STUDIES ON THE ECOLOGICAL PHYSIOLOGY OF *PORPHYRA ABBOTTAE* AND *PORPHYRA TORTA* (RHODOPHYTA): DEVELOPMENT OF NEW SPECIES FOR MARICULTURE IN ALASKA

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Studies on the ecological physiology of *Porphyra abbottae* and *Porphyra torta* (Rhodophyta): Development of new species for mariculture in Alaska

Abstract

Environmental factors affecting the distribution and growth of *Porphyra abbottae* and *Porphyra torta* were studied in a research project on the feasibility of nori mariculture in southeast Alaska. *In situ* abundance of *Porphyra abbottae* was compared at sites in Sitka Sound, Cross Sound and Chatham Strait. Growth of laboratory cultured *Porphyra torta* gametophytes was studied in response to nutrients, salinity, crowding and substrate. The seasonal progression of abundance in *Porphyra abbottae* varied between study sites, with associated differences in water motion and temperature. Double sampling techniques improved accuracy of *in situ* abundance estimates. Germination and initial growth of cultured *Porphyra torta* gametophytes were dependent on plant density, and substrate texture affected recruitment. Nitrate positively affected growth and pigment concentration at environmental levels; negative effects of low nitrate were reversible. *Porphyra torta* gametophytes tolerated low salinity and inorganic carbon at least to half of normal levels.

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CHAPTER 1

GENERAL INTRODUCTION

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General Introduction

Significance of *Porphyra* to the region

Porphyra is a conspicuous member of the rocky intertidal of southeast Alaska, as it is on most of the world's temperate coasts. Coastal peoples worldwide are familiar with this seaweed as a subsistence food and, in Asia, as a commercial crop (Lobban & Harrison, 1994; Schiel & Nelson, 1990; Mumford & Miura, 1988). To the Tlingit people of Southeast Alaska, *Porphyra* is known as *laak'usk* (black seaweed) and one of many *Tlene di* (things of low tide) (Betts, 1991). Specific sites, usually rocky reefs and headlands, are identified as traditional harvest beds, and harvest takes place during a narrow 2 - 3 week window at the peak of the leafy growth in the spring, the specific time varying from site to site. Seaweed including *Porphyra* is harvested as a subsistence item in 29 out of 30 communities in southeast Alaska, at a rate of up to 14 pounds per capita (Betts, 1991). Since *laak'usk* is common only in outer coastal areas, it has traditionally been valued for trade with people living in inside coastal areas or the interior. Recent interest in commercial harvest of *Porphyra* is of concern to many traditional users. To date there is no management plan for commercial harvest or knowledge of its population biology. Mariculture may be a more acceptable form of production in this region, creating a new economic enterprise without

disrupting natural populations and traditional harvesting areas (Matthew Kookesh, Alaska Department of Fish and Game, Angoon; personal communication).

Porphyra life-cycle

Porphyra generally has a biphasic, dimorphic life cycle with a foliose, haploid gametophyte and a filamentous, diploid sporophyte called the conchocelis (Fig. 1). The gametophytes normally produce male and female gametes, in the same or separate blades depending on species, and upon fertilization these non-motile zygotospores are released and readily germinate into the diploid conchocelis (Cole & Conway, 1980; Conway & Cole, 1977). The conchocelis is inconspicuous and bores into the calcareous shells of molluscs and barnacles. Little is known of this phase in nature, but it is probably perennial in many species, allowing their persistence through unfavorable environmental conditions (Lobban & Harrison, 1994; Conway & Cole, 1977). After varying lengths of time individual cells along the filaments mature into conchosporangia, responding to environmental cues such as temperature and photoperiod. The release of conchospores from the sporangia is also cued by environmental signals. In some species, both gametophyte and conchocelis phase are capable of reproducing asexually by monospores (Guiry, 1990; Cole & Conway, 1980; Conway & Cole, 1977). Since the conchocelis is so inconspicuous and different in form from the gametophyte, it was once thought to be a different genus, called *Conchocelis*.

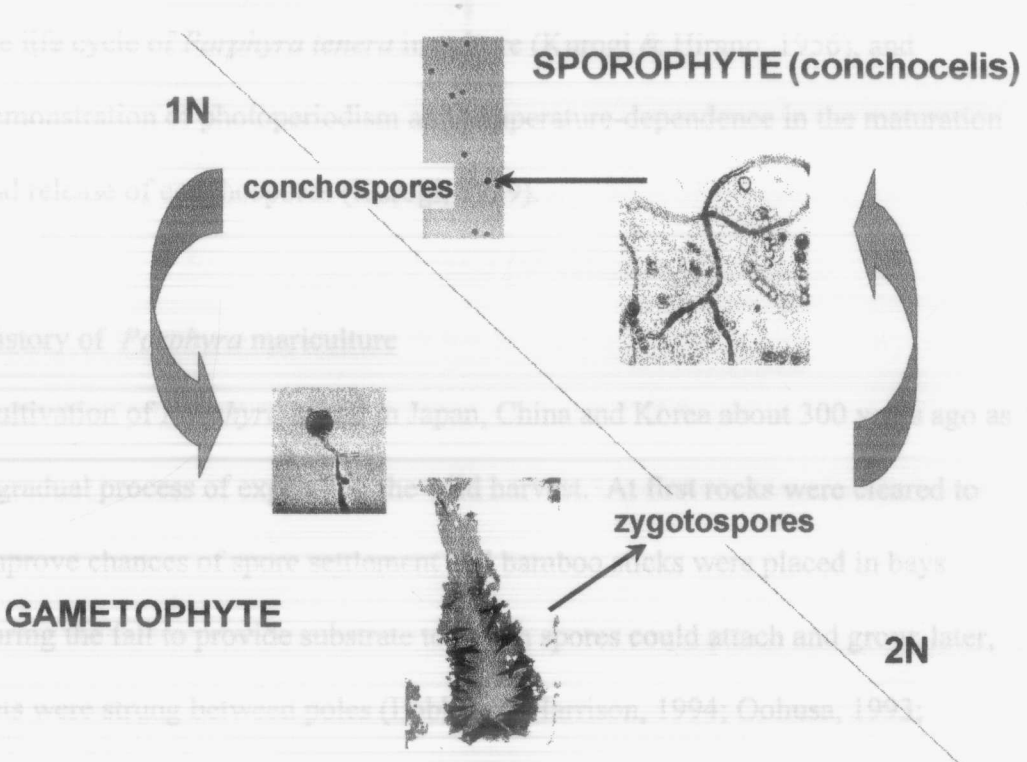

FIG. 1-1. Generalized *Porphyra* life cycle. Counter-clockwise from top: *P. torta* conchospores; *P. torta* sporeling, 2-cell stage; mature *P. abbottae* gametophyte, with fertile margins (dark areas); vegetative conchocelis of *P. abbottae* (thin filaments) with conchosporangia (rounded cells).

The life cycle and alternating generations of *Porphyra* were first elucidated by Drew (1949). This important discovery was soon followed by the completion of the life cycle of *Porphyra tenera* in culture (Kurogi & Hirano, 1956), and demonstration of photoperiodism and temperature-dependence in the maturation and release of conchospores (Kurogi, 1959).

History of *Porphyra* mariculture

Cultivation of *Porphyra* began in Japan, China and Korea about 300 years ago as a gradual process of expanding the wild harvest. At first rocks were cleared to improve chances of spore settlement and bamboo sticks were placed in bays during the fall to provide substrate to which spores could attach and grow; later, nets were strung between poles (Lobban & Harrison, 1994; Oohusa, 1993; Mumford & Miura, 1988). The seasonal crop was harvested and processed by hand, finely chopping the blades and spreading a slurry on bamboo mats to air dry, and the standard Japanese nori sheet became established (Mumford & Miura, 1988). Polypropylene and cremona fiber nets for growing *Porphyra* were developed and came into widespread use in the 1960's (Mumford & Miura, 1988). All cultivation originally was done in the intertidal where periodic drying at low tides helped to kill competing organisms. When it was discovered that continuous immersion did not harm larger blades, floating rafts were developed, thus allowing cultivation in deeper offshore areas (Oohusa, 1993; Mumford &

Miura, 1988). The most important scientific development in this century was the discovery of the conchocelis phase (Drew, 1949) and control over the production of conchospores (Kurogi, 1959; Kurogi & Hirano, 1956).

Production of nori in Asia is supported by a high level of research on genetics, physiology, biochemistry and food value (Mumford & Miura, 1988; Noda & Iwata, 1978). Other recent research has focused on strain selection, alternative methods of propagation, and the causes and control of fungal and bacterial diseases (Lobban & Harrison, 1994; Xin, 1991; Saga, 1990; Mumford & Miura, 1988). Nori is one of the highest valued fishery or mariculture products in the world, with an annual retail value of around $2 billion; Japan produces about 60% of the world's nori, but China and Korea also produce significant amounts (Lobban & Harrison, 1994). Nori worth at least $25 million annually is exported to the U.S. where the market is mostly in Asian restaurants and Asian and natural food stores. Annual consumption of nori in the U.S. was slightly less than 1 sheet per capita in the early 1990's, compared to per capita consumption of around 80 sheets in Japan and Korea, and the U.S. market is far from saturation (Merrill, 1993).

In contrast to the long history of *Porphyra* mariculture in Asia, interest in and research on *Porphyra* mariculture on the North American Pacific coast has been

substantial only in the last 30 years (Merrill, 1993; Bergdahl, 1990; Waaland *et al.*, 1986; Freeman, 1985). *Porphyra* has been cultivated on a pilot scale in Washington state and British Columbia using both local and Japanese species (Mumford, 1987; Waaland *et al.*,1986; Byce *et al.,* 1984), but efforts to establish commercial mariculture there have been stalled by opposition to permitting and lack of financing (Lobban & Harrison, 1994; Dickson, 1992; Freeman, 1985).

Research on *Porphyra* mariculture in southeast Alaska

Southeast Alaska is an ideal prospective location for *Porphyra* mariculture, with pristine coastal waters, many sheltered bays and inlets, over 25 native species and a state aquatic farm permit system. State law prohibits the importation of non-native species, so the development of species native to Alaska is important. The research project in which I participated during this thesis project was the first research on cultivation requirements for native species and strains of *Porphyra* in Alaska. The key project objective was to grow several species of *Porphyra* through their life cycle in the laboratory and gain control over the production of conchospores for the seeding of nori nets. The species were all indigenous to the region and had been previously identified as having acceptable properties, such as flavor, color and texture, for edible nori production (Lindstrom, 1993; Waaland *et al.*, 1986).

Research objectives and development of laboratory methods

An important objective of the *Porphyra* mariculture study in southeast Alaska was to determine tolerance limits and optima in physical ecological conditions for the growth of the gametophytes. My thesis research was directed toward this objective, along two different but related pathways. I first wanted to know how the distribution and growth of *Porphyra* was affected by physical factors in the marine environment of southeast Alaska. Besides being of ecological interest, knowledge of *in situ* growth limits and requirements is also essential for decisions about location, physical set-up and seasonal timing of production for future mariculture activities. Ecological and physiological effects identified or suggested in these studies would be then investigated further in controlled laboratory experiments. Laboratory experiments were designed to investigate the effects of salinity, nutrients, and irradiance on early growth of *Porphyra* blades. Many preliminary trials were necessary, however, to create cultures of juvenile blades of consistent quality under controlled conditions that simulated natural conditions to the extent possible, and to choose response variables that could be measured reliably and accurately. This process also included modifying experimental procedures to work with microscopic *Porphyra* blades, and identifying factors and levels of greatest interest and relevance. Much of the value of this research lies in the development of methods for laboratory

experiments with microscopic and very small *Porphyra* blades, methods that could be applied to the study of other small seaweeds.

Species used in this study

Porphyra abbottae was studied in the field surveys, and *Porphyra torta* was used for laboratory experiments. *Porphyra torta* and *Porphyra abbottae* are indigenous to the outer coast of Southeast Alaska and are considered sibling species based on similar morphology and genetics (Lindstrom & Cole, 1993). *Porphyra torta* appears during winter months in the high intertidal, while *Porphyra abbottae* appears in the same or similar locations during the spring in the mid-intertidal zone (Lindstrom & Cole, 1993; Conway & Cole, 1977). In both species the maturation and release of conchospores are precisely timed by photoperiod and temperature (Lindstrom & Cole, 1993; Waaland *et al.*, 1990; Waaland *et al.*, 1987). In laboratory cultures from Washington state, *P. abbottae* released conchospores following a change to long daylength (16 hrs light: 8 hrs dark) at low temperature and irradiance (Waaland *et al.*, 1990). Gametophytes of this species appear on beaches in Washington and British Columbia in spring, becoming reproductive in May through June, and are absent in fall and winter (Conway & Cole, 1977). In laboratory cultures of *P. torta* from Washington, conchospore release followed a change to short daylength (less than 12 hrs light: greater than 12 hrs dark) at moderate temperature and low irradiance (Waaland *et al.*, 1990). *Porphyra torta* gametophytes appear on British Columbia and

northern Washington shores from late November until April or May, and are absent during the summer and early fall (Conway & Cole, 1977). In our laboratory, *P. torta* was the more easily manipulated species, at least with respect to obtaining conchospores. *Porphyra abbottae* is the preferred subsistence species in southeast Alaska, partly because it is more abundant and can be gathered during the spring when the weather is better (Betts, 1991). However, *P. torta,* with its similar morphology and highly palatable taste and texture, should be as well suited to mariculture production as its sibling species.

Environmental factors included in study

Even a casual observer will notice the very specific spatial distribution patterns of *Porphyra abbottae* and *Porphyra torta.* They are present on rocky shores with moderate exposure along the outer coast, and generally do not penetrate far into inside waters. Each species forms dense, nearly monospecific patches in a band at a specific tidal elevation.

Temperature is an important determinant in the geographic distribution of many temperate and high latitude algal species (Kain & Norton, 1990). Temperature is more uniform and warmer in winter on the outer coast of southeast Alaska. In inside waters, the fresh water input and cold interior air lower water temperature in winter. Summer surface water temperatures may fluctuate widely, with cooling

in some areas from high volumes of cold glacial meltwater and heating from insolation in sheltered areas during prolonged sunny periods. But the seasonal effects of temperature on the initiation and growth of *Porphyra* gametophytes are already known (Waaland *et al.*, 1990), and temperature had no significant effect on growth or pigmentation in laboratory-cultured *P. abbottae* blades (Hannach, 1989; Hannach & Waaland, 1989). Highest growth rates for conchocelis of *P. pseudolinearis* and *P. torta* occurred at 7°C, and for *P. abbottae* at 11°C (Stekoll *et al.*, 1999). Temperature was considered in analyzing field survey data, but was not included as a factor for study in the laboratory experiments beyond keeping it constant and within the natural range for this region.

Salinity fluctuations cause osmotic stress and changes to ion concentration gradients in seaweeds, which in turn can have major effects on photosynthesis and respiration, and cell damage if extreme (Lobban & Harrison, 1994). Species diversity in algae usually declines with salinity along a geographical salinity gradient (Kain & Norton, 1990; Kautsky & Kautsky, 1989). On the other hand, many red algae are tolerant of fairly wide ranges of salinity, and adaptation to reduced salinity has been demonstrated (Kain & Norton, 1990). Conchocelis of *Porphyra abbottae* grew best at salinities from 20 to 30 ppt while conchocelis of *P. torta* and *P. pseudolinearis* grew best at 30 ppt. Conchocelis growth in all three species was inhibited at or below 15 ppt (Lin, 1999; Stekoll *et al*, 1999).

Salinity was a factor of primary interest in the laboratory blade growth experiments.

Growth-limiting nutrients in seaweeds include nitrogen, phosphorus, carbon and iron (Lobban & Harrison, 1994). Carbon, in the form of bicarbonate, is the most important nutrient for seaweeds, but would normally be limiting in natural seawater only where it is diluted by fresh water or possibly in intensive culture areas (Kain & Norton, 1990). In this study, bicarbonate levels were measured in local seawater and tested at comparable levels in factorial experiments involving salinity. Nitrogen is the nutrient most often limiting to seaweed growth (Kain & Norton, 1990; Lobban & Harrison, 1994). Effects of nitrogen on growth and pigmentation have been demonstrated with cultured *Porphyra abbottae* blades (Hannach, 1989; Hannach & Waaland, 1989), but not with *P. torta*. Many laboratory growth experiments use media containing nitrogen levels far exceeding those in the marine environment. In this study, nitrogen levels were monitored throughout the year in local seawater, and ecologically relevant levels were used in experiments. Seaweeds can store nitrogen in various forms and use reserves for growth during the summer period when nitrogen is very low (Lobban & Harrison, 1994). The possibility of nitrogen storage in *P. torta* blades was also investigated.

Water motion is intimately connected with nutrition in seaweeds, for flow over the plant surface greatly increases the supply of available nutrients and rates of diffusion (Kain & Norton, 1990). Water motion enhanced growth of *Porphyra abbottae* blades at all nutrient levels in culture, with the greatest effect at the lowest nutrient level (Hannach & Waaland, 1989), but was associated with reduced pigment concentration (Hannach, 1989). Strong tidal currents are beneficial to growth of seaweeds, but heavy wave action can be damaging (Lobban & Harrison, 1994; Kain & Norton, 1990). Since the effects of water motion have already been investigated with *P. abbottae* and are generally well known, water motion was not tested as an independent factor in this study, but it was provided to experimental cultures as an aid to spore dispersal and growth. In the field surveys, the degree and nature of water motion was a factor that varied among the field study sites.

Although the biotic factors, competition and herbivory, are highly important in structuring algal communities in nature (Kain & Norton, 1990; Santelices, 1990; Kautsky & Kautsky, 1989), they were outside of the scope of this study and not included as factors for investigation. However density (Harper, 1977) in natural *Porphyra* beds and in cultures may affect growth of individual plants and confound analysis of other factors, so density was investigated separately.

Selection of response variables for experiments

I estimated both relative abundance and size of individual blades in field surveys of *Porphyra abbottae*. Abundance provides information about net production of *Porphyra* at a site, while size and growth of individual blades are indicators of physiological condition. Photosynthesis is potentially a good indicator of instantaneous growth and physiological condition. Determining change in oxygen concentration in closed containers was effective for measuring photosynthesis in larger field-collected blades, but unsuitable for fine measurements on very small blades in the laboratory without specialized equipment (Geider & Osborne, 1992), so after much effort I had to abandon the method. Measures of average growth rate involve differences in size or weight measurements over a time period. Microscopic blades could not be weighed, but their flat, thin shape made accurate surface area measurements possible with digital image analysis. To characterize growth over a longer time period, and determine if rates changed over time, I made repeated measurements on the same cultures, but sampled individual blades randomly within those cultures. I also used phycoerythrin concentration as an indicator of physiological status. Phycoerythrin is the most abundant photosynthetic pigment in *Porphyra* and possibly serves as a nitrogen reserve compound (Hannach, 1989; Amano & Noda, 1987).

