameters K_s (half saturation constant) and V_m (maximum velocity or maximum uptake rate). The assumptions of the model are that 1) growth rate remains constant, and 2) uptake follows Michaelis-Menten saturable kinetics with constant kinetic parameters. The model does not consider changes in the rate of uptake due to factors other than substrate concentration, e.g., nutrient stress, utilization of internal pools or water motion.

The model is applied to the NO_3^- concentrations of f/20 and f/2 cultures using initial conditions and growth rate data from Chapter 4. The kinetic parameters were either derived from a set of measurements

of uptake rate vs. concentration (described below) or drawn from the literature.

The model

At any time t, nitrogen uptake (U) is directly proportional to the total plant surface area in the vessel:

$$U_t = V A_t \qquad (1)$$

where U_t = uptake at time t (moles h^{-1}), V = instantaneous uptake rate (moles m^{-2} d^{-1}) and A_t = area at time t (m^2).

If growth is exponential, i.e., $dA/dt = \mu A$, then

$$A_t = A_o e^{\mu(t-t_o)} \quad \text{and}$$

$$U_t = V A_o e^{\mu(t-t_o)} \qquad (2)$$

where A_o = area at time t_o (m^2) and μ = specific growth rate (d^{-1}).

The N remaining in the vessel at time t:

$$N_t = N_o - \int_{t_o}^{t} U_t \, dt \qquad (3)$$

where N_o = N in vessel at t=0, and N_t = N in vessel at time t.

Replacing (2) in (3) and solving:

$$N_t = N_o - V A_o/\mu \, (e^{\mu(t-t_o)} - 1) \, . \qquad (4)$$

If uptake follows Michaelis-Menten kinetics, then

$$V = \frac{V_m \, [N_t]}{K_s + [N_t]} \qquad (5)$$

where V_m = maximum uptake rate, K_s = half-saturation constant, and $[N_t] = N_t$/vessel volume.

The concentration at time t is obtained by replacing (5) in (4) and solving for $[N_t]$:

$$[N_t] = 1/2\ (([N_o] - a\ V_m/vol - K_s) \pm \sqrt{b^2 + 4[N_o]K_s}) \qquad (6)$$

where $a = A_o/u\ (e^{\mu(t-t_o)} - 1)$

$$b = K_s - [N_o] + a\ V_m/vol\ .$$

The time it takes for the concentration to reach a value [Nt] is then:

$$t = 1/\mu\ \ln\ (1 + \frac{\mu\ ([N_o]-[N_t])\ (K_s+[N_t])}{A_o\ V_m/vol\ [N_t]}) \qquad (7)$$

and the time it takes for the concentration to equal the K_s is:

$$t = 1/\mu\ \ln\ (1 + \frac{2\mu\ ([N_o]-K_s)}{A_o\ V_m/vol})\ . \qquad (8)$$

Eq. (8) shows that for $No>>K_s$, i.e. saturating initial concentration, the time until the uptake rate is reduced to 1/2 V_m is nearly independent of the parameter K_s.

Kinetic parameters

Plants grown in f/20 and f/2 at high water motion (Chapter 4) were incubated at 3 (if f/20-grown) or 4 (if f/2-grown) NO_3^- concentrations: 13, 26 (unenriched seawater), 130 (f/20) and 970 µM (f/2). At each concentration, 4 detached blades were incubated in separate dishes at 70 µmol photons m^{-2} s^{-1} and 100 rpm. Samples for NO_3^- analysis were taken at 2, 4 and 6 h. At the two lowest concentrations, the sampling volume was replaced each time with new medium and the resulting concentration thus calculated. For each 2 hr

period, K_s and V_m were obtained by linear regression of V vs. S/V, where V is the uptake rate (as μmol g fresh weight^{-1} h^{-1}) and S (μM) the mean of the initial and final concentrations in each period (Table A.1). For model predictions (eqs. 6-8) V_m values were converted to μmol m^{-2} d^{-1} assuming 61 g m^{-2} (Chapter 2) and 16h light d^{-1}.