Statistical methods

Sampling with quadrats along transects or at selected points, either randomly or systematically, is common practice among researchers studying algae in the field (Lobban & Harrison, 1994). Obtaining unbiased samples that are representative of the population as a whole is difficult since most algae, including *Porphyra*, have clustered or patchy distribution patterns and extreme local variation in abundance (Lobban & Harrison, 1994). Alternative sampling methods were investigated in this study. Double sampling designs, which make use of auxiliary information, can greatly improve the efficiency and accuracy of estimates at all levels (Thompson, 1992). Multistage sampling and the use of natural habitat units (Hankin, 1984) provides a possible way to handle the large-scale variation in *Porphyra* abundance along a coastline.

In laboratory growth experiments, repeated measurements were made on the same cultures in order to monitor growth and changes in growth rate over a several-week period. While only a single outcome per unit is measured in a typical factorial design, in longitudinal studies change can be studied directly since repeated observations are made on the same unit (Diggle *et al.*, 1994). Longitudinal studies differ from time series analyses in that they usually include a larger number of units, can accommodate various factorial designs and cover a shorter time period. Special statistical methods are required because the repeated

measurements on the same individual or unit are not independent (Diggle *et al.*, 1994). Several methods of repeated measures and longitudinal data analysis were considered in this study.

Growth models for *Porphyra* and other seaweeds are rare to nonexistent. Most researchers assume that growth over a short time period is exponential (Kain & Norton, 1990; Hannach & Waaland, 1989), and use the relative growth rate formula,

$$RGR = (\ln w_t - \ln w_{t0})/ (t - t_0),$$

where w_t is plant weight at time t and w_{t0} is plant weight at time zero (t_0). The assumption of exponential growth is not necessarily valid. If growth is not truly exponential, the relative growth rate equation gives an estimate of average growth rate which depends on the time interval used and the amount of measurement error or noise (Parchevsky & Parchevsky, 1998). A cubic spline regression has been used to model instantaneous growth rates; it is mathematically complex but was very sensitive to short term responses to environmental fluctuations in data from microplankton and from the red alga *Gracilaria* (Parchevsky & Parchevsky, 1998). Fitting growth curves to biological data rather than *vice versa* (Parchevsky & Parchevesky, 1998) and fitting growth curves to large numbers of individual plants (Weiner, 1995) are ideas that would allow powerful new analyses of the influence of environmental factors on

physiological and population processes. This study only touched upon the need for such methods. Development of new methods for analysis of growth responses in *Porphyra* or other seaweeds would be a highly worthwhile goal for future studies.

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CHAPTER 2

SEASONAL AND ENVIRONMENTAL VARIATIONS IN THE GROWTH AND ABUNDANCE OF *PORPHYRA ABBOTTAE* IN SOUTHEAST ALASKA AND IMPROVED SAMPLING METHODS[1]

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Chapter 2

Seasonal and environmental variations in the growth and abundance of *Porphyra abbottae* in southeast Alaska and improved sampling methods

Abstract

The leafy annual gametophytic phase of *Porphyra abbottae* (Krishnamurthy) (Rhodophyta) is a dominant member of rocky intertidal communities along the outer coast of Southeast Alaska; it is historically and currently an important subsistence food, and a potential species for commercial mariculture. Seven sites were surveyed from April through July 1995 in Sitka Sound, Cross Sound and Chatham Strait. Peak abundance occurred in May or June, depending on location and possibly on water temperature. High abundance, in terms of percent cover, was associated with high water motion. The sites with largest blades were not necessarily those with the highest abundance, but sites with large blades had strong tidal current or moderate wave action. Although certain relationships between abundance, size and physical parameters were suggested, lack of precision in abundance and mean blade weight estimates and inability to isolate effects of specific physical factors in the environment prevented statistical testing of relationships. Better methods of estimating abundance and mean weight estimates were sought for future studies. Two double-sampling methods were tested and found to be efficient in improving the precision of estimates while minimizing sampling effort and cost. A multistage sampling design is proposed

to estimate abundance over larger areas and permit statistically valid between-site comparisons.

Introduction

Porphyra abbottae, a dominant mid-intertidal alga occurring on moderately exposed rocky shores of the northeastern Pacific ocean, has been studied as a candidate for commercial mariculture in Washington and British Columbia (Bergdahl, 1990; Mumford & Miura, 1988; Waaland *et al.*, 1986). It is also a traditional subsistence food for coastal peoples from Washington to Alaska, and is currently harvested and processed in a traditional manner in at least 29 southeast Alaska communities (Betts, 1991). No surveys or estimates of abundance of *P. abbottae* have been conducted in southeast Alaska, and some subsistence users are concerned about the possibility of commercial harvest of *P. abbottae* and the pressures it would put on this resource. On the other hand, there is interest in *Porphyra* mariculture in the region; among those expressing interest are some southeast Alaska Native organizations (Lindstrom, 1993a). Since *P. abbottae* has not been cultivated commercially, and has never been cultivated in Alaska, gaining an understanding of its natural distribution, abundance, seasonality and response to environmental factors in Alaska is a necessary first step in developing the species for mariculture.

Environmental factors affecting the growth and abundance of marine algae include substrate, irradiance, photoperiod, temperature, emersion and dessication, salinity, nutrients, inter- and intraspecific competition and grazing (Kain & Norton, 1990). *Porphyra abbottae* has a wide geographical distribution, from Washington state to the Commander Islands (Lindstrom & Cole, 1993); there is some evidence of interpopulation genetic variation (Lindstrom, 1993b). It occupies a well-defined zone in the mid-intertidal, and precise environmental cues, including temperature and photoperiod, regulate reproduction (Lindstrom & Cole, 1993; Waaland *et al.*, 1990). Nutrients in combination with water motion, irradiance and temperature influenced the growth and condition of juvenile *P. abbottae* blades in controlled culture experiments (Hannach, 1989; Hannach & Waaland, 1989). The responses of natural populations to environmental conditions such as temperature, salinity, nutrients and water motion and the factors controlling the horizontal distribution of *P. abbottae* have not yet been investigated.

In situ abundance estimates of seaweeds and other marine plants have been used to compare abundance of species between several areas (Fillit, 1995; Naito & Russell, 1989; Zavodnik, 1987), to study seasonal changes in growth and reproduction (Schoschina *et al.*, 1996; Fillit, 1995; Gunnarsson & Ingolfsson, 1995; Hansen, 1977), to examine community structure (Jagtap, 1996; Ballesteros, 1991; Kautsky & Kautsky, 1989), to test hypotheses about zonation and geographical distribution (Carter & Anderson, 1991; Santelices, 1990; Foster, 1982), and to assess the impacts of environmental change

(Hawkins & Hartnoll, 1983) and commercial harvest (Chopin *et al.*, 1992). One study used an estimate of fractal dimension to compare the habitat size and complexity provided by several types of seaweed in the intertidal community (Gee & Warwick, 1994). Primary production of seaweeds has also been estimated and compared between species or areas (Fillit, 1995; Ballesteros, 1991, 1989; Naito & Russell, 1989; Zavodnik, 1987). Typically, rectangular quadrats are located systematically or randomly along a transect (Jagtap, 1996; Gunnarsson & Ingolfsson, 1995; Lobban & Harrison, 1994; Chopin *et al.*, 1992; Hawkins & Hartnoll, 1983; Foster, 1982). Sampling must be representative of the population or community and mathematically random, or at least unbiased, as in systematic samples along a transect; both requirements are difficult to achieve given the irregularities of the terrain and the vegetation (Lobban & Harrison, 1994). Abundance has been estimated visually as the percent cover of a given species within a quadrat (Littler & Littler, 1985), as biomass by destructively sampling all the algae within a quadrat (DeWreede, 1985), or by counting individuals (Lobban & Harrison, 1994; Hawkins & Hartnoll, 1983). The method chosen must be appropriate to the type of algae being measured and the question being asked. For example, widely spaced individuals can be easily counted, but percent cover would be difficult to determine, while in a turf or dense bed, counting individuals may be impossible (Lobban & Harrison, 1994). Less commonly, individual plants have been sampled for length and weight measurements (Schoschina *et al.*, 1996), or individually tagged for growth monitoring (Ballesteros, 1989; Hansen, 1977).

In situ abundance estimation of aquatic plants should be useful for a wide range of purposes, including ecological studies, environmental monitoring and resource management. Demonstration of statistically significant comparisons or trends, however, is difficult because of high local variation and patchiness at all scales (Lobban & Harrison, 1994) and the high level of background noise caused by the many unmeasured variables in the environment (Gunnarsson & Ingolfsson, 1995; Hawkins & Hartnoll, 1983; Foster, 1982). Often the sampling methods described in phycological literature are simplistic and inadequate to handle the complexities of the intertidal environment, but the use of more powerful sampling methods is rare.

In this study, the field surveys and sampling of *Porphyra abbottae* in southeast Alaska were conducted to examine variation at different sites in abundance and growth in relation to physical factors including temperature, salinity and water motion. The data were analyzed for precision, sample size and cost efficiency, and two alternative sampling methods using auxiliary data or stratification were tested. A sampling design is proposed which could improve abundance estimates and permit statistical comparisons between sites.

Study sites

Three areas in southeast Alaska were chosen for field surveys: Sitka Sound, Cross Sound and Chatham Strait (Fig. 2-1). Each area was known to have abundant *Porphyra abbottae* and a local subsistence harvest. Because of their geographical

locations the areas were assumed to differ at least in wave exposure, tidal current, and water temperature.

Sitka Sound

Three sites were selected in Sitka Sound. John Brown's Beach is on the northern tip of Japonski Island (57°03'N, 135°22'W). It is a gradually sloping beach with gravel in the high intertidal, near the back of Sitka Sound and facing west with moderate wave exposure. Chaichei Islands (57°04'N, 135°28'W) are a group of small islands in the middle of Sitka Sound. The study site is a steep bedrock incline with full southwestern exposure to the Gulf of Alaska and heavy wave action, especially during storms. Pirate Cove (56°59'N, 135°22'W) is on the southwest corner of Sitka Sound near the mouth. The study site is in a small cleft in the rocky shore. Although the shoreline is exposed to the Gulf of Alaska to the west, the many offshore rocks and reefs break the swell and the site is protected at low tide by a rock ridge, receiving only moderate surge except during big storms.

Cross Sound

Two sites were selected on the George Islands (58°12'N, 136°23'W) near Elfin Cove. The North George Island site is a gravel bottomed pocket in a northwest-facing cove with exposure to Cross Sound and moderate to heavy surge. The South George Island site is on the south side of Seiner's Pass, a narrow pass

between two of the islands with strong tidal current. It is protected by the islands from wave exposure.

Chatham Strait

Two sites were visited near Angoon (57°30'N, 134°35'W), but data were collected only from Gaa-Dax. Gaa-Dax is a roughly level shelf along Chatham Strait about two miles north of the entrance to Mitchell Bay, with full exposure in three directions to the Strait. The other site was inside Mitchell Bay, with very strong tidal currents but protection from wave exposure.

Materials and methods

Sampling and measurements

Porphyra abbottae was the dominant alga at all study sites in a band between 0 to 2.5 m above mean lower low water, broken horizontally into discrete patches according to beach topography and substrate. Typically, *Alaria maginata* dominated the zone below *P. abbottae* and *Fucus gardneri* dominated the zone above it and the margins of the *P. abbottae* zone were distinct. A horizontal transect was placed through the center along the entire length of the most prominent patch of *Porphyra abbottae* at each site. The patch containing the transect was mapped by measuring the distance from the transect line to the top and bottom of the patch and the tidal elevation at 5 m intervals along the transect. Ten random points were chosen along the transect, and a rectangular quadrat

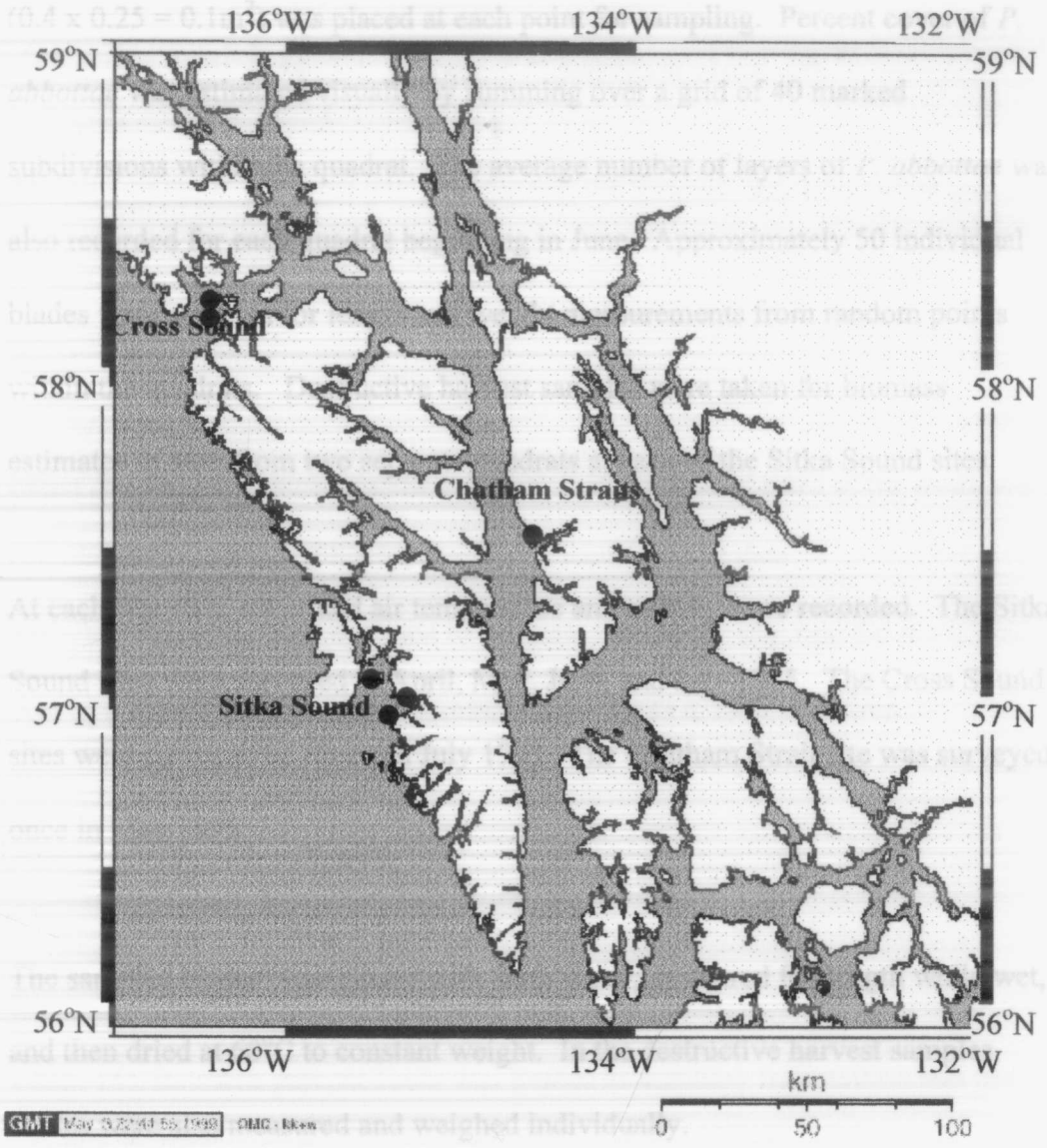

FIG. 2-1. Map of northern southeast Alaska showing location of study sites.

(0.4 x 0.25 = 0.1m^2) was placed at each point for sampling. Percent cover of *P. abbottae* was estimated visually by summing over a grid of 40 marked subdivisions within the quadrat. The average number of layers of *P. abbottae* was also recorded for each quadrat beginning in June. Approximately 50 individual blades were sampled for length and weight measurements from random points within the quadrats. Destructive harvest samples were taken for biomass estimates in May from two separate quadrats at each of the Sitka Sound sites.

At each site visit, water and air temperature and salinity were recorded. The Sitka Sound sites were surveyed in April, May, June, and July 1995. The Cross Sound sites were surveyed in June and July 1995. The Chatham Strait site was surveyed once in May 1995.

The sampled blades were rinsed with fresh water, measured for length while wet, and then dried at 60°C to constant weight. In the destructive harvest samples, blades were also measured and weighed individually.

Data analysis

The mean percent cover, sample standard deviation (s), and standard error of the mean (se) were calculated for each site and date. The coefficient of variation ($cv = se \div$ mean) was also calculated and used to estimate the sample size (n) required to give $cv \leq 10\%$, according to the formula

$$n = \left[\frac{1}{N} + \left(\frac{X}{\gamma} \right)^2 \right], \text{ where } X = \text{desired } cv(y) \text{ and } \gamma = \frac{s}{y}$$

The area of the *P. abbottae* patch containing the transect was determined and the population size N was designated as the total area in m^2 divided by a single quadrat area (0.1 m^2).

Mean dry weight, sample standard deviation, and standard error of the mean were calculated for individual blades sampled at each site and date. The coefficient of variation was calculated and used to estimate the sample size and the associated cost in terms of sampling and processing time for $cv \leq 10\%$, as above.

Alternative sampling methods

Double sampling for ratio estimation

Biomass was estimated using double sampling ratio estimation (Thompson, 1992; Chap. 14), in which percent cover was treated as auxiliary information and per quadrat dry weight as the variable of interest. Data from Pirate Cove and Chaichei Islands in May were used to test this method. Notation and equations are listed in Fig.2-2. The first sample consisted of the percent cover estimates from 12 quadrats; the second sample, a subsample of the first sample, consisted of the total dry weight estimates from 2 quadrats. Estimates of total biomass and average biomass per unit area, with associated variances, were obtained.

To further test this method, a synthetic data set was constructed and sampled, as follows. A map of the Pirate Cove sampling area and the transect was drawn to scale, and approximate percent cover levels to the nearest 10% (*i.e.* 0-10%, 11-20%, etc.) were assigned to areas of the map based on the May field data and notes. A first sample of n' quadrats was selected using randomly numbered coordinates (distance along transect, distance from transect) for percent cover estimates. The approximate percent cover level was read from the map and a random number 0-9 was assigned to the second place. A subsample of n quadrats was then selected and their total dry weight was estimated by summing individual dry weights from a random sample with replacement of the pool of all blades sampled at Pirate Cove and Chaichei Islands in May. The number of dry weights summed for total per quadrat weight was the % cover x 120 (estimated mean number of blades per quadrat). The sample size n' and subsample size n were determined from the formula for minimizing variance at a set cost (Fig. 2-2). The total cost C, in terms of sampling and processing time per site, was assumed to be 960 minutes; the cost per quadrat c' for percent cover estimates was set at 10 min and the cost per quadrat c for total dry weight estimates was set at 120 min.

Double sampling for stratification

A revised estimate of mean dry weight per blade with associated variance was obtained for each site sampled in May using double sampling for stratification (Thompson, 1992; Chap.14; Cochran, 1977). Notation and equations are listed in

Fig. 2-3. A first sample of *n'* blades was sorted into 5 strata according to length. A second sample of *n* blades was selected by stratified random sampling from the first sample. The population mean dry weight was estimated from the weighted mean dry weights for each stratum, and the estimated variance of mean was calculated. The sample sizes *n'* and *n* were based on the number of blades for which data was available, and were not the ideal sizes for the method. Optimal sample sizes were calculated using the sample variances of the field samples (Fig. 2-3). The calculations were based on a total cost of 480 min of laboratory time, with cost per blade of sorting to length strata at 0.5 min and cost per blade of obtaining a dry weight at 5 min. Time for collection of samples in the field was not considered in these estimates.