Because uptake of f/2-grown plants was not saturated in f/20 (ca. 120 μM), values for the kinetic parameters based on the ca. 2-120 uM range were lower than those based on the full range of concentrations (Table A.1). This might be the result of a significant contribution of passive diffusion to the total uptake , or of biphasic uptake kinetics, although evidence for the latter in macroalgae is still lacking (Lobban et al.). The fast initial uptake rate of f/2 grown blades resulted in abnormally high K_s and V_m values at 0-2 hr. The values obtained from the 2 to 4-h period are in better agreement with published values for macroalgae (Table A.1), except for the very high K_s (56 μM) of f/2-grown plants when calculations are based on the full range of concentrations. Because these parameters are being estimated in order to predict the rate of disappearance of NO_3^- from f/20 and f/2 cultures, the full range of concentrations will be used to estimate the parameters of f/2-grown plants.

Predictions of the model

Eq. 6 predicts that for high initial N concentrations ($>>K_s$) the rate of decrease is largely dependent on the initial amount of plant material (A_o) and on the maximum uptake rate (V_m). Estimates of A_o in

the cultures indicate a range of 300-2500 mm^2 (due to growth and occasional thinning), with mean values of 1300-1700 mm^2. The different growth rates of these cultures (0.059-0.143 d^{-1}, Table 4.3) have a relatively much smaller effect on the rate of depletion. The magnitude of K_s is important at low concentrations only and, therefore, it does not affect the initial phase of the depletion curve.

The time it takes for a culture to reach a concentration of K_s is nearly independent of the magnitude of K_s (eq. 8). This time interval is thus a useful parameter to estimate how long the plants are subjected to a limiting N supply before a medium change or a physiological measurement.

f/20 cultures: According to the model, the concentration of f/20 cultures usually dropped sharply to <10 µM during the first day (Fig. A.1A) and reached 0.5-1.5 µM by day 5 (inset Fig. A.1A). This agrees with measurements that indicated total depletion of N by day 5. For a mean initial area and V_m values ranging from 2.5 to 17 µmoles g^{-1} h^{-1} the K_s would be reached between days 5 and 1, respectively (Fig. A.2A).

f/2 cultures: Based on mean initial area values the model predicts a steady drop in concentration up to day 4 (Fig. A.1B), when it reaches ca. 200 µM. Even at high initial areas the concentration remains above 30 µM until day 5. The difference in the curves predicted for slow and fast water motion are mainly due to differences in growth rates. These differences are not enough to account for the large measured difference in consumption between the two water motions: mean measured concentrations at 5 days were 670 and 200 µM at slow and

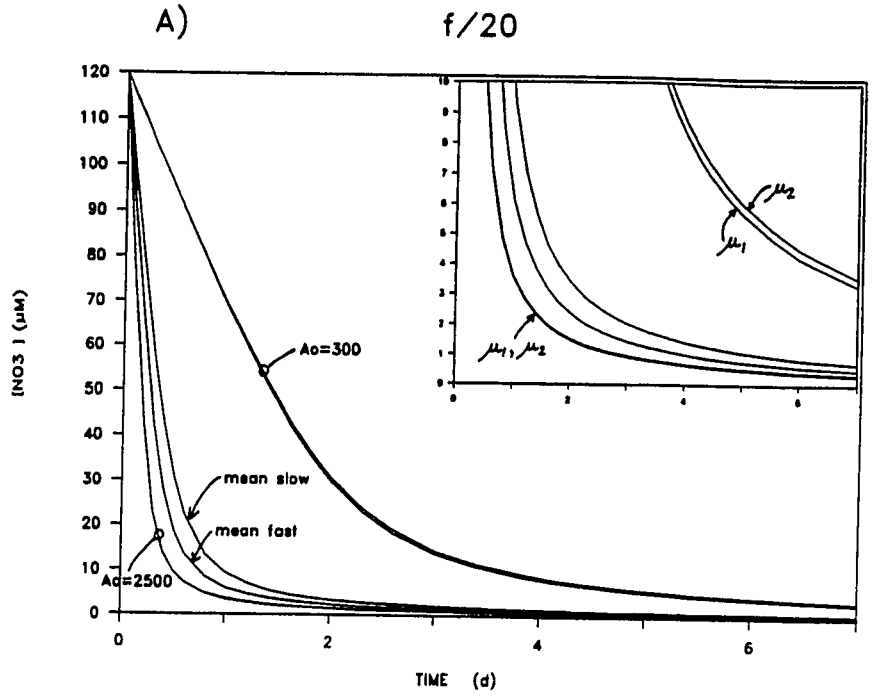
fast water motion, respectively (Fig. A.1B). This suggests differences in the uptake capacities of the two groups of plants which have not been included in the model. The model predicts that only under conditions of very high initial area and V_m would the concentration drop to K_s before day 5 (Fig. A.2B). In this case the plants could be severly nitrogen limited for about 1 day, unless internal reserves were being utilized.