N = total number in population

n' = number of units in first sample

n = number of units in second sample or subsample

y_i = variable of interest, observed in second sample only

x_i = auxiliary variable, observed in first and second samples

$r = \dfrac{\sum_{i=1}^{n} y_i}{\sum_{i=1}^{n} x_i}$, for y_i and x_i in second (smaller) sample

$\hat{\tau}_x = \dfrac{N}{n'} \sum_{i=1}^{n'} x_i \qquad \hat{\tau}_r = r\hat{\tau}_x$

$$\hat{\text{var}}(\hat{\tau}_r) = N(N-n')\frac{s^2}{n'} + N^2\left[\frac{n'-n}{n'n(n-1)}\right]\sum_{i=1}^{n}(y_i - rx_i)^2$$

C = total cost of sampling (ignoring fixed costs)

c' = cost per unit for first sample $\qquad$ c = cost per unit for second sample or subsample

$C = c'n' + cn$

For given C, min. variance when $\dfrac{n}{n'} = \sqrt{\dfrac{c'}{c}\dfrac{s_r^2}{(s^2 - s_r^2)}}$

FIG. 2-2. Notation and equations used in double sampling ratio estimation method (Thompson, 1992; Chap.14).

N = total number of units in population

n' = number of units in first sample

n = number of units in second sample (subsample of first sample)

$W_h = \frac{N_h}{N}$ = proportion of population in stratum h, for $h = 1,....,L$

n_h' = number of units in first sample observed to be in stratum h

$w_h = \frac{n_h}{n}$ = proportion of first sample in stratum h (unbiased estimator of W_h)

n_h = number of units in second sample selected from stratum h in first sample

y_{hi} = variable of interest measured in second sample

$\bar{y}_h = \sum_{i=1}^{n_h} y_i / n'$, sample mean in stratum h

$\bar{y}_d = \sum_{h=1}^{L} w_h \bar{y}_h$, estimator of population mean

s_h^2 = sample variance in stratum h

$$\hat{\text{var}}(\bar{y}_d) = \sum_{h=1}^{L}\left(\frac{n_h' - 1}{n' - 1}\right)\frac{w_h s_h^2}{n_h} + \frac{1}{n'}\sum_{h=1}^{L} w_h(\bar{y}_h - \bar{y}_d)^2$$, estimator of variance of $\bar{y}_d$

(for large population, where $N >> n'$ and $N \to \infty$)

C = total cost of sampling (ignoring fixed costs)

c' = cost per unit for first sample

c = cost per unit for second sample or subsample (assuming equal cost per sample in all strata)

$$C = c'n' + cn'\sum v_h w_h, \text{ where } v_h = s_h\sqrt{\frac{c'}{c(s^2 - \sum w_h s_h^2)}}$$

FIG. 2-3. Notation and equations used in double sampling for stratification method (Thompson, 1992; Chap.14, Cochran, 1977; p.331-334).

Results

Field surveys and analysis

Abundance, using percent cover estimates, varied with season with peak abundance in mid-May to mid-June. Abundance varied from site to site across all three survey areas (Fig. 2-4). Taking into account the number of layers of *P. abbottae* in the cover estimates (3-D cover) changed the scale but not the relative differences in abundance between sites and months. A subsistence harvest had occurred only one to two weeks prior to sampling at Gaa-Dax, which could explain the low abundance observed there in May. Fully reproductive blades and the beginning of senescence were noted at all sites in the month following peak abundance.

There was minimal variation in salinity between sites and months. Surface water temperature increased between April and July. Temperature was similar between sites although slightly lower at the Cross Sound sites than at the Sitka Sound sites in the months when it was measured (Table 2-1).

At most sites and most months, the variation in the percent cover estimates was high. In order to test for differences between sites, a coefficient of variation of 10% or less was desired. This level was approached at the sites of highest abundance during peak months of the growing season, but in most cases the *cv*

was over 20%. Sample sizes of greater than 50 would have been required in many of these cases to reduce the *cv* to 10% (Table 2-2).

There were seasonal trends in the dry weights of individual *P. abbottae* blades, similar to those for percent cover. No clear relationship between site and mean blade weight persisted across all months and mean individual blade weight and percent cover did not vary in any direct relationship. The peak dry weights for the sites occurred in different months, but were of similar magnitude for several of the sites (Table 2-3). The blade dry weights were highly variable, with coefficients of variation as high as 50%, except for Pirate Cove in May with a very large sample size. In order to reduce variance, extremely large sample sizes would be needed in most cases, greatly raising the cost in sampling and processing time for each site (Table 2-3).

Alternative sampling methods

Double sampling for ratio estimation

Although a direct comparison was not possible between the original percent cover abundance estimates and the double sampling ratio estimates of biomass, the alternative method produced a biomass estimate for Pirate Cove with reasonable precision. The variance in the Chaichei Island biomass estimate was high, but the sample sizes for both estimates were very small. The estimates obtained from the two simulations had close agreement and acceptable precision. The optimal

sample size estimates were similar for all cases, and would enable sampling at a reasonable cost in time and effort per site (Table 2-4).

Double sampling for stratification

A direct comparison was possible between the original blade dry weight estimates and those made using double sampling for stratification. In all cases, the means were similar between the two methods, and the variance was reduced when double sampling for stratification was used (Fig. 2-5). Although a large primary sample of blades is needed for length stratification, the secondary sample for individual weights is reduced to about half the number needed to achieve the same level of precision using simple random sampling, and the cost in sampling time is similarly reduced (Fig. 2-6).

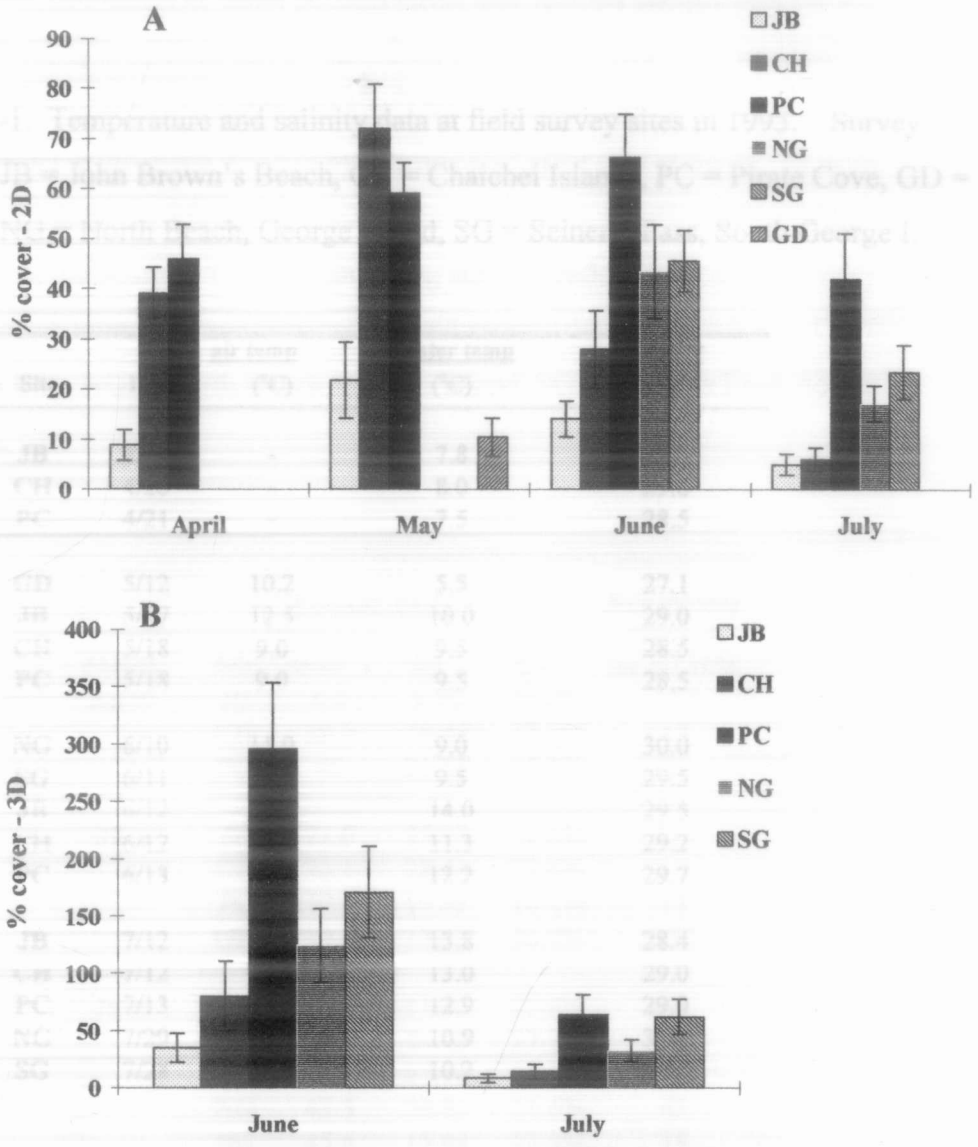

FIG. 2-4. Percent cover of *Porphyra abbottae* April – July, 1995. Data are from 5 locations in Southeast Alaska, with 2-dimensional, flat estimates of canopy layer (A), and 3-dimensional flat estimates of cover times average no. of layers (B). Sites in Sitka Sound: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove. Sites in Cross Sound: NG = North Beach, George Island, SG = Seiner's Pass, South George Island. Site in Chatham Straits: GD = Gaa Dax, north of Angoon. Bars represent mean of n = 10 cover estimates $\pm$ SE (0.1 m^2 quadrat).

TABLE 2-1. Temperature and salinity data at field survey sites in 1995. Survey sites are: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove, GD = Gaa Dax, NG = North Beach, George Island, SG = Seiner's Pass, South George I.

Month	Site	Date	air temp (°C)	water temp (°C)	salinity (ppt)
April	**JB**	4/19	-	7.8	29.5
	CH	4/20	-	8.0	29.8
	PC	4/21	-	7.5	28.5
May	**GD**	5/12	10.2	5.5	27.1
	JB	5/17	12.5	10.0	29.0
	CH	5/18	9.0	9.5	28.5
	PC	5/18	9.0	9.5	28.5
June	**NG**	6/10	11.0	9.0	30.0
	SG	6/11	19.2	9.5	29.5
	JB	6/12	13.5	14.0	29.5
	CH	6/12	12.8	11.3	29.2
	PC	6/13	11.8	12.2	29.7
July	**JB**	7/12	13.2	13.8	28.4
	CH	7/12	16.0	13.0	29.0
	PC	7/13	13.5	12.9	29.0
	NG	7/29	10.5	10.9	30.1
	SG	7/28	15.0	10.2	30.5

TABLE 2-2. Estimates of mean percent cover and optimal sample size for *P. abbottae* by simple random sampling. Percent cover is mean and standard deviation (SD) of $n = 10$ quadrats (0.1 m^2) from 1995 samples. Coefficient of variation (CV) is the mean divided by the standard error (not shown). Sample size in final column is number of quadrats that would be required to lower the coefficient of variation to 10% or less, using simple random sampling. Survey sites are: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove, GD = Gaa Dax, NG = North Beach, George Island, SG = Seiner's Pass, South George Is.

		Percent cover			Sample size required
Month	**Site**	**mean**	**SD**	**CV**	**for cv≤10%**
April	**JB**	8.8	9.75	35.0%	117
	CH	39.2	16.48	13.3%	17
	PC	46	21.85	15.0%	22
May	**JB**	21.9	23.98	34.6%	114
	CH	72.1	28.40	12.4%	15
	PC	59.2	19.48	10.4%	11
	GD	10.4	13.08	36.2%	153
June	**JB**	14.0	11.45	25.8%	65
	CH	27.9	24.49	27.7%	74
	PC	66.3	27.58	13.1%	17
	NG	43.5	30.03	21.6%	43
	SG	45.6	19.64	13.5%	18
July	**JB**	4.9	6.59	42.4%	168
	CH	6.0	7.45	39.5%	143
	PC	42.1	29.12	21.8%	46
	NG	17.2	11.14	20.3%	38
	SG	23.5	17.24	23.0%	49

TABLE 2-3. Mean dry weights of individual *Porphyra abbottae* blades. Data are mean and standard deviation (SD) for *n* samples. Coefficient of variation (CV) is the mean divided by the standard error (not shown). Sample size in second column from right is number of samples that would be required to lower the coefficient of variation to 10% or less, using simple random sampling. The sampling cost is the time in hours required to collect and process all samples, assuming 5 min per sample (final column). Survey sites are: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove, GD = Gaa Dax, NG = North Beach, George Island, SG = Seiner's Pass, South George I.

			Dry wt.(g)				
Month	**Area**	**n**	**Mean**	**SD**	**CV**	**n for cv ≤ 10%**	**Cost in hours**
April	**JB**	13	0.181	0.1434	58.1%	1997	166
	PC	50	0.083	0.1380	63.2%	439	37
May	**JB**	26	0.381	0.4597	34.9%	316	26
	CH	132	0.205	0.1311	15.4%	313	26
	PC	289	0.227	0.0620	6.4%	120	10
	GD	22	0.349	0.3381	35.5%	277	23
June	**JB**	50	0.096	0.0772	41.0%	840	70
	CH	71	0.106	0.1096	37.2%	981	82
	PC	57	0.498	0.5054	18.9%	204	17
	NG	50	0.184	0.2740	40.3%	811	68
	SG	46	0.209	0.3027	38.8%	692	58
July	**JB**	65	0.035	0.0261	57.7%	2166	181
	CH	63	0.082	0.0723	41.2%	1072	89
	PC	56	0.247	0.1741	22.5%	285	24
	NG	49	0.128	0.1527	43.5%	927	77
	SG	37	0.494	0.6083	25.9%	249	21

TABLE 2-4. Biomass estimates of *Porphyra abbottae* by double sampling ratio method. Data are from Pirate Cove (PC) and Chaichei Islands (CH) in May 1995, and two simulations from a synthesized data set (PC sim.1, PC sim.2). Percent cover is mean ± SE of *n'* primary samples; biomass is ratio estimate ± SE from the *n'* primary samples and *n* secondary samples. Optimal sample sizes were calculated based on cost and minimum variance (see Methods section); numbers in parentheses for PC and CH sample sizes are actual sample sizes used. Cost in hours is based on 10 min per primary sample and 120 min per secondary sample.

			biomass		optimal sample sizes		
site	area (m^2)	% cover	total (kg)	per unit area (kg m-2)	% cover n'	biomass n	cost (hrs)
PC	150	64 ± 9.0	35.2 ± 17.3	0.234 ± .115	17(12)	7(2)	16.8
CH	165	75 ± 6.1	35.6 ± 4.3	0.216 ± .026	26(12)	6(2)	16.3
PC sim.1	150	69 ± 5.2	28.1 ± 2.9	0.187 ± .019	21	7	17.5
PC sim.2	150	69 ± 5.2	26.7 ± 2.3	0.178 ± .016	21	7	17.5

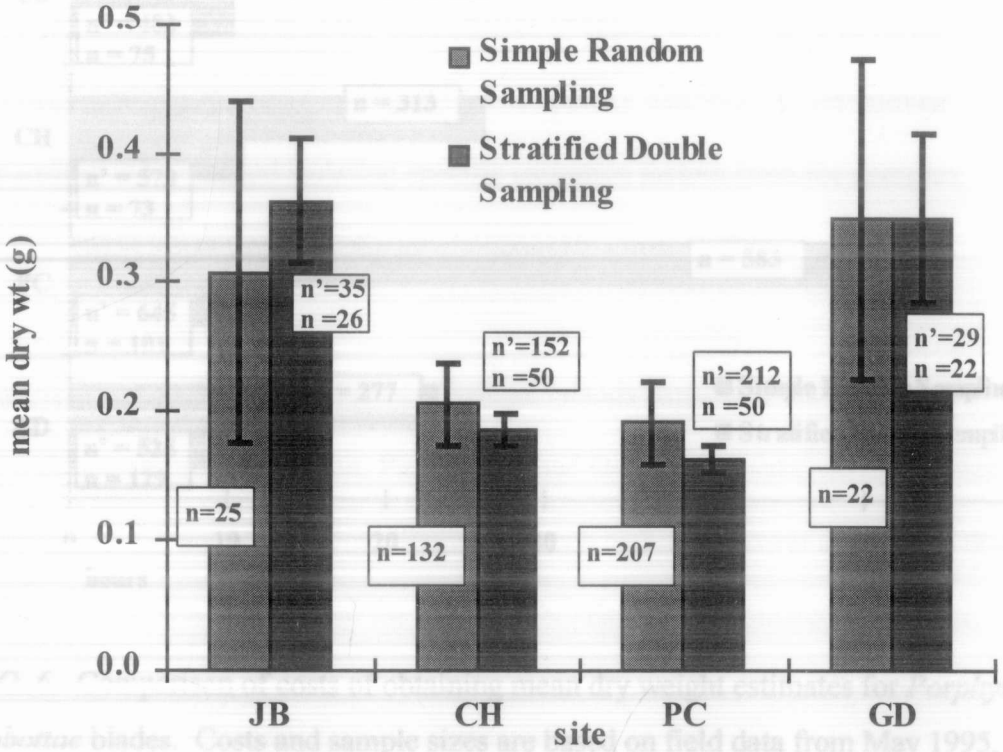

FIG. 2-5. Comparison of simple random sampling and stratified double sampling mean dry weight estimates for *Porphyra abbottae* blades. Bars for simple random sampling estimates indicate mean ± SE of *n* samples (*n*-size shown on chart); bars for stratified double sampling indicate weighted mean ± SE of *n* samples selected from *n'* samples stratified by length (*n'* and *n* shown on chart). Blades sampled from: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove, GD = Gaa Dax; May 1995.