Application to measurements of photosynthetic parameters

In order to determine whether the decrease in the N concentration of the cultures affected the photosynthetic response, the concentrations at the time of each photosynthetic measurement were estimated from the model and correlated with the parameters α and P_s. Simple correlation coefficients were non-significant in all cases. Rank correlations showed a significant positive correlation between N concentration and the photosynthetic parameters in f/20 cultures (Fig. A.3, Table A.2). This indicates that a portion of the variance associated with these parameters is accounted for by the loss of N from the medium. Still, there is almost no overlap in the parameter values between f/20 and f/2 cultures. Rank correlations were not significant for f/2 cultures (Fig. A.4, Table A.2).

Correlations between the number of days the N concentration was $<K_s$ and the P-I parameters were non-significant for all cultures. This suggests that as N becomes limiting, the photosynthetic parameters are affected gradually and that this occurs before the concentration reaches the K_s.

Fig. A.1. Time course of change in NO_3^- concentration as modeled from eq. 6, where A_o varies between 300 and 2500 mm^2. A) f/20 cultures: N_o=120 µM , K_s=12 µM, V_m=16 µmoles g fresh weight^{-1} h^{-1}, μ_1=0.059 d^{-1}, μ_2=0.075 d^{-1}. Inset: detail of curves at <10 µM. B) f/2 cultures: N_o=966 µM, K_s=56 µM, V_m=12.5 µmoles g fresh weight^{-1} h^{-1}, μ_1=0.088 d^{-1}, μ_2=0.143 d^{-1}. μ_1 and μ_2 are the specific growth rates in slow or fast water motion, respectively. "mean slow" and "mean fast" curves are based on mean values for A_o and µ obtained from the slow and fast water motion cultures, respectively. Symbols indicate means of concentrations measured at 5 days.