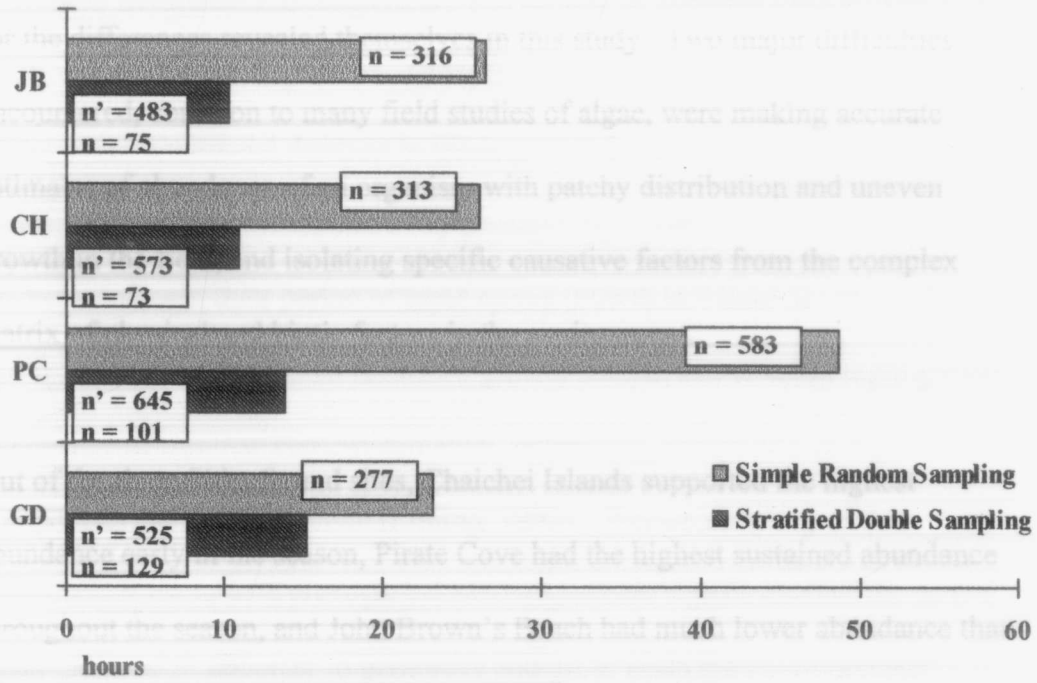

FIG. 6. Comparison of costs of obtaining mean dry weight estimates for *Porphyra abbottae* blades. Costs and sample sizes are based on field data from May 1995, using simple random sampling and stratified double sampling. Bars are estimated time in hours to collect and process samples, at 5 min per dry wt estimate and 0.5 min per blade for stratification by length in the double sampling method. Sample sizes for simple random sampling are those sufficient to give a coefficient of variation ≤ 10%; optimal sample sizes for stratified double sampling are calculated by assuming a maximum total cost and minimizing variance (see Methods section). Estimates based on samples from: JB = John Brown's Beach, CH = Chaichei Islands, PC = Pirate Cove, GD = Gaa-Dax; May 1995.

Discussion

Site-specific differences in seasonal abundance and individual blade size of *Porphyra abbottae* were evident from the field data, but no simple explanations for the differences revealed themselves in this study. Two major difficulties encountered, common to many field studies of algae, were making accurate estimates of abundance of an organism with patchy distribution and uneven growth in the field, and isolating specific causative factors from the complex matrix of physical and biotic factors in the environment.

Out of the three Sitka Sound sites, Chaichei Islands supported the highest abundance early in the season, Pirate Cove had the highest sustained abundance throughout the season, and John Brown's Beach had much lower abundance than the other two sites throughout the season. Abundance, as estimated by percent cover, did not correspond directly with individual plant size. The highest mean blade weights occurred at John Brown's Beach and Gaa-Dax in May, when percent cover was lowest at those sites. South George Island had the second highest individual dry weight for all months and sites in late July, when abundance had declined at all sites. Blade condition was associated with both abundance and individual dry weight. In general, dark green, reproductive blades with high photosynthetic output were associated with high weight and abundance (unpublished data).

The seasonal initiation of rapid blade growth in higher latitude marine algae is associated with an increase in irradiance above a critical level in early spring, while a decline in growth or the onset of senescence often coincides with summer maximum irradiance and temperature and decline in nutrients (Schoschina *et al.*, 1996; Gunnarsson & Ingolfsson, 1995; Ballesteros, 1991, 1989; Hansen, 1977). Endogenous circannual rhythms in blade growth have been discovered in some kelp species and are synchronized by photoperiod. These internal clocks cause the kelps to begin their period of rapid spring growth in winter or very early spring, anticipating the most favorable growth season, and to cease rapid growth in advance of summer nutrient depletion, redirecting energy into storage compounds for winter survival (Lüning, 1993). Precise coupling of the reproductive cycle with photoperiod and temperature signals enables the annual blade phase of *P. abbottae* to germinate and grow when the environmental conditions are the most favorable (Lobban & Harrison, 1994; Waaland *et al.*, 1990).

John Brown's Beach had the highest air and water temperatures in May through July, while water temperatures in Cross Sound remained lower than those in Sitka Sound late in the season. Possibly the lower temperatures are optimal for *Porphyra abbottae* or are associated with other beneficial conditions, such as upwelling or higher nutrient levels. A prolonged period of sunny weather in early May may have had a detrimental effect on the condition of *P. abbottae* at

John Brown's Beach, Gaa-Dax, and possibly Chaichei. Photoinhibition or uncoupling of the photosystems can occur when emersed blades are exposed to full sunlight (Lobban & Harrison, 1994). Temperature tolerances and optima are important in regulating local distributions in many red algae, along with irradiance at higher latitudes. Water temperature affects respiratory and photosynthetic activity, determining in part the compensation point and photosynthetic maximum, and influences growth rates (Lobban & Harrison, 1994; Kain & Norton, 1990). Although salinity is an important factor in determining the distribution of many red algae, salinity was nearly constant at 28-30 ppt at all sites in this study throughout the season.

Chaichei Islands and North George Island were the sites most exposed to heavy wave action, and although abundance was high at these sites, mean individual blade size was smaller. *Porphyra* has thin, flexible blades that can yield to wave motion, but excessive motion can cause tearing or removal of blades and stunted form, as stresses increase with length (Kain & Norton, 1990). John Brown's Beach and North George Island were the only sites having loose gravel and so would be subject to abrasion during big storms. The sites supporting the largest blades, Pirate Cove and South George Island, were more protected from wave battering, but a strong tidal current passed along the South George Island site. Blades at the Mitchell Bay site off Chatham Strait (surveyed, but not included in the data) also grew very large and elongated in a strong tidal current. Moderate

water movement is beneficial to seaweeds, because the moving water brings in a constant supply of nutrients and gases and removes waste and silt. The water motion in a steady tidal stream presents the blade more perpendicularly to the incident light (Kain & Norton, 1990).

Disturbance such as wave action and stress such as low salinity, nutrients or light interact with interspecific competition in structuring algal communities. A higher level of disturbance prevents competitive exclusion by one species, but the highest level of species diversity is found where disturbance and primary productivity are at intermediate levels (Ballesteros, 1991; Kautsky & Kautsky, 1989). Many species of *Porphyra* are opportunistic, fast-growing and have high reproductive outputs, but are also relatively tolerant of emersion stresses, enabling them to colonize and persist, through their growing season, on cleared patches of rocky shores (Kain & Norton, 1990; Santelices, 1990). On the other hand, these species are probably less competitive with other species in calmer environments with higher species diversity, or when low salinity and/or low nutrient stress exists. Horizontal distribution of *Porphyra abbottae* along shorelines is likely controlled by an interaction of stress, especially salinity and nutrients, and competitive interactions with other algae. Requirements of the cryptic sporophyte, or conchocelis stage, may also play a role in determining horizontal distribution. Vertical zonation is likely controlled at the upper end by abiotic factors such as dessication and at the lower end by dominance of the larger,

space-holding kelps or perennial crusts and coralline algae (Lobban & Harrison, 1994; Carter & Anderson, 1991; Santelices, 1990; Foster, 1982).

Biomass estimates using percent cover as auxiliary data provide an efficient and potentially more useful method of estimating abundance. The percent cover method is quick and non-destructive, but accuracy is a problem; patchiness and the presence of multiple layers add complication. Furthermore, for physiology studies or stock assessment, biomass incorporates a measure of net production that is missing in cover estimates. Double sampling provides a reasonable compromise between the advantages of the percent cover method, and the greater accuracy of directly measuring abundance by weight. Since auxiliary data are used, the number of destructive samples needed is smaller, and the precision of the estimates is improved. The double sampling for stratification method was highly effective in improving estimates of mean dry weight of individual blades. Stratification by length is quick and easy, even with a large initial sample, the subsample that must be individually dried and weighed is much smaller, and precision is improved dramatically. The relationship between biomass and mean individual dry weight is not constant, and may be affected by density, a factor that must be taken into account when comparing growth between sites (Chopin, 1992; Kain & Norton, 1990).

Many commonly used methods of sampling and abundance estimation do not adequately address the complexity of intertidal algal communities. Many studies have used quadrats, placed haphazardly (Hansen, 1977), in spots with the densest cover (Naito & Russell, 1989), randomly (Jagtap, 1996), or systematically (Gunnarsson & Ingolfsson, 1995; Fillit, 1995; Chopin, 1992; Hawkins & Hartnoll, 1983). Percent cover has been commonly estimated (Gunnarsson & Ingolfsson, 1995; Ballesteros, 1991; Naito & Russell, 1989; Hawkins & Hartnoll, 1983; Foster, 1982), as well as biomass (Jagtap, 1996; Fillit, 1995; Ballesteros, 1991; Naito & Russell, 1989; Hansen, 1977) and sometimes primary productivity (Ballesteros, 1989). Growth has been estimated as a difference in biomass (Naito & Russell, 1989; Hansen, 1977) or difference individual length or weight (Schoschina *et al.*, 1996; Hansen, 1977) per unit time. Consideration of optimum sample size and alternative sampling methods which may be better suited to organisms with a clustered distribution (Thompson, 1992; Hawkins & Hartnoll, 1983) is rare in the literature on algal ecology.

For ecological studies or biomass and productivity estimates, a sampling design is needed that will handle patchiness or clustering and provide greater accuracy. A multistage sampling design could be applied to marine algae along a stretch of coastline. A given coastline is broken up into smaller units, which are sampled in the first stage, but rather than creating first stage units of equal length, as is commonly done, the area is broken into "natural habitat units" that vary in size

(Hankin, 1984). On typical southeast Alaska coastline, individual small islets or projecting rocky points separated by streams or gravel or sand beaches form natural habitat units for *Porphyra* or other algae that are abundant on the projecting rocky shores, but absent from stream mouths and beaches in between. The first stage units are mapped and measured, and then selected either by simple random sampling or with probability proportional to size. A strong correlation often exists between sampling unit size and abundance, when habitat units are similar in type and quality. The precision of the estimate is improved by taking advantage of this correlation using ratio estimation or selection with probability proportional to size in the first stage (Hankin, 1984). The variable of interest is estimated within the selected sampling units and projected over the entire sampling area according to the method chosen. In a three-stage design, a finer level of natural habitat units, such as the discrete bands or patches of *Porphyra* within the selected first stage unit would be designated and sampled in the second stage. In the third stage sampling for the variable of interest is conducted within the selected secondary units (Thompson, 1992; Chap13).

Estimation of size and growth of individual blades can provide information about the influence of environmental factors on physiological and population processes. A promising new approach of fitting growth curves to individual plants has been tested in terrestrial plants, which may permit, if many individuals are measured

over time, a large number of parameters to be accurately estimated and analyzed and different growth models compared (Weiner, 1995).

In conclusion, it is possible to obtain good estimates of biomass and mean individual weight using specialized sampling methods. Such estimates are good indicators of the quality of environmental conditions at a site in relation to the particular population of algae, but identifying specific factors that have positive or negative impacts remains a challenging task. It is likely the distribution and growth of *P. abbottae* results from the interaction of many factors, all highly variable in space and time. For mariculture purposes, the grower could simply select outplanting sites where wild *Porphyra* is abundant and healthy, or seek to reproduce such conditions to the extent possible at another location. For greater understanding of the ecology of *Porphyra*, improved sampling and estimation in the field needs to be combined with results from controlled laboratory and field experiments to sort out the most influential factors from the complex matrix of physical and biotic factors in the natural environment.

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CHAPTER 3

ECOLOGICAL PHYSIOLOGY OF *PORPHYRA*: EFFECTS OF DENSITY AND SUBSTRATE TYPE ON RECRUITMENT AND GROWTH OF *PORPHYRA TORTA* GAMETOPHYTES[2]

[2] Prepared for submission to the *Journal of Applied Phycology*.

Chapter 3

Ecological physiology of *Porphyra*: effects of density and substrate type on recruitment and growth of *Porphyra torta* gametophytes

Abstract

Germination of *Porphyra torta* (Krishnamurthy) (Rhodophyta) conchospores was dependent on initial density, with the highest germination rates at the intermediate density. Mortality occurred over time in experimental cultures regardless of initial density and blade size increased with the decrease in density, tending toward the –1.5 power self-thinning rule. Spores released over a variety of substrate types, including strands of Japanese nori net, fine Nitex net fabric, and glass microscope slides, settled in highest numbers on Nitex net with 60 μm mesh. Attention must be given to both density of settled conchospores and to substrate in experimental cultures of *Porphyra* blades, as well as in mariculture. Further study is warranted on the relationship of substrate and density to establishment and growth of the early life history stages of *Porphyra* gametophytes in natural populations.

Introduction

Mariculture of *Porphyra* has been practiced for over 300 years in Japan, China and Korea, and is supported today with a highly-refined body of scientific and practical knowledge. By contrast, interest in *Porphyra* mariculture on the Pacific coast of North America is only very recent, and the commercial potential of the genus is still

undeveloped (Lobban & Harrison, 1994). One of the challenges in the development of *Porphyra* mariculture in North America is to bring species native to the eastern Pacific coast into cultivation, as an alternative to introducing non-native species from Asia. Research at the University of Alaska has focused on gaining control of the life history of native Alaskan *Porphyra* species in culture, and experimentally defining environmental conditions required for optimum growth. In preliminary experimental cultures, the density at which juvenile *Porphyra torta* blades were grown and the substrate material used were identified as having possible confounding effects with other variables being tested.

Recent studies on the effects of density-dependent growth in marine algae have focused on natural populations, using sampling in intact stands (Flores-Moya *et al*, 1996; Cousens & Hutchings, 1983; Schiel & Choat, 1980) and in harvested areas (Chopin *et al.*, 1992), thinning and clearing of adult stands (Kendziorek & Stekoll, 1984; Reed, 1990b; Ang & DeWreede, 1992; Creed *et al,* 1996; Scrosati & DeWreede, 1998), and evaluation of recruitment and growth of juveniles on settling plates (Reed, 1990a; Ang & DeWreede, 1992; Kendrick, 1994; Creed *et al,* 1996; Andrew & Viejo, 1998). Observational studies have also been used to compare density-dependent growth in submerged freshwater plants to that in terrestrial plants and marine macroalgae (Duarte & Kalff, 1987). In terrestrial plants, it is established that intraspecific competition results in reduced growth at moderate density and increased mortality, or self-thinning, at high density (Yoda *et al.,*

1963; Harper & White, 1974; Harper, 1977; Gorham, 1979; Antonovics & Levin,1980; White, 1981). These general rules of density-dependent growth have been extended to marine macroalgae, with some modifications. In contrast to the controlled cultivation experiments and agronomic trials with terrestrial plants, many studies on natural stands of marine macroalgae introduced uncontrolled factors confounding the relationship between density and growth, such as genetic variability and natural selection (Antonovics & Levin, 1980), the interaction of and differential effects on different life-history stages (Kendrick, 1994; Creed *et al.*, 1996; Andrew & Viejo, 1998), patterns of dispersal and settlement of propagules in the marine environment (Roughgarden *et al.*, 1988; Reed *et al.*, 1988), substrate type and availability and the effect of microhabitat on juveniles (Reed, 1990a; Scrosati & DeWreede, 1998; Andrew & Viejo, 1998), and other ecological interactions such as herbivory (Antonovics & Levin, 1980; Reed, 1990b). Studies on experimental populations of marine macroalgae are most valuable in elucidating these relationships, but are less common than natural population studies (Creed *et al.*, 1998). Some applied studies on density-dependent growth have been conducted with cultivated *Porphyra* in Japan (Yoshida, 1972a,b), and practical knowledge guides the production of seeded nets at the correct density for nori cultivation (Noda & Iwata, 1978).

Few studies have examined the specific effects of substrate type on the attachment, survival and growth of macroalgae, although substrate has been implied as a factor affecting the survival and density of natural populations (Cousens & Hutchings, 1983;

Reed, 1990a; Brawley & Johnson, 1991). Optimal surface relief dimension in artificial substrates was investigated for naturally recruiting algae in an intertidal environment (Harlin & Lindbergh, 1977), and *Fucus* recruitment was compared between different groove dimensions on settling plates (van Tamelen *et al.*, 1997). *Porphyra torta* naturally grows on bare, exposed bedrock. If developed for mariculture, it would be grown on the nori cultivation nets currently used in Japan. However, it is common to grow experimental cultures of juvenile *Porphyra* and other algae on glass microscope slides, due to ease of observation and measurement. In preliminary experiments, *P. torta* blades grown on glass slides showed excessive or abnormal rhizoid development. Since it was unknown whether glass slides as substrate adequately simulated either natural field conditions or mariculture conditions, several other substrate materials were tested.

The purpose of this investigation was to determine whether density-dependent growth or self-thinning of juvenile *Porphyra torta* blades occurs in culture, and whether substrate type has any effect on successful attachment and germination of the conchospores.

Materials and Methods

Culture Material

Porphyra torta conchocelis (culture PtCH13a), cloned from a single carpospore from a blade collected at Chaichei Islands, Alaska (20 April, 1995; 57°4'N, 135°28'W), was induced to release conchospores, from which experimental cultures were grown.

Density Effects Experiment

Three levels of initial spore density were produced in batch cultures by varying the amount of conchocelis used to "seed" the cultures. Each batch consisted of 24 half microscope slides (25 mm x 37.5 mm) stuck with silicon grease to the bottom of a clear, 2.4 L flat-bottom container (15 cm x 15 cm) with Provasoli's enriched seawater medium (Provasoli, 1968) at ¼ concentration (PES/8), seeded with 0.002, 0.02, and 0.2 g (fresh weight) of conchocelis filaments, respectively, for the low, medium, and high density cultures. The batch cultures were incubated for 3 weeks in conchospore releasing conditions, under a photoperiod of 8 hrs light: 16 hrs dark (8:16 L:D) at 40-45 μmol $m^{-2}s^{-1}$ photon flux density (PFD) and11°C, with water motion at 30 rpm provided by 3-D rotary platforms. Spores were released after one week, and the majority of spores had germinated after 3 weeks. At 3 weeks, the half microscope slides were transferred to individual 60 mm x 15 mm petri dishes with 10 ml enriched seawater (PES/8), and the individual cultures were incubated under 16:8 L:D at 45 μmol $m^{-2}s^{-1}$ PFD and11°C, without water motion.

Initial counts of attached spores, sporelings and juvenile blades were made immediately following transfer to individual culture dishes, by randomly sampling 12 areas from a grid overlaid on each slide, in each of which all ungerminated spores and all blades (including germinating spores) were counted separately in one field of view (2.54 mm^2). To test the effectiveness of the seeding method in providing three distinct initial densities,

recruitment densities were estimated as the mean total count (spores, sporelings and blades) per unit area. To test for density effects on germination, the germination rates for the three density groups were compared as the average ratio of blades to total attached spores and blades. Differences in recruitment and in germination rates between treatment groups (low, medium and high density) were analyzed using one-way analysis of variance. The count data were square-root transformed to meet the assumptions for one-way analysis of variance. Multiple comparison tests were conducted using the Tukey method (S-Plus 4, 1997).

At 2, 4½, 7½, and 12 weeks following transfer to individual culture dishes, 12 cultures were selected at random from each treatment group for counts and blade area measurements. Videotape recordings were made of microscopic images of all blades found along each long edge and three lengthwise transects on each slide. Blade counts were normalized as number of blades cm^{-2}. Blade surface areas were measured digitally from the recordings at a later date using Optimas 4.0 image analysis software, calibrated with a micrometer scale recorded on each sampling date. The relationship of blade size to density was analyzed for each treatment group using linear regression of $\log_{10}$ blade area against $\log_{10}$ density on the combined data from all four sampling dates. The linear regression assumptions and fit were tested using exploratory data analysis (S-Plus 4, 1997; Neter *et al.*, 1983). Blade surface area, rather than weight, was used as a measure

of size to permit non-destructive sampling; blade surface area was found to be significantly correlated with weight ($r = 0.897$, $P = 0.0001$).

Substrate Experiment

Six materials were tested for attachment and germination of conchospores: Japanese nori net strands, double-strand and single-strand; Nitex brand plankton net of mesh size 60, 20 and 10 μm; and plain glass microscope slides. All materials were sterilized in boiling water, except the double nori net strand, which was treated with hot tap water only due to its tendency to unravel in boiling water. After sterilization, 9 pieces of each material were placed into each of two 2.4 L seeding tubs. Each tub was "seeded" with 0.25 g fresh weight of *P. torta* conchocelis. Cultures were grown for 3 weeks in conchospore releasing conditions, under 8:16 L:D at 50-60 μmol $m^{-2}s^{-1}$ PFD and11°C, in sterile seawater enriched with Guillard's f/2 (Guillard & Ryther, 1962), and with water motion provided by 3-D rotary platforms at 30 rpm. Conchospore release began within 4 days, and was 90-95% complete after 10 days. After 17 days, the majority of conchospores had attached to the substrate and begun to germinate. After 3 weeks, the 18 substrate pieces per substrate type with attached spores and sporelings were transferred to individual culture dishes and placed in growth conditions under 16:8 L:D at 75-100 μmol $m^{-2}s^{-1}$ PFD and 11°C, in enriched sterile seawater. After 3 days in growth conditions, attached spores and blades were counted in 6 randomly selected fields of view (2.54 mm^2) in each of 12 cultures randomly selected from the 18 total cultures per

substrate type. Counts were normalized to number (attached spores, germinating spores, and juvenile blades) · mm^{-2}. The surface area of the nori net strands was estimated as the half-cylindrical surface, $\pi \cdot d/2 \cdot l$. Differences in density of settled spores and blades between substrate types were evaluated using one-way analysis of variance. Data were $\log_e$ transformed to meet the assumptions for one-way analysis of variance. Multiple comparison tests were conducted using the Tukey method (S-Plus 4, 1997).

Results

Density effects experiment

Initial densities of settled spores were positively dependent on conchocelis "seeding" weight ($P<<0.001$), and differed significantly between all treatment groups (Fig.3-1). The effect of density on germination was significant ($P<<0.001$), with the highest average germination rate in the medium density group. The germination rates were significantly different between the low and medium density groups and between the medium and high density groups, but germination rates were not significantly different between the low and high density groups (Fig. 3-2).

As blades grew and increased in surface area, there was a corresponding decrease in density in all treatment groups (Fig. 3-3). Linear regression of $\log_{10}$ transformed blade area against $\log_{10}$ transformed density yielded slopes of –1.47, -1.39, and –0.99 for the low, medium and high density groups, respectively. However, the variance of the slope

estimates was very high and consequently the power for significance tests was much less than 0.5, too low to allow meaningful inferences (Neter *et al.*, 1983). In testing whether the fit and residuals of the linear model conformed to the assumptions for regression (Neter *et al.*, 1983; S-Plus 4, 1997) several problems were revealed. The most serious of these was that the model explained less than half of the variation in the data (r-f plots; S-Plus 4, 1997). In the medium density treatment, the error terms were non-normally distributed. In both the high and medium density treatments, the distribution of the response ($\log_{10}$ blade area) was distinctly bimodal.

Substrate experiment

The number of settled spores per unit area varied according to substrate type, in the following order: Nitex net, 60μm mesh > Nitex net, 20μm mesh > nori net double strand > Nitex net, 10μm mesh > glass slides > nori net single strand (Fig. 3-4). Density of settled spores was significantly different ($P<<0.001$) between all substrate types except between Nitex 20μm and nori net double strand, Nitex 10μm and nori net double strand, and glass slides and nori net single strand. The development of the juvenile *Porphyra torta* blades could not be followed past the recruitment stage in this experiment, due to severe bacterial contamination in some of the cultures.

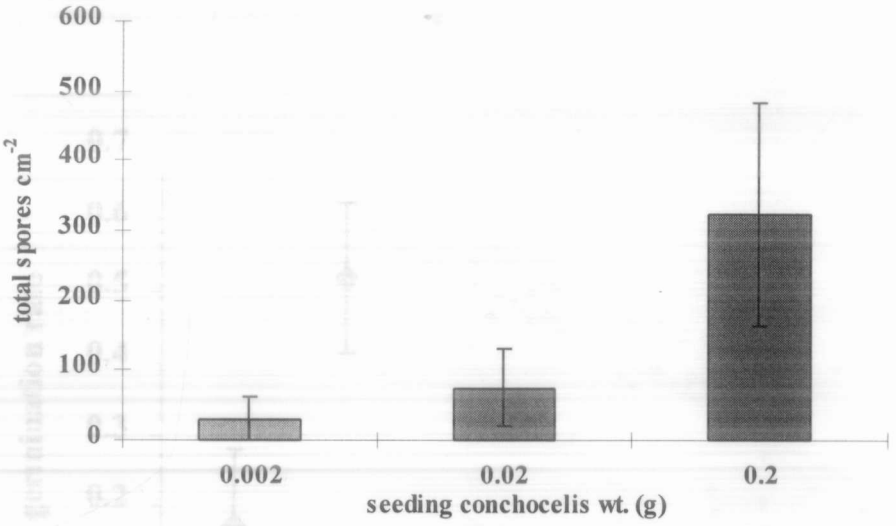

FIG. 3-1. Recruitment, or initial density, of *Porphyra torta* conchospores settled onto glass slides in culture. Three separate batch cultures were "seeded" with 0.002, 0.02, 0.2 g. of fertile conchocelis filaments; slides were separated into individual culture dishes and counts were made after germination began. Data are mean $\pm$ SE of all n=24 cultures in each treatment group. All means were significantly different from each other (Tukey method, P = 0.05).

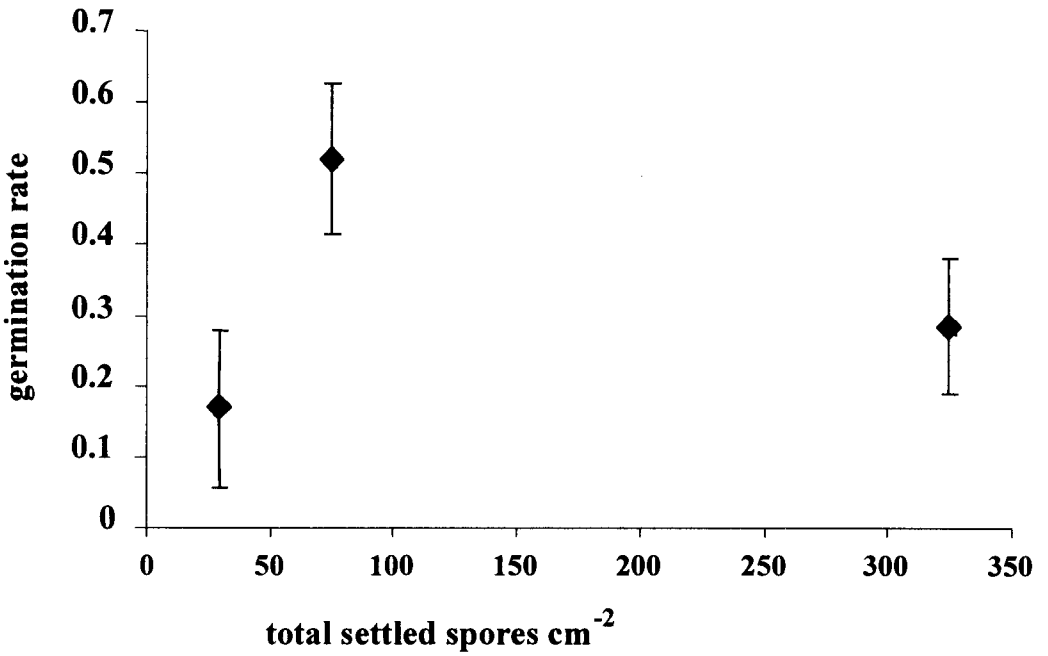

FIG. 3-2. Germination rates vs. recruitment levels of *Porphyra torta* conchospores "seeded" at three different levels: A – 0.002 g., B – 0.02 g., C – 0.2 g. (fresh weight fertile conchocelis filaments, per batch of 24 slides). Germination rates are the proportion of germinated spores to total spores. Counts are mean $\pm$ SE of n=24 cultures from each treatment group. Means of treatment group A (low density) and treatment group C (high density) were not significantly different (Tukey method, P = 0.05).

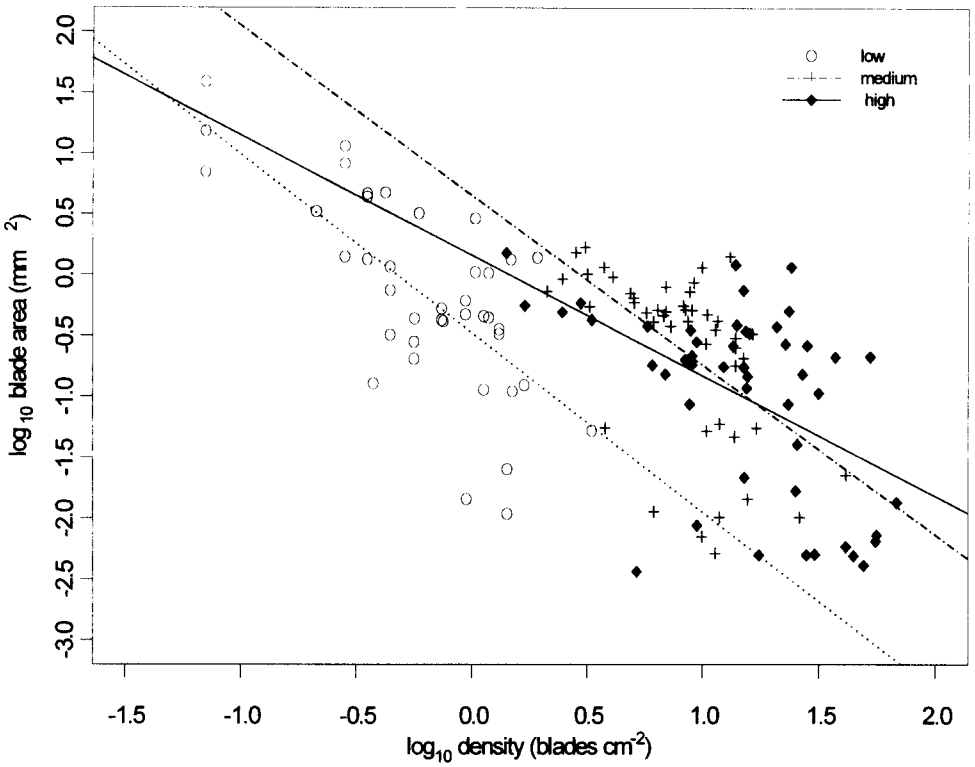

FIG. 3-3. Relationship of blade surface area to blade density over 10 wk experimental period. Blade counts and surface area measurements were made on n=12 randomly-selected units from each treatment group at approximately 2-1/2 week intervals. Data are counts and average blade area for each unit at all sampling dates. Least-square linear regression fits are shown for: low initial density (circles, dotted line); medium initial density (crosses, dashed line); and high initial density (diamonds, solid line).

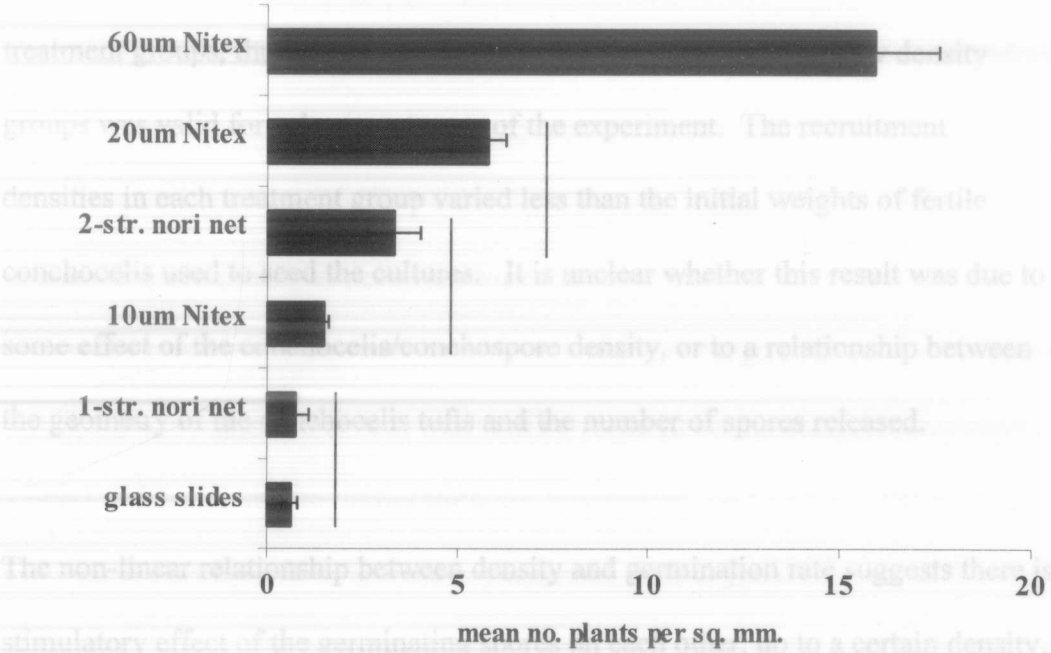

FIG. 3-4. Settlement and attachment of *Porphyra torta* conchospores on six different substrates (vertical axis labels). Data are mean counts ± SE from n=12 cultures per substrate type. Bars connected by vertical lines were not significantly different (Tukey method, P = 0.05).

Discussion

Recruitment density was significantly different between each of the three treatment groups, thus the distinction between high, medium and low density groups was valid for subsequent parts of the experiment. The recruitment densities in each treatment group varied less than the initial weights of fertile conchocelis used to seed the cultures. It is unclear whether this result was due to some effect of the conchocelis/conchospore density, or to a relationship between the geometry of the conchocelis tufts and the number of spores released.

The non-linear relationship between density and germination rate suggests there is some stimulatory effect of the germinating spores on each other, up to a certain density. In terrestrial plants, positive effects of density on germination have sometimes been observed (Harper, 1977). Germination and salinity tolerance in *Plantago coronopus* were enhanced when seeds were sown in clumps, with optimum clump size determined in part by salinity and the nature of the substrate (Antonovics and Levin, 1980). Positive density-dependence in germination may be caused by the release of germination stimulators, such as respiratory CO_2 in closed cultures (Harper, 1977) or changes in water movement and ion and water absorption (Antonovics & Levin, 1980). Although positive density-dependence in germination necessarily results in intense competition at later stages of growth, the benefits to the early life stages, including stabilization of the microenvironment and exclusion of

other species, may give denser initial populations an overall adaptive advantage (Antonovics and Levin, 1980). An interaction between crowding and dessication was observed in *Mazzaella cornucopiae*, a turf-forming, clonal red alga that occupies the rocky upper intertidal in British Columbia. Natural stands are dense, resulting in reduced net photosynthesis due to shading when fully immersed, but in protection from desiccation and bleaching during emersion (Scrosati and DeWreede, 1998). In some brown algae, a minimum spore density is necessary for successful recruitment, in part because fertilization is induced by a pheromone with a maximum effective range of about 1 mm. Even after fertilization, a minimum density may still be required for successful recruitment (Reed, 1990a). Although neither desiccation nor fertilization were factors relevant to the cultures of juvenile *Porphyra torta* in these experiments, little is yet known about the mechanisms of density-dependence in germination, so neither environmental nor chemical factors can be ruled out.

In density-dependent plant growth, individual growth rate and size are negatively dependent on density at medium initial densities, and the law of constant final yield (carrying-capacity) applies. At high initial densities density-dependent mortality, or self-thinning occurs (Antonovics & Levin, 1980). Applicability of the –1.5 power law has been confirmed for natural stands of *Ecklonia radiata*, *Sargassum sinclairii*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Saccorhiza polyschides*, and *Chordaria flagelliformis* (Cousens and Hutchings, 1983), for experimental cultures of *Laminaria digitata* and *Fucus serratus*

(Creed *et al.*, 1998), and for the slow-growing annual kelp *Phyllariopsis purpurascens* (Flores-Moya *et al.*, 1996). For *Asparagopsis armata*, a bushy, low intertidal red alga, self-thinning proceeded at a higher than predicted rate during the early growth stages, but ceased as the population reached reproductive stage (Flores-Moya *et al.*, 1996). Harvest of *Chondrus crispus* in Nova Scotia resulted in higher densities of small, non-reproductive blades and a decrease in biomass and in density of reproductive blades, compared to undisturbed beds (Chopin *et al.*, 1992). Similarly in Bristol Bay, Alaska, thinning to simulate harvest of *Fucus* for herring-roe-on kelp resulted in high recruitment in the small size classes (Kendziorek & Stekoll, 1984). In cultivated Japanese *Porphyra tenera*, yield (total dry weight per unit length of *hibi* string) was positively dependent on density during the early stage of growth, but for mature fronds greater than 5 cm in length, average individual dry weight was negatively dependent on density, and yield was independent of density (Yoshida, 1972a,b).

The juvenile, non-reproductive *Porphyra torta* blades in this experiment clearly experienced mortality in all cultures over time, and growth and density appeared to follow the –1.5 power relationship (Yoda *et al.*, 1963; Harper, 1977). The –1.5 power slope of $\log_{10}$ blade area to $\log_{10}$ density could not be confirmed because the linear model only explained 50% or less of the relationship between mean blade area and density over time. A large number of blades in the high and medium density treatments remained very small throughout the experiment, as could be seen in the bimodal distribution of mean blade

areas. Since the regressions could not be statistically tested, it is not possible to determine whether mortality was density-dependent or density-independent (Harper, 1977). If some spores or germlings were not viable and would have died anyway, then mortality was density-independent. But even if density-independent mortality occurs, stress from crowding can weaken individuals and increase their chance of death (Harper, 1977).