A)
f/20
[NO3] (µM)
TIME (d)
Ao=300
Ao=2500
mean slow
mean fast
µ2
µ1
µ1, µ2

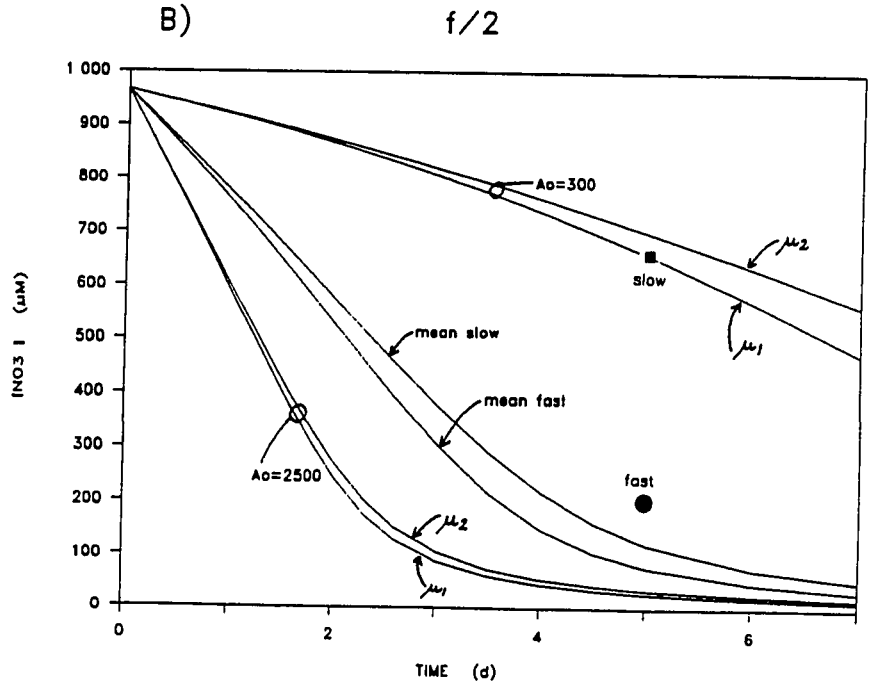
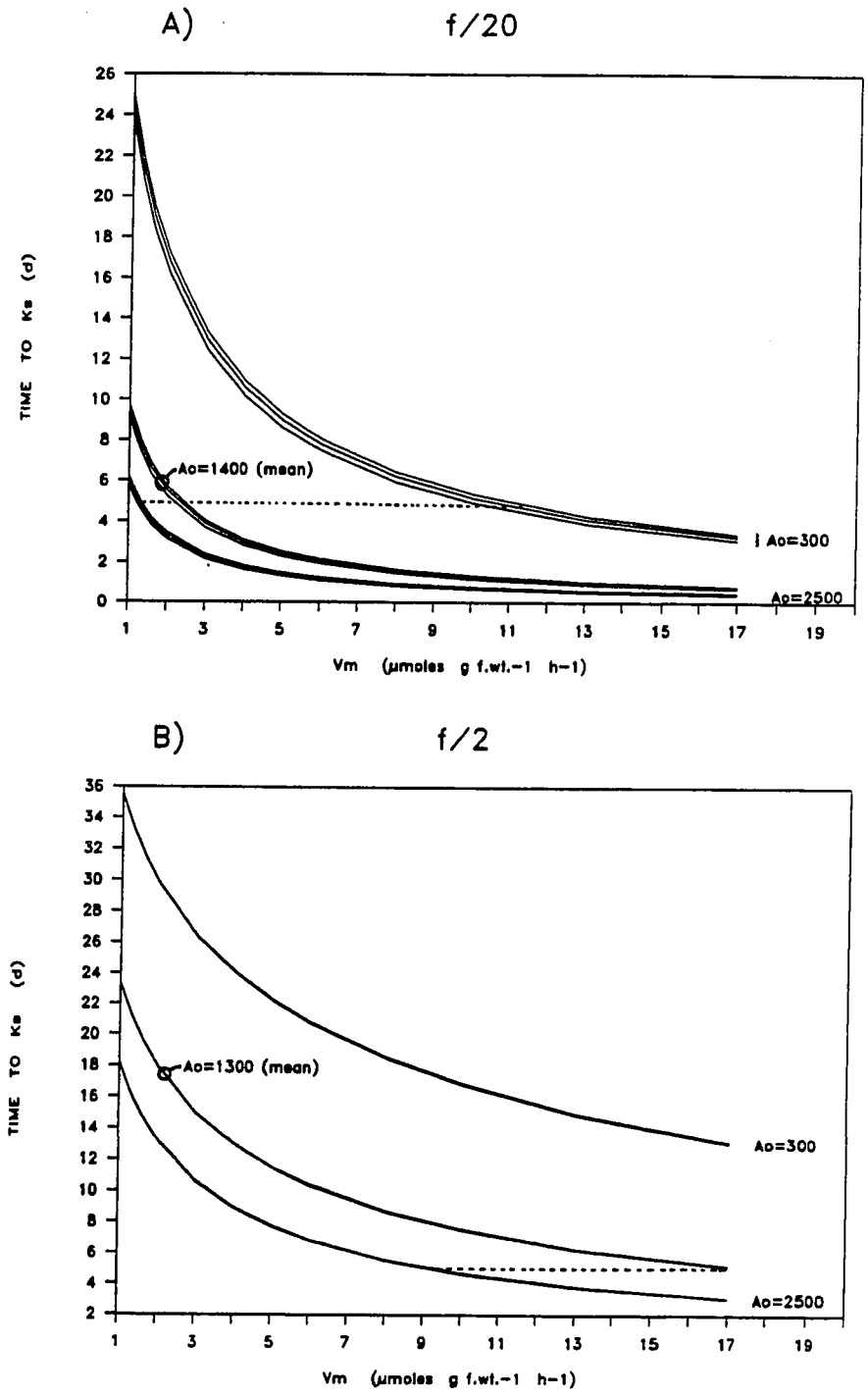