Initial density and survival is affected by the substrate on which *Porphyra* conchospores settle and attach. A relationship between spore size and size of interstitial spaces is likely. *Porphyra torta* conchospores are about 10μm in diameter. The best substrates had interstitial spaces large enough to accommodate several conchospores. By contrast, substrates with no interstitial spaces or spaces less than or equal to the diameter of one conchospore were poorer substrates with respect to recruitment. Normal rhizoid development occurred where fine net strands were of an equivalent size to and allowed attachment of rhizoids. Preferential settlement and growth on surfaces with larger-size texture has been demonstrated for other species of macroalgae. In an experiment using discs divided into quadrants coated with 0.1-0.5, 0.5-1.0 and 1.0-2.0 mm particles or left smooth, most of the thirteen species of macroalgae recruiting onto these surfaces in the field showed a preference for the larger particle sizes (Harlin & Lindbergh, 1977). *Fucus* in Prince William Sound, Alaska had higher recruitment and survival in deeper-grooved acrylic settling plates, possibly because of the protection grooves give germlings from dessication, grazing and sweeping of the adult canopy (van Tamelen *et al.*, 1997). Larger-

size textures provide greater surface area for settlement of spores and provide resistance to desiccation and refuge from grazers (Kain & Norton, 1990). Interstitial spaces may cause formation of small eddies which facilitate spore settlement, and also can trap detritus, which may influence algal settlement through increasing electrical charge from the adsorbed organic particles, or possibly encouraging the growth of a microbial (bacterial, microalgal) film that is required for the attachment of germlings in some species (Harlin & Lindbergh, 1977). Development was also affected by substrate in *Corallina officinalis*, in which only spreading encrustations appeared on smooth surfaces, while erect, articulated forms grew on rough surfaces (Harlin & Lindbergh, 1977). In outplanting experiments with the fucoid alga *Pelvetia fastigiata*, survival of embryos was highest under a canopy of adult *Pelvetia*, probably due to protection from exposure to heat and light from the sun during tidal emersion, yet recruitment in nature occurs mostly within red algal turfs and only rarely under adult canopies. The bare rock surface beneath the adult canopy probably does not provide enough protection from water motion for settling embryos; grazing may also be higher under a canopy (Brawley & Johnson, 1991). In other experiments, the canopy of adult *Fucus gardneri* provided protection to embryos from heat and dessication but swept many of the small plants off the substrate (van Tamelen, 1997). In this study, environmental stresses such as desiccation during emersion, grazing and high water motion were absent, but in the presence of limited, constant water motion, *Porphyra* conchospores settled preferentially on surfaces with greater relief. The non-motile conchospores appeared to settle in greatest numbers where they became trapped, such as within the slightly larger

mesh openings. These spaces also collected detritus and had high levels of bacterial growth. Whether detritus and bacteria had any effect on conchospore settlement is unknown, but the high bacterial growth within the larger mesh sizes appeared to be detrimental to the later growth of juvenile blades. In the high energy intertidal environment, effects of substrate on settlement of spores would be expected to have even greater importance, while high water motion may be expected to reduce fouling with bacteria and competing algae.

Demonstration of density-dependent relationships in cultured *Porphyra* gametophytes and the effect of substrate type is important for future experimental work and the development of new species for mariculture. A larger range of initial densities and repeated measurements of individual blades are needed in future experiments on growth and density. The application of these results to ecological questions is tantalizing; little is known about the processes of conchospore release, attachment and germination in *Porphyra* under natural conditions, and in particular the question of how the non-motile spores become attached and survive even under heavy surf remains unanswered.

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CHAPTER 4

ECOLOGICAL PHYSIOLOGY OF *PORPHYRA*: RESPONSE OF *PORPHYRA TORTA* GAMETOPHYTES TO NITRATE, SALINITY AND INORGANIC CARBON IN CULTURE[3]

[3] Submitted to *Marine Biology*.

Chapter 4

Ecological physiology of *Porphyra*: response of *P. torta* gametophytes to nitrate, salinity, and inorganic carbon in culture

Abstract

The leafy gametophytic phase of *Porphyra torta* (Krishnamurthy) (Rhodophyta), a candidate species for mariculture in Alaska, grows in winter and early spring along the outer coast of Southeast Alaska. To answer questions about environmental limits to growth, and under what conditions this species could be cultivated outside of its natural range, growth and phycoerythrin concentration in juvenile gametophytes were compared under combinations of three physical factors that could be easily manipulated in culture. Twelve combinations of nitrate (88 and 2.2 μmol L^{-1}), salinity (30, 15, 7.5 ppt), and inorganic carbon (2.0, 1.0 and 0.5 mmol L^{-1}) were tested, with the highest level of each factor considered "normal" with respect to the natural conditions under which *P. torta* grows. In three successive recovery experiments, blades grown for 2, 4 and 6 weeks in the experimental media combinations were returned to normal media for 10 days. Low nitrate had a significant, negative effect on both growth and phycoerythrin concentration. Blades exposed to low nitrate regained growth rates and pigment concentrations comparable to or higher than those in unexposed blades, when returned to normal growth medium. Reduced blade growth was observed under low salinity, but could not be statistically confirmed, while inorganic carbon had no observed effect on blade growth. Neither salinity nor inorganic carbon had a significant effect on

phycoerythrin concentration. Growth of *Porphyra torta* gametophytes in nature occurs in winter and early spring when nitrogen is usually not limiting. Salinity stress combined with other, unknown factors may prevent growth of *P. torta* in inside coastal areas.

Introduction

Porphyra torta is one of at least 70 *Porphyra* species known worldwide (Stiller & Waaland, 1993), a winter-early spring inhabitant of the upper intertidal zone of rocky beaches along the northeastern Pacific (Lindstrom & Cole, 1993; Waaland *et al.,* 1990; Conway & Cole, 1977). *Porphyra torta* and its spring-early summer counterpart, *P. abbottae*, called *laak̲'usk* (black seaweed), are part of the traditional diet of the Tlingit people of Southeast Alaska, (Betts, 1991). *Porphyra* is one of the world's oldest and most important maricultural crops; cultivars have been selected and developed over a three hundred year period in Japan, China and Korea (Lobban & Harrison, 1994). These countries are the world's leading producers, and support research on strain selection, genetics, and tissue culture (Mumford & Miura, 1988; Ikenoue & Kafuku, 1992). *Porphyra* has been cultivated on a pilot scale in Washington state and British Columbia (Byce *et al.,* 1984; Mumford, 1990, 1987; Waaland *et al.,* 1986), but its potential as a commercial crop in North America remains undeveloped (Merrill, 1993). *Porphyra torta* is among several west coast North American species identified by Waaland *et al.*

(1986) for their commercial potential, and by Lindstrom (1993) for commercial potential in Alaska.

The life cycle of *Porphyra torta* is biphasic with a foliose, haploid gametophyte and a filamentous, diploid sporophyte, or conchocelis (Burzycki & Waaland, 1987; Conway & Cole, 1977; Drew, 1949). Maturation and release of conchospores require a short day photoperiod of 12 hours or less (Waaland *et al.*, 1987). This stimulus is probably coupled with the environmental requirements for blade growth in *P. torta* (Waaland *et al.*, 1990).

Porphyra torta is restricted to outer coastal areas where salinity is stable throughout the year at about 30 ppt. In inside waters salinity undergoes seasonal fluctuations to 10 ppt or less. The role of salinity in the distribution of *P. torta* is unclear. Species of the mostly subtidal red algal genus *Gracilaria* from a variety of habitats tolerated a broad range of salinities in culture, indicating that salinity is not critical in determining distribution of *Gracilaria* (Bird & McLachlan, 1986). Complete osmoacclimation and adaptation over several generations between marine, estuarine, and freshwater environments has been observed in the genus *Bangia,* which is closely related to *Porphyra* (Hartog, 1972; Geesink, 1973; Reed, 1980, 1985; Sheath & Cole, 1980). Estuarine and upper intertidal algae generally have a broader tolerance to salinity (Kirst, 1989; Russell, 1987), and can often tolerate short-term changes in salinity without complete osmotic adjustment (Kirst, 1989, 1995; Reed,

1990). Photosynthesis and respiration are inhibited by severe osmostic stress in all algae, but the severity of stress depends on acclimation (Kirst, 1989). The dilution of inorganic carbon can have a greater effect than salinity on photosynthesis of seaweeds in hyposaline media (Ogata & Matsui, 1964; Hammer, 1968; Ogata & Schramm, 1971; Ohno, 1976).

Nutrients, especially nitrogen, are expected to affect both growth and quality of *Porphyra torta* blades. Nitrogen supply in coastal waters fluctuates strongly with season, and is correlated with seasonal growth patterns in algae (Hanisak, 1983). The gametophyte phase of *P. torta* occurs during the winter when nitrogen concentrations are high, but the feasibility of year-round cultivation needs to be determined. Most of the available dissolved inorganic nitrogen, primarily nitrate (NO_3^-) and ammonium (NH_4^+), is consumed by spring phytoplankton blooms in Alaska coastal waters (Ziemann *et al.*, 1989). Peak macroalgal growth occurs at the same season but is not a simple function of nitrogen availability, because macroalgae have many possible pathways of nitrogen uptake, utilization, and storage. Nitrogen beyond structural needs, such as DNA and membrane proteins, and physiological requirements, such as enzymes and photosynthetic pigments, can be stored; amino acids and phycobiliproteins serve this function in *Porphyra* and other red algae. These reserves are used for growth and physiological processes in times of nitrogen limitation (Naldi & Wheeler, 1999; Lobban & Harrison, 1994; Hwang *et al.*, 1987; Thomas & Harrison, 1985; Hanisak, 1983; Bird *et al.*, 1982). Color and concentration of photosynthetic pigments were used as indicators of nitrogen deficiency

and recovery upon experimental fertilization in *Porphyra yezoensis* (Amano & Noda, 1987), and as indicators of water column N availability using *Gracilaria* spp. (Horrocks *et al.*, 1995).

Porphyra torta can be reliably cultured through its life cycle, but the effects of specific environmental factors on blade growth and physiology are mostly unknown. The requirements of the critical early stage of blade growth will determine net seeding and outplanting strategies for mariculture (Hansen, 1983; Dawes, 1982). Blade growth was measured in field outplant trials on *P. torta* in Washington state (Waaland *et al.*, 1986), and effects of specific factors on growth of juvenile blades in culture were determined for *P. abbottae* (Hannach & Waaland, 1989; Hannach, 1989). This study examines the dependence of growth and phycoerythrin concentration in juvenile *P. torta* blades on nitrate, salinity, and inorganic carbon at a range of *in situ* levels, the possible interactions among these factors, and the ability of *P. torta* blades to recover from limitations in any of these factors.

Materials and Methods

Porphyra torta conchocelis (culture i.d. PtEI02a1) was cloned from a single carpospore from a blade collected on April 9, 1997 at Elovoi Island, Alaska (56°49'N, 135°24'W). A 0.025 g (fresh weight) tuft of free conchocelis filaments was fragmented and incubated under a photoperiod of 8 hrs light: 16 hrs dark

(8:16 L:D) at 90-110 μmol m^{-2} s^{-1} photon flux density (PFD) and 12°C. The culture medium was an artificial seawater base (AW; Harrison *et al.*, 1980) enriched with Guillard's f/2 (Guillard & Ryther, 1962). Water motion was provided by a Labline 3D rotator at 30 rpm. Spores were released in a single pulse after 3 1/2 weeks; germination and attachment were first observed one week later. Fine (20 μm) Nitex brand mesh was used as a substrate for the germinating blades, which were seeded in a single batch to achieve uniform settlement. During the germination and initial growth period, the medium used was enriched AW with nitrate equivalent to f/20 (other nutrients at f/2 level), changed weekly. Four weeks after spore release, individual mesh pieces with newly germinated blades attached were placed in separate 25 ml Erlenmeyer flasks containing 10 ml media at one of 12 experimental treatment combinations of salinity, bicarbonate, and nitrate. Four replicate cultures were grown in separate 25-ml Erlenmeyer flasks for each treatment. Three salinity levels (30, 15, 7.5 ppt) were made by diluting the AW base with distilled H_2O or distilled H_2O containing 2.0 mM L^{-1} $NaHCO_3$. Thus, within each salinity level bicarbonate was either kept constant at the full seawater concentration of 2.0 mM, or diluted (2.0, 1.0, 0.5 mM) in the same proportion as the salinity. To each of the six artificial seawater base mixtures, enrichment media containing nitrate at either 88 or 2.2 μM was added (equivalent to Guillard's f/20 and f/800 in nitrate; other nutrients at f/2 level). The combination with the highest level of each factor (salinity = 30 ppt, bicarbonate =

2.0 mM, nitrate = 88 μM) was considered the "normal" or control level. (Note that the two levels of bicarbonate, full and diluted, are actually the same when salinity = 30 ppt, but were treated as separate levels in order to preserve a balanced factorial design.) The individual cultures were grown under the same controlled environmental conditions as provided for spore release, listed above. The medium for each treatment group was changed every week, after sampling.

The date at which blades were separated into individual culture flasks and experimental treatments started was considered week 0; a random sample of 3 blades per culture flask was harvested on this date and once per week for the following 6 weeks (weeks 1-6), for measurement and phycoerythrin (PE) analysis. Microscopic images of the sampled blades were recorded on videotape, and blade surface areas were later measured digitally from the images, using Optimas 4.0 image analysis sofware, calibrated with micrometer scale image recorded on the sampling date. The blades themselves were frozen in 0.2 M NaCl with 0.2 M $H_2PO_4^-$/ HPO_4^{2-} buffer at pH 6.7, for PE analysis. Prior to extraction, the stored blades were thawed, removed from the buffer, air-dried (28°C) to constant weight (approx. 24 hr), weighed, and returned to the buffer. Two replicate samples of three blades had to be combined from each treatment to give sufficient material for the extraction. The outer cuticle and cell wall required enzyme digestion prior aqueous extraction of PE. Purified papain (Sigma) was used at a final

concentration of 13 units per ml to digest the cuticle, and a filter-sterilized extract ($H_2PO_4^-$/ HPO_4^{2-} buffer) of abalone digestive enzymes (Sigma, abalone acetone powder, crude), at a final concentration 1.0 mg ml^{-1} and pH 6.7, was added for cell wall digestion (Polne-Fuller & Gibor, 1990). Spectra were recorded prior to extraction of the experimental samples to ensure that the PE spectrum was not qualitatively affected by the enzymes. Cell wall digestion and PE extraction required about 14 hr at room temperature, after which the samples were frozen again, then thawed and filtered through glass microfiber filters (Advantec MFS). Absorbance of the PE extract was read on a Beckman DU-64 spectrophotometer in the visible light range at 700, 588, 565 and 455 nm, against a blank containing 1.0 mg ml^{-1} abalone enzyme extract in $H_2PO_4^-$/ HPO_4^{2-} buffer. Final PE concentrations were calculated using the formula,

$$PE = [(A_{565} - A_{588}) - (A_{455} - A_{588}) \cdot 0.20] \cdot 0.12$$ (Beer & Eshel, 1985)

and were standardized to fresh (air dry) weight.

At the end of weeks 2, 4, and 6, a subsample of blades was removed from each culture flask and returned to the normal or control level medium for 10 days. At the end of this recovery period, blades were sampled, measured, and analyzed for PE concentration.

Data for the growth experiment were analyzed using two-stage or derived variable analysis (Diggle *et al.,* 1994). Using exploratory data analysis methods (Cleveland, 1993; Hoaglin *et al.,* 1983) an inverse fifth-root (-0.2 power) transformation of the blade surface area data was found to yield a linear relationship between time and area for each treatment. In the first stage of the analysis, robust regression (S-Plus 4, 1997) was performed on the data from each treatment (n = 4 replicates at t = 7 measurement times) and the slope coefficients were obtained. In the second stage, the main effects of nitrate, salinity, and carbon nested within salinity, and the combined effect of nitrate and salinity, were tested on the robust regression coefficients using ANOVA (Neter *et al.,* 1996). Fitted values were transformed back to their original dimensions; instantaneous growth rates could then be obtained from the model by differentiation. The main effects of nitrate, salinity, and carbon nested within salinity, and the combined effect of nitrate and salinity on phycoerythrin concentration were analyzed on the data from each week using ANOVA. For the recovery data, differences in blade surface areas (growth recovery) and in phycoerythrin concentration (PE recovery) between the end of the 10 d recovery period and the last day of experimental treatment were analyzed using ANOVA. The assumptions of the ANOVA model were tested for each data set using diagnostic and exploratory data analysis methods (S-Plus 4, 1997; Cleveland, 1993; Hoaglin *et al.,* 1983).

Results

Blade Growth

The *Porphyra torta* blades grew in all treatments over the 6 week experimental period, but growth varied according to treatment (Fig. 4-1). The F-tests on the robust regression coefficients were significant only for nitrate, at P = 0.021. The slope values were lower for the low nitrate groups at all levels of salinity and carbon within salinity. The mean slope values declined with salinity, but the difference was not significant (P = 0.079). The power of the test was low since there was no replication in the regression coefficients. The model for blade size at time t was,

$$y' = \beta_{0i} + (\mu.. + \alpha_i) \cdot t + \varepsilon, \; i = 1,..,m,$$

where y' is the -0.2 power transformed blade area, β_{0i} is the -0.2 power transformed initial area for group i, $\mu..$ is the grand mean over all groups, α_i is the explanatory variable for nitrate at level i, and ε is an error term. Blade area was predicted by the equation,

$$\text{area} = (y')^5.$$

The instantaneous growth rate at time t was predicted by the equation,

$$\text{growth rate} = d(\text{area})/dt = -5(\mu.. + \alpha_i) / [\beta_{0i} + (\mu.. + \alpha_i) \cdot t]^6.$$

According to this model, growth rate increased with time during the initial stage, and the rate of increase was dependent on nitrate level. Beyond 8 - 10 weeks, the

exponential increase predicted by the model becomes unrealistic, suggesting that other factors must mediate growth at later stages.

Phycoerythrin concentration

The phycoerythrin concentrations calculated from absorbance data are shown in Table 4-1. Fluctuations in weights and background absorbance readings in the very small samples caused high variability, and there were only 2 replicates per treatment for each week, since samples had to be combined to yield sufficient material for the analysis. A square-root transformation of the concentration values produced a slightly better fit in most weeks, based on diagnostic plots of residuals. As expected, there were no significant effects at week 0, prior to exposure of blades to experimental conditions. F-tests were significant for nitrate at $P = 0.009$ and $P = 0.016$ for weeks 1 - 2, respectively, and highly significant for nitrate at $P << 0.001$ for weeks 3 - 6. There were no significant effects in any week for salinity, carbon within salinity, or nitrate · salinity interaction.

Growth recovery

With few exceptions, blade surface areas increased over the 10 day recovery periods following 2 and 4 week exposures to experimental treatments (Fig. 4-2). Growth did not vary significantly between treatment groups during this period.

Following the 6 week exposure, low nitrate had a significant, negative effect on growth during recovery at $P = 0.043$, and a significant interaction with salinity, at $P = 0.042$ (groups exposed to low salinity and low nitrate had higher growth during recovery).