B)
f/2
[NO3] (µM)
TIME (d)
Ao=300
Ao=2500
slow
fast
mean slow
mean fast
µ2
µ1

Fig. A.2. Number of days before a culture reaches a concentration equal to the K_s, calculated according to eq. 8. A_o varies between 300 and 2500 mm^2. Ranges of kinetic parameters are drawn from the literature, with K_s values set at 2,7, and 13 µM. A) f/20 cultures: N_o=120 µM, μ=0.067 d^{-1}. B) f/2 cultures: N_o=966 µM, μ=0.116 d^{-1}. The dotted lines delimit the range of initial areas and V_ms which could reach the K_s before the 5th day.


A)
f/20
TIME TO Ks (d)
Ao=1400 (mean)
Ao=300
Ao=2500
Vm (µmoles g f.wt.-1 h-1)
B)
f/2
TIME TO Ks (d)
Ao=1300 (mean)
Ao=300
Ao=2500
Vm (µmoles g f.wt.-1 h-1)

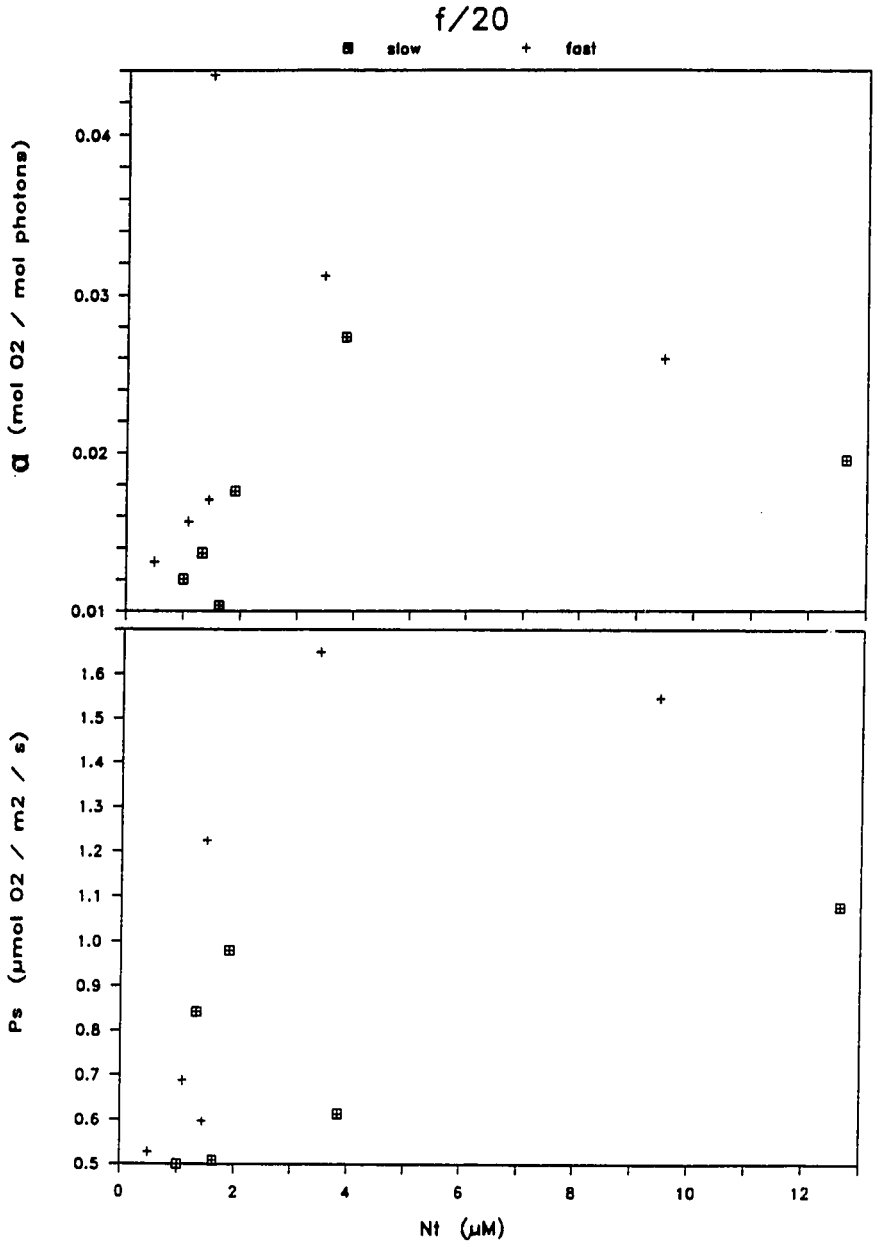


Fig. A.3. f/20 cultures: relationship between the photosynthetic parameters (α , P_s) and the predicted NO_3^- concentration (N_t) on the day of measurement. For significance of correlations see Table A.2.