Phycoerythrin recovery

Differences in phycoerythrin concentrations between the beginning and end of the 10 day recovery period were greater for those groups exposed to low nitrate for 2, 4 and 6 weeks (Fig. 4-3). Phycoerythrin concentrations in the nitrogen-depleted blades often surpassed those in non-depleted blades during the recovery period. Low nitrate had a large, positive effect on recovery of phycoerythrin, significant at $P = 0.020$ for the 2 week exposure, and at $P << 0.001$ for the 4 week and 6 week exposure. Salinity had a significant effect on recovery of phycoerythrin concentration at $P = 0.014$ for the 4 week exposure, a result that is difficult to interpret given that salinity did not have a significant effect on phycoerythrin concentration at any time during the 6 week exposure period. In the third recovery experiment, there was a significant nitrate · salinity interaction ($P = 0.002$). Recovery of phycoerythrin concentration in groups exposed to low nitrate was greatest at the middle level of salinity.

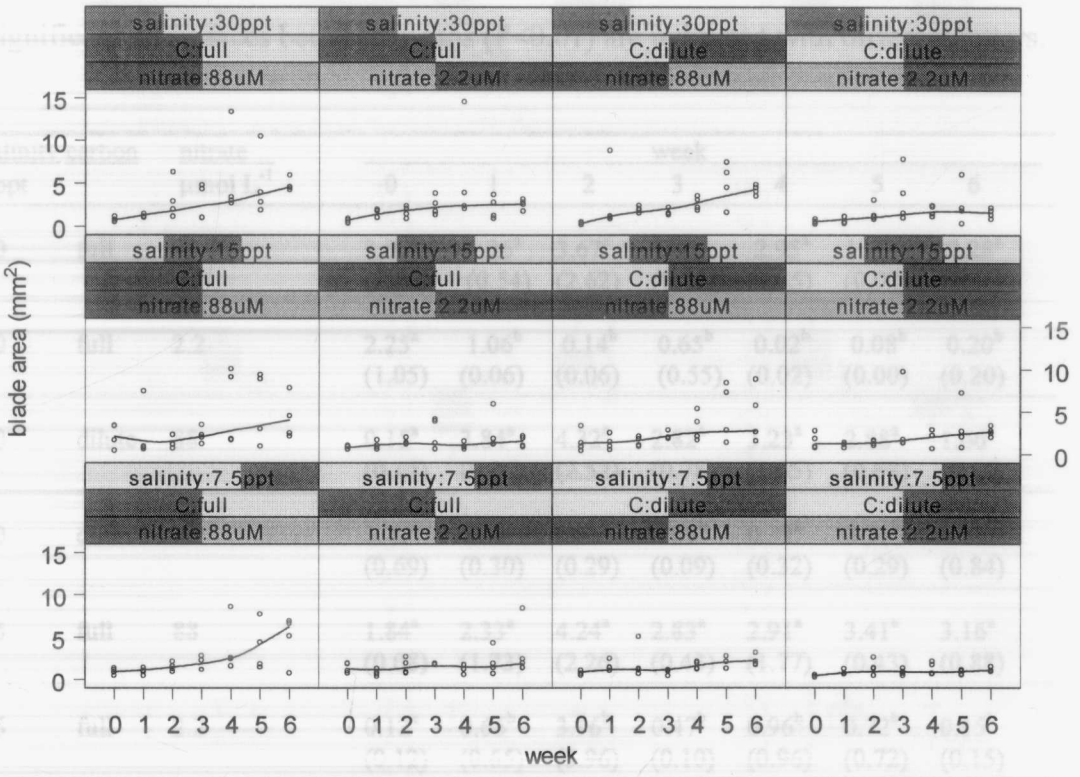

FIG. 4-1. Growth of juvenile *P. torta* blades over 6 wks. Blades were grown in media containing levels of salinity, inorganic carbon, and nitrate indicated in the bars above individual graphs. Levels of carbon are: full = 2.0 mM at all levels of salinity; dilute = diluted with salinity to 2.0, 1.0, and 0.5 mM, at 30, 15 and 7.5 ppt, respectively. Scatter plots show surface areas of n=4 replicates per treatment per week; different blades were selected each week using random sampling. Trend in growth is indicated by a loess smooth curve.

TABLE 4-1. Phycoerythrin concentration (PE) of juvenile *P. torta* blades, measured at beginning of experiment and once a week for 6 wks. Blades were grown in media containing levels of salinity, carbon, and nitrate listed in first 3 columns (see Fig. 1). PE was calculated from absorbance measurements as mg · g fw^{-1}, using 2 blades per sample; data are mean (SE) of n=2 samples per treatment. Significant differences between means ($P<0.01$) are indicated with different letters.

salinity ppt	carbon	nitrate µmol L^{-1}	week 0	1	2	3	4	5	6
30	full	88	3.00^a (1.80)	1.26^a (0.54)	3.67^a (2.62)	1.80^a (0.84)	2.95^a (1.85)	1.80^a (0.02)	2.28^a (0.46)
30	full	2.2	2.25^a (1.05)	1.06^b (0.06)	0.14^b (0.06)	0.65^b (0.55)	0.02^b (0.02)	0.08^b (0.00)	0.20^b (0.20)
30	dilute	88	0.15^a (0.15)	3.84^a (0.96)	4.32^a (2.52)	2.82^a (0.78)	3.23^a (1.05)	2.88^a (0.68)	1.66^a (0.68)
30	dilute	2.2	1.71^a (0.69)	0.30^b (0.30)	0.45^b (0.29)	0.09^b (0.09)	0.32^b (0.32)	0.36^b (0.29)	0.84^b (0.84)
15	full	88	1.84^a (0.08)	2.33^a (1.73)	4.24^a (2.26)	2.83^a (0.43)	2.91^a (1.77)	3.41^a (0.43)	3.16^a (0.88)
15	full	2.2	0.12^a (0.12)	0.65^b (0.65)	3.96^b (3.96)	0.47^b (0.10)	0.96^b (0.96)	0.72^b (0.72)	0.15^b (0.15)
15	dilute	88	0.84^a (0.84)	4.81^a (1.27)	12.48^a (2.88)	4.26^a (1.23)	4.62^a (2.85)	5.11^a (1.05)	4.29^a (0.73)
15	dilute	2.2	2.16^a (1.10)	0.51^b (0.51)	1.70^b (1.10)	0.47^b (0.14)	0.40^b (0.40)	0.00^b (0.00)	0.25^b (0.25)
7.5	full	88	2.55^a (2.55)	1.96^a (0.65)	6.61^a (4.59)	3.08^a (1.48)	4.08^a (2.66)	3.73^a (0.13)	2.77^a (0.16)
7.5	full	2.2	0.80^a (0.80)	2.16^b (0.24)	3.23^b (2.57)	1.26^b (0.69)	1.57^b (0.83)	0.56^b (0.31)	0.08^b (0.08)
7.5	dilute	88	1.17^a (0.83)	3.03^a (2.01)	5.40^a (3.00)	1.37^a (1.03)	2.75^a (1.15)	3.52^a (0.16)	1.02^a (1.02)
7.5	dilute	2.2	0.60^a (0.20)	1.63^b (1.03)	5.08^b (3.21)	1.01^b (0.41)	1.20^b (1.20)	0.48^b (0.48)	0.20^b (0.20)

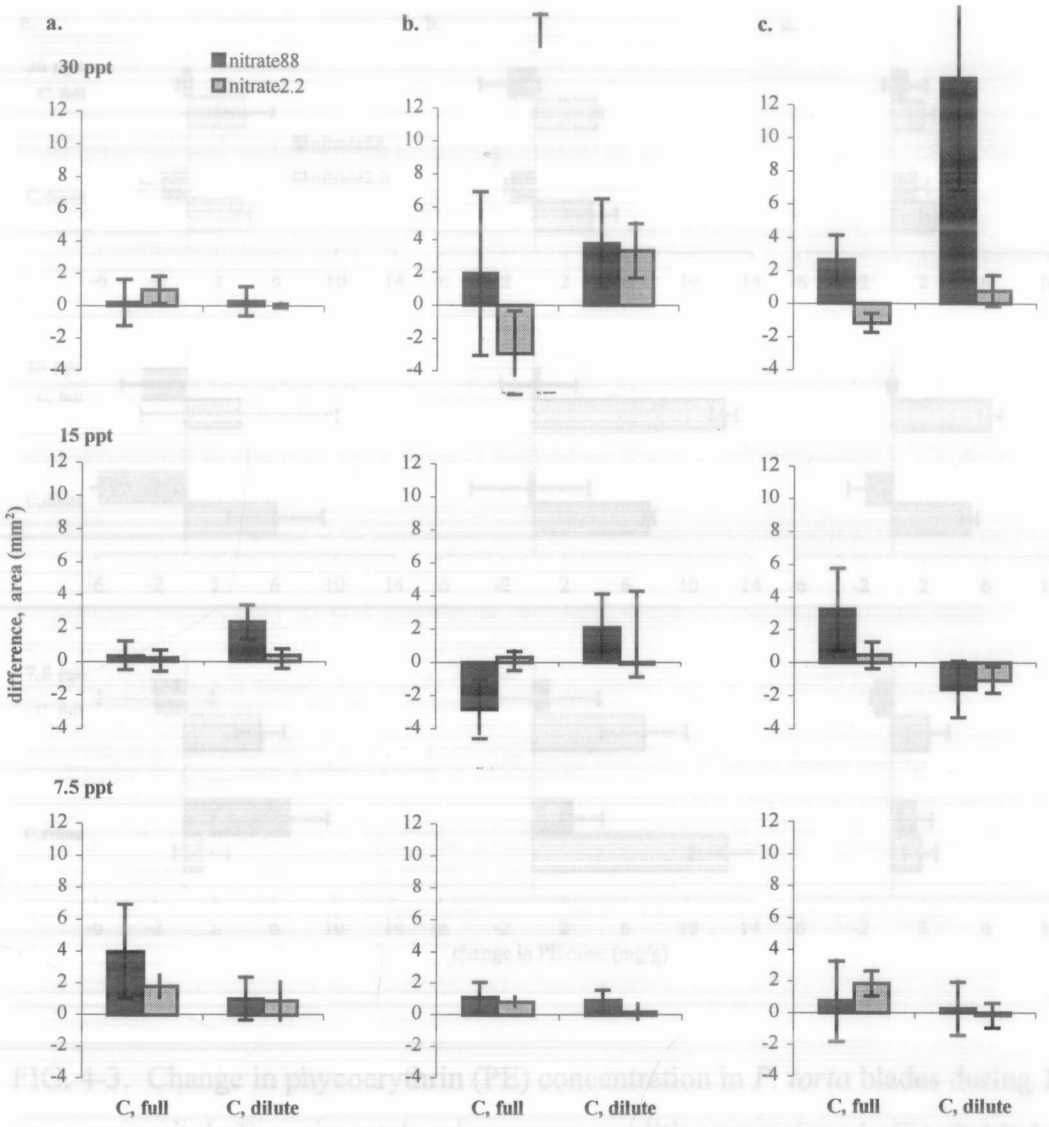

FIG. 4-2. Growth of *P. torta* blades during 10 d recovery period in normal (control) medium (salinity=30ppt, bicarbonate=2.0mM, nitrate=88μM), expressed as difference in blade area. Blades were exposed to one of 12 treatment combinations of salinity (30, 15, 7.5 ppt), bicarbonate (full=2.0 mM at all salinities, dilute=2.0, 1.0, 0.5 mM at 30, 15, 7.5ppt, respectively), and nitrate (88, 2.2μM) for: (a) 2 weeks, (b) 4 weeks, (c) 6 weeks, prior to the 10 d recovery period. Data are mean of n=4 blades ± SE; different blades from the same experimental unit were sampled by random sampling at beginning and at end of recovery period.

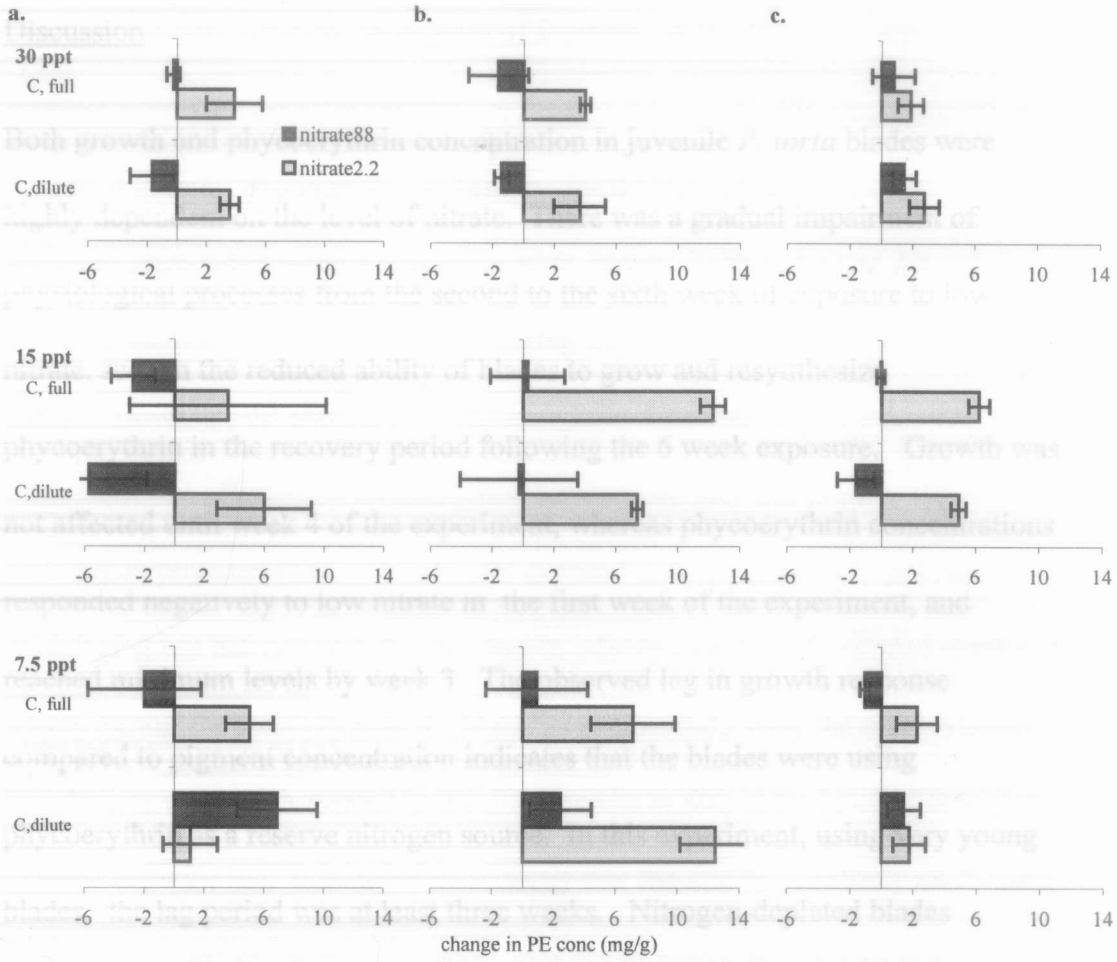

FIG. 4-3. Change in phycoerythrin (PE) concentration in *P. torta* blades during 10 d recovery period. Experimental and recovery conditions are given in Fig. 2; blades were exposed to experimental conditions for (a) 2 weeks, (b) 4 weeks, (c) 6 weeks, prior to recovery period. PE concentration calculated as mg · g fw^{-1}, using 2 blades per sample, at beginning and end of recovery period; data are mean differences of n=2 samples ± SE.

Discussion

Both growth and phycoerythrin concentration in juvenile *P. torta* blades were highly dependent on the level of nitrate. There was a gradual impairment of physiological processes from the second to the sixth week of exposure to low nitrate, seen in the reduced ability of blades to grow and resynthesize phycoerythrin in the recovery period following the 6 week exposure. Growth was not affected until week 4 of the experiment, whereas phycoerythrin concentrations responded negatively to low nitrate in the first week of the experiment, and reached minimum levels by week 3. The observed lag in growth response compared to pigment concentration indicates that the blades were using phycoerythrin as a reserve nitrogen source. In this experiment, using very young blades, the lag period was at least three weeks. Nitrogen-depleted blades responded with a strong increase in phycoerythrin concentration when nitrate was resupplied in the medium.

The growth rate decreased with salinity, and the magnitude of the effect was almost as large as, but independent of, the effect of nitrate. The F-test was most likely non-significant because of the low power of the analysis of variance. The two-stage analysis required by the longitudinal design used the replicates for modeling linear regression in the first stage, thus eliminating replication in the

second stage and reducing the degrees of freedom. A larger sample size could increase power by reducing variation in the first stage or by allowing replication in the second stage. Exposure to low salinity for 6 weeks limited the ability of blades to grow when salinity was returned to 30 ppt. Salinity had no detectable effect on phycoerythrin concentration during the 6 week experimental treatment period. However, the recovery of phycoerythrin following the experimental treatments was affected by an interaction between salinity and nitrate; the highest recovery in phycoerythrin was in blades exposed to low nitrate at 15 ppt salinity. A changing ion balance with the increase in salinity may have facilitated uptake of nitrate during the recovery period. The effects of salinity were the same whether bicarbonate was supplemented in the dilute media or not, at the level of detection in these experiments. It is therefore unlikely that the effects of salinity on *P. torta* blades are caused by carbon limitation; they are probably due instead to ion loss and the extra energy required to maintain ionic balance. In addition to reduced growth, deformities, cell death, and easily fragmented tissue were observed microscopically in blades exposed to low salinity, in both this and previous (unpublished) experiments.

The non-constant growth rates in the juvenile blades could have been caused by build-up and storage of amino acids and proteins in the first few weeks of growth, followed by a rapid increase in structural components and cell division as growth

progressed. The appearance of reproductive tissue at about 6-8 weeks (Waaland *et al.*, 1990) would likely reverse the increase in growth rate, and may be linked to senescence processes (Flores-Moya *et al.*, 1997).

High variability in blade size and random sampling increased the residual error term in the F-tests for all analyses involving blade size. Blades were also of different ages at the start of the experiment since attachment and germination of conchospores from the same batch were spread over a 3 week period. These sources of variability could be reduced or eliminated by following growth in individual blades, rather than a random sample of all blades in a culture, but would require a different method of analysis to handle the autocorrelation arising from repeated measures on the same individual (Diggle *et al.*, 1994).

Nitrogen is known to be an essential and growth-limiting nutrient for the seaweeds, but parameters of N availability and use vary with taxon and are not well-known for all groups or species (Lobban & Harrison, 1994; Reed, 1990; Hanisak, 1983). In *Gracilaria*, the red alga in which uptake, utilization and storage of nitrogen and their relationship to growth have been most thoroughly studied, the growth rate dropped to zero after an 8 week exposure to external NO_3^- + NO_2^- below 1 μM. During the N-depletion period, internal reserves of total

organic N dropped faster than those of amino acids or inorganic nitrogen; phycobiliproteins were suggested as reserve compounds (Hwang *et al.*, 1986). *Gracilaria* could grow at non-nutrient-limiting rates if 100 μM NO_3^- was provided for one 48-hr period every 2 weeks. The unenriched seawater used in this experiment, however, still contained 8-12 μM NH_4^+ + NO_3^- (Ryther *et al.*, 1981). N-limitation only occurred at moderate to high light intensities in *Gracilaria,* and did not manifest itself in reduced growth rates until after 19 days (Lapoint & Duke, 1984). N-deficient *Gracilaria* took up NH_4^+ more quickly and in greater amounts than NO_3^- when they were resupplied (D'Elia & DeBoer, 1978; Ryther *et al.*, 1981; Bird *et al.*, 1982). Uptake of NO_3^- + NO_2^- in N-depleted *Gracilaria* became saturated at an external concentration of 84 μM (Hwang *et al.*, 1987). Nitrate reduction within the cell may be a limiting step in the utilization of new nitrate in N-depleted algae; nitrate reductase activity depended on external and internal concentrations of NO_3^- in *Porphyra* (Thomas & Harrison, 1985) and *Gracilaria* (Hwang *et al.*, 1987), and was inhibited by increasing external NH_4^+ (Hernandez *et al.*, 1993).

Phycoerythrin is strongly implicated as a nitrogen storage compound in *Gracilaria*, because of its pattern of depletion and regeneration in response to decreasing or increasing external N supply (Ryther *et al.*, 1981; Bird *et al.*, 1982; Lapoint & Duke, 1984). Phycoerythrin comprised 20% of total N in *Gracilaria*

tikvahiae, but decreased markedly under N-limitation in the 2.5 week lag period before observable decline in growth (Lapoint & Duke, 1984). Phycoerythrin was metabolized as an N source during N-limited growth in *Gracilaria* after reserves of inorganic N and amino acids were used; it was depleted sooner and resynthesized later than other proteins in response to additional external NO_3^- (Bird *et al.*, 1982). In a recent quantification in *Gracilaria pacifica* (Naldi & Wheeler, 1999), soluble proteins and free amino acids were the largest nitrogen storage pools, at 50% and 16%, respectively, of total nitrogen, while the phycoerythrin pool was smaller, representing about 5-6% of total nitrogen. The relative contributions of different nitrogen compounds to the storage pools, however, vary with species; phycoerythrin can comprise up to 60% of total soluble protein in some red algae (Naldi & Wheeler, 1999).

In our study, phycoerythrin was a good indicator of nitrogen limitation in *Porphyra torta* blades; it is probably a major nitrogen storage compound in this species. Since phycoerythrin concentration responded to nitrogen limitation within one week, it could serve as a rapid and reliable way of assessing the nitrogen status of both wild and cultivated *Porphyra*. Quality in the cultivated *Porphyra* product, or nori, depends on properties such as color and texture. The desired black color results from an abundance of the photosynthetic pigments, chlorophyll, carotenoids, phycoerythrin and phycocyanin (Kudoh, 1987; Kato &

Aruga, 1984); the relationship of this property to the availability of nitrogen has been recognized by Japanese mariculturists and researchers for over 40 years (Amano & Noda, 1987). In a fertilization experiment, a 6-8 fold increase in phycoerythrin concentration was observed within 2 days after nitrogen was replenished, at 20 ppm, to nitrogen-deficient *Porphyra* blades. Ammonium was absorbed faster and resulted in higher levels of pigment than nitrate, but both were effective. Recovery of pigmentation was possible after an N-deficient period of up to 8 days, in 5 cm long plants (Amano & Noda, 1987).

Porphyra torta occurs mainly in areas where the seawater salinity is constant throughout the year at around 30 ppt; however, as a high intertidal species, it is subject to high salinity during dessication and freezing and low salinity from rainfall and runoff. The salinity tolerance of the sporophyte, or conchocelis phase of *P. torta* may be a factor in its distribution. Best growth of *P. torta* conchocelis in laboratory experiments occurred at 30 ppt (Lin, 1999; Stekoll *et al.*, 1999). *Porphyra umbilicalis* was able to maintain osmotic balance in media between 0.2 and 1.5x natural seawater, mainly through adjustment of internal K^+ concentration (Wiencke & Lauchli, 1981). The primary cost to the cell of low salinity in *P. perforata* blades is the transport of K^+ into the cell in order to maintain essential functions such as the extrusion of Na^+ (Eppley & Cyrus, 1960). In *Porphyra purpurea* both K^+ and Cl^- concentrations decreased to a new equilibrium level

within 6 hrs of a salinity change from 1 to 0.25x. The energy required to maintain the balance between internal and external ion concentrations, and the cost of accumulation of nitrate, increased with decreasing salinity in *P. purpurea*; the concentration of amino acids decreased with salinity (Reed *et al.*, 1980). These responses would limit growth of *Porphyra* at low salinity. The increased energy costs might also make *P. torta* less competitive ecologically in areas where low salinity is a factor (Russell, 1987). This does not mean that *P. torta* could not be cultivated in areas with lower salinity than in its natural range. Within-species variation in salinity tolerance is documented for many algae (Russell, 1987). Marine-adapted *Bangia* could not survive an abrupt change in salinity in one generation, but transition to near-fresh water could be accomplished over several generations in increments of 10-20% at each generation (Hartog, 1972; Geesink, 1973). Growth of third generation cultured germlings of marine *Bangia* increased with salinity across a range of 1/32 to 1x seawater media , but was not much different between 1/4 and 1x salinity (Reed, 1980). First generation germlings produced by asexual sporulation of freshwater *Bangia* from Lake Ontario were able to grow at all salinities from 1 to 26 ppt, but grew best at the lower salinities. In subsequent generations, growth was best at the salinity level in which the preceding generation had been cultured (Sheath & Cole, 1980).

Nitrogen levels would definitely present limitations for mariculture of *Porphyra torta* in Alaska, but adequate nitrogen is usually available from September through April. Salinity may limit mariculture in areas with regular, high freshwater input, but it is possible that tolerant strains could be developed through selection and adaptation. Periodic emersion of net-grown *P. torta*, or placement of nets in the intertidal as was formerly done in Japan, would have the effect of increasing salinity to the blades in non-rainy weather. Fertilization to increase nitrogen levels would have the undesirable effect of promoting growth of epiphytic and competing algae, particularly during the late spring and summer when light is abundant. A pulsed fertilization scheme would probably provide sufficient nitrogen to *Porphyra torta*, while limiting N input to competing algae. The negative impacts of artificially raising the nitrogen load on the nearshore environment need to be carefully considered. Siting *Porphyra* farms near other mariculture activities, such as oyster cultivation, may provide sufficient nutrient input without additional fertilization. The effective growing season could be extended, or unfavorable conditions avoided, by growing juvenile blades under controlled conditions in large indoor or outdoor tanks for the first 6-8 weeks. While initial growth of juvenile blades is critical for successful mariculture, experiments are also needed to test responses of mature blades to varying salinities, exposure periods, and nutrient levels in the ocean.

The laboratory methods for *P. torta* developed in this research can be applied to other species and conditions, in ecology, physiology and mariculture research. These techniques will be essential for strain selection and development if new species of *Porphyra* are to be brought into cultivation in the northeast Pacific.

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CHAPTER 5

CONCLUSIONS

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Field Survey

Porphyra abbottae follows strict seasonal, geographic and vertical zonation patterns, but the determining factors have not yet been isolated in the field. There are variations in abundance and growth within the normal season and range of *P. abbottae* associated with water temperature, water motion, exposure to high irradiance and heat during low tides in sunny weather and probably nitrogen availability. High abundance occurs in areas with moderate to high water motion, but scouring may limit growth where gravel is present. *Porphyra abbottae* persists longer into the summer in areas with cooler water temperature and tidal currents or moderate wave action. Abundance and blade size are not necessarily correlated. High abundance but reduced blade size is associated with heavy wave action, while very large blade size is associated with tidal current or moderate wave action. Isolating the effects of specific factors in the natural environment is difficult. Field experiments are needed in which factors of interest can be manipulated.

Effective sampling of *Porphyra* in its natural habitat requires more than simple random sampling along a single transect. Where biomass estimates are needed, a double sampling ratio estimate can be used, using cover as an auxiliary variable (Thompson, 1992); this method increases accuracy while reducing sample size. A

multistage sampling design with primary and secondary units following natural habitat boundaries can deal with large-scale patchiness (Hankin, 1984). Estimation of mean blade weight is more accurate and sample size reduced if a larger sample is first stratified by length and then subsampled for weight determination, as in the double sampling for stratification method (Cochran, 1977). Accurate biomass estimates for *P. abbottae* and *P. torta* are needed to assess the impact of actual or potential harvests and other human activities, while size and growth of individual blades are of interest in growth studies and ecological physiology experiments. The relationship between growth and density in natural *Porphyra* beds needs to be investigated. Possible increases in density and abundance must be considered if the gametophytes reproduce asexually by monospores (Cole & Conway, 1980; Conway & Cole, 1977; Yoshida, 1972a,b).

Pilot studies for mariculture will require some methods similar to those in field studies. Experiments using *Porphyra* on cultivation nets will permit study of its responses to the varying conditions in the natural environment, with greater control over study sites and timing.

Recruitment, substrate and density

The initial stages of *Porphyra* gametophyte growth from conchospore release and attachment through germination and early blade growth are not thoroughly understood, in part because of the difficulty of observation and measurement of

the microscopic stages. *Porphyra torta* conchospores in my laboratory cultures required at least 3 weeks for attachment and germination. Conway and Cole (1977) reported a period of 12 weeks from conchospore release to germination in *P. torta* and 6-8 weeks in *P. abbottae* in laboratory cultures. They reported that *P. abbottae* released conchospores readily, while in other *Porphyra* species conchospore release was infrequent or did not occur. In our laboratory, conchospore release from *P. abbottae* was infrequent. These processes are extremely difficult to observe in nature, so it is still unknown if conditions for conchospore release, attachment and germination in culture differ from those in nature.

Germination of *Porphyra* conchospores is bipolar (Guiry, 1990; Conway & Cole, 1977), with a rhizoid developing at one pole. As the gametophyte develops, rhizoids branch out and elongate from the base of the blade, attaching to the substrate. Abnormal rhizoid development was observed in cultures of *P. torta* gametophytes kept suspended by air bubbling in which no substrate was provided. Many blades in these cultures had large numbers of small rhizoids, growing not only from the base of the blade but all around the blade margins. Gametophytes grown on glass slides sometimes also exhibited this abnormal development. Substrate must therefore affect gametophyte development. The type of substrate affected recruitment of *P. torta* in this study. Highest recruitment was observed on substrates in which the size of interstitial spaces was several times the diameter

of a conchospore. In nature, small cracks and grooves in rock surfaces probably protect algal spores from high water flow initially, and from heat and dessication as they germinate and grow (van Tamelen, 1997; Kain & Norton, 1990; Harlin & Lindbergh, 1977). Maximum spore deposition in some species is associated with low water flow rates, but the time period for firm attachment to the substrate is not obviously correlated with water motion (Kain & Norton, 1990). The time for attachment varied among the red algal species studied, but the strength of attachment in almost all species increased with time (Kain & Norton, 1990). In this study, many conchospores in culture remained unattached after 2-3 weeks, although most eventually became attached. It seems unlikely that spores in nature could remain unattached for even a few days without being swept away by waves or currents.

Germination in *P. torta* blades in culture was dependent on density, with the highest germination rate at medium density. In terrestrial plants, density can positively affect germination by stabilizing the microhabitat, excluding other species, or releasing stimulators, even though it will result in intense competition later (Antonovics & Levin, 1980; Harper, 1977). Respiratory CO_2 has been observed to stimulate germination in closed culture containers (Harper, 1977). Positive effects of density on germination have been observed in kelps (D.C. Reed, 1990). These effects are possible in cultures of *Porphyra*, but specific mechanisms are still unknown.

Growth in *P. torta* blades in culture was also density dependent, with a negative relationship between density and blade size. Density decreased over time regardless of initial density. The relationship between blade size and density suggests that *P. torta* blades approached a final constant yield, or carrying capacity (Antonovics & Levin, 1980; Harper, 1977; Yoda *et al.*, 1963). The observed mortality may or may not have been density-dependent. The cultures may not have been sufficiently dense to meet the boundary condition for self-thinning, and mortality occurred for other reasons (Cousens & Hutchings, 1983; Schiel & Choat, 1980). Density-dependent growth and mortality results from competition for resources, usually light, nutrients or space (Harper, 1977). Although the nature of these limitations is different for seaweeds than for terrestrial plants, most seaweeds are also regulated by density-dependent stress (Creed *et al.*, 1996). Large canopy blades can shade smaller or younger blades (Creed *et al.*, 1998, 1996; Scrosati & DeWreede, 1998; Flores-Moya *et al.*, 1996; Cousens & Hutchings, 1983), and nutrients could be limiting in high density natural stands or cultures, although this has not been specifically tested (Creed *et al.*, 1998, 1996). Space limitation, as well as light limitation, can be seen in increased recruitment of small individuals following a canopy-removing disturbance or harvest (Creed *et al.*, 1996; Chopin *et al.*, 1992; Kendziorek & Stekoll, 1984). It is unlikely that either light or space would have been limiting for *P. torta* blades in this experiment until the blades were 3-5 mm long.

Nutrients should not have been limiting either since the blades were bathed in nutrient-enriched media that was periodically changed; however, nutrients may have become locally depleted in the unstirred cultures, especially in areas of high density. Bacteria may also have affected the nutrient status of the cultures. Density-independent mortality could occur for genetic reasons (Ang & DeWreede, 1992); for example, some spores may not be viable. Large aggregations of two- to four-cell germlings were observed in some experiments during this study that persisted in the cultures for some time but did not grow. Self-thinning could not be statistically confirmed in this study, but growth and mortality of *P. torta* blades in the experimental cultures appeared to follow the pattern of the self-thinning law. More investigation is needed on this question, in the lab and in the field.

Effects of salinity

Low salinity resulted in some reduction in growth in experimental *P. torta* cultures, but the effect was not statistically significant. In cultures at the lowest salinity tested (7.5 ppt) deformities, cell death, and easily fragmented tissue were observed (Fig. 5-1). Reduced salinity causes stress in algal cells by increasing the osmotic pressure from the medium, the turgor pressure inside the cell, and the ionic concentration gradients between the cell and the medium. High turgor pressure can disrupt cell structure and ultimately rupture the cell. Maintaining physiological levels of ions, particularly K^+, within the cell, and accumulating and

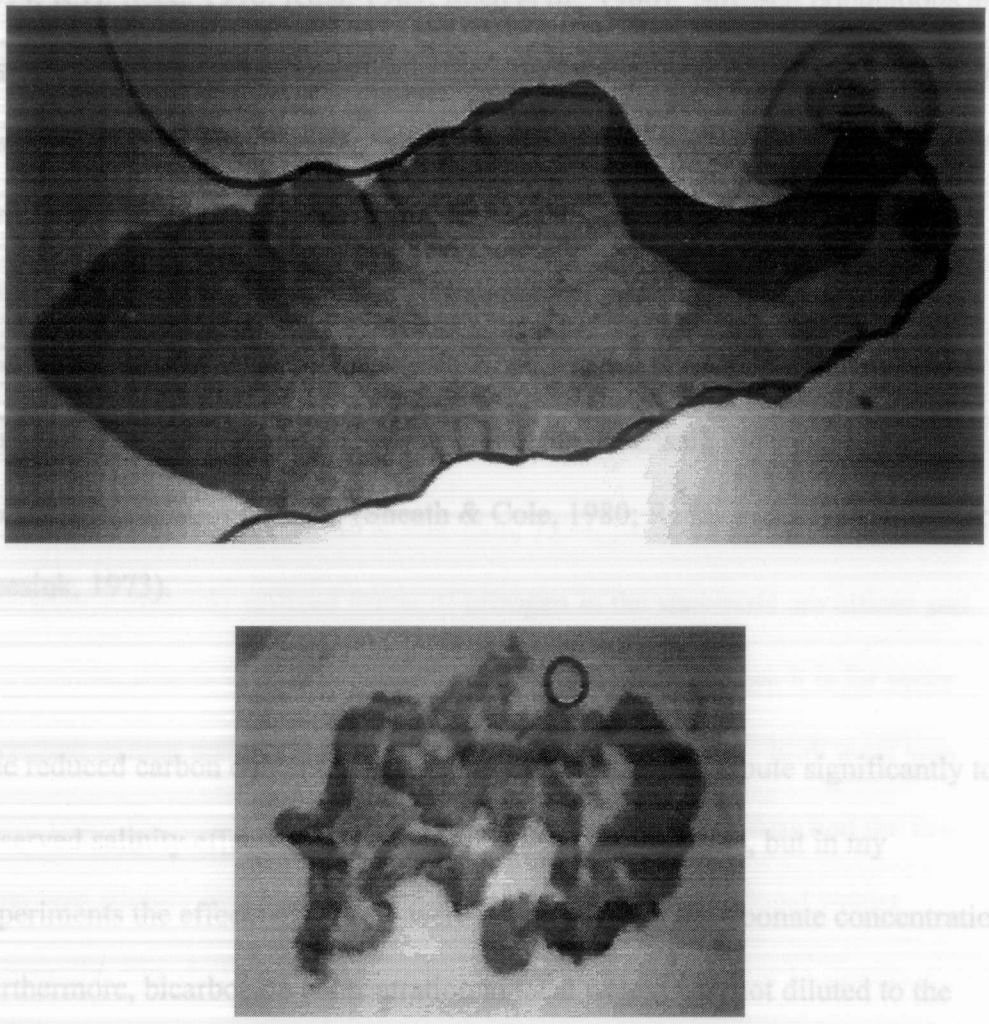

FIG. 5-1. *Porphyra torta* gametophytes grown at 2 different salinity levels for 6 weeks: 30 ppt (A) and 7.5 ppt (B). Microscopic images were captured on videotape at 16x magnification. The blade in (A) has a smooth surface and uniform cell pattern, while the blade in (B) is broken and disrupted with irregular cell patterns.

maintaining proper concentrations of nutrients and cellular organic compounds requires additional energy for marine algae in low salinity (Lobban and Harrison, 1994; R.H. Reed, 1990; Kirst, 1989; Reed *et al.*, 1980). Stressed populations are less able to compete for resources in the environment with other algae (Kautsky & Kautsky, 1989; Russell, 1987), which may explain in large part the absence of *P. torta* and P. *abbottae* in areas of low or fluctuating salinity. However, wide ranges of salinity tolerance and acclimation to moderate changes have been documented for some red algae (Kirst, 1989; Russell, 1987). Within-species variation in salinity tolerance is also documented (Russell, 1987) and long term genetic adaptation is possible (Sheath & Cole, 1980; Reed, 1980; Hartog, 1972; Geesink, 1973).

The reduced carbon content of low salinity water may contribute significantly to observed salinity effects (Kain & Norton, 1990; Ohno, 1976), but in my experiments the effects of salinity were independent of bicarbonate concentration. Furthermore, bicarbonate concentration in local waters was not diluted to the same extent as salinity during the summer when freshwater input was high (Appendix, Fig. A-1).

Porphyra torta gametophytes could tolerate reduced salinity to at least 15 ppt, so it is likely that both *P. torta* and *P. abbottae* could be cultivated in areas where

salinity is sub-optimal for at least part of the year (Appendix, Fig. A-2). Interspecific competition would be greatly reduced for cultivated *Porphyra*, and other conditions could be optimized to reduce the impact of salinity stress. There is also a good possibility that strains tolerant to reduced salinity could be obtained after cultivation for a number of generations.

Effects of nitrate

Nitrate had a strong effect on growth and pigment