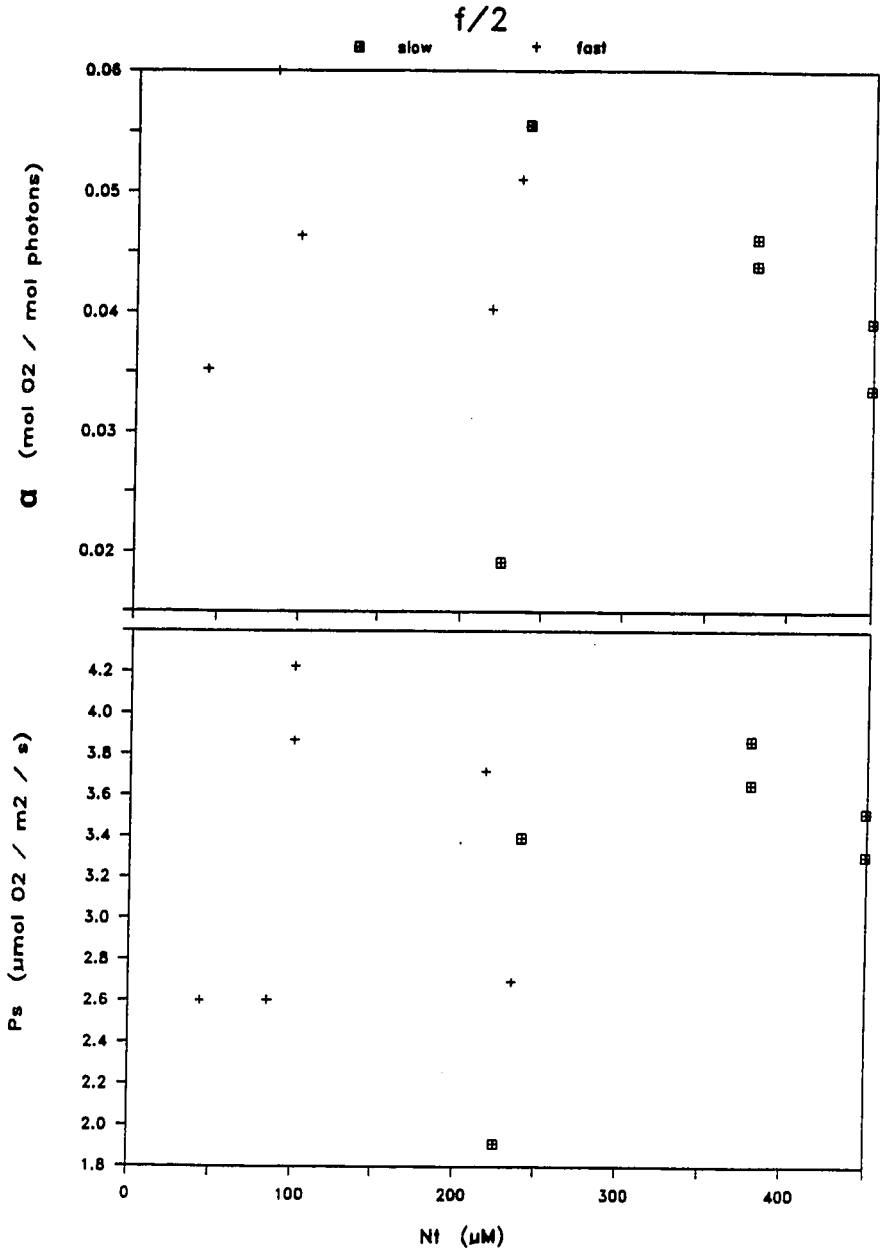


Fig. A.4. f/2 cultures: relationship between the photosynthetic parameters (α , P_s) and the predicted NO_3^- concentration (N_t) on the day of measurement. For significance of correlations see Table A.2.

Table A.1. NO_3^- uptake kinetic parameters for a) various macroalgae, and b) from *Porphyra abbottae* uptake experiment.

	K_s (µM)	V_m (*) (µmol g FW^{-1} h^{-1})	Reference
a)			
Laminariales	4.1 - 13.1	0.7 - 3.1	(1)
Fucus germlings	2 - 5	2.5	(2)
Gigartinales	2.5 - 4.9	1.0 - 2.9	(1)
Enteromorpha			
low tissue N	13.3	16.9	(3)
high tissue N	2.3	7.5	(3)

b)

Porphyra abbottae

	K_s	V_m	time of incubation (h)	conc. (µM)
grown in f/20	29	34	0 - 2	7.2 - 124
	12	16	2 - 4	1.9 - 109
	36	31	4 - 6	1.8 - 97
grown in f/2	11	6	0 - 2	7.3 - 123
	334	37	0 - 2	7.3 - 950
	11	6	2 - 4	1.6 - 116
	56	12.5	2 - 4	1.6 - 898
	-8	1	4 - 6	1.5 - 112
	no uptake at 960 µM		4 - 6	1.5 - 914

(*) conversion to fresh weight (FW) assuming 1 g dry weight = 10 g FW.
(1) in Lobban et al. 1985
(2) Thomas et al. 1985
(3) O'Brien and Wheeler 1987

Table A.2. Rank correlation coefficients (r_s) for the photosynthetic parameters α and P_s vs. the predicted NO_3^- concentration ($[N_t]$).

		α vs. $[N_t]$	P_s vs. $[N_t]$	n
f/20	slow	0.77	0.71	6
	fast	0.77	0.89*	6
	all	0.63*	0.62*	12
f/2	slow	-0.06	0.31	6
	fast	0.26	0.47	6
	all	-0.27	0.13	12

* $p \leq 0.05$

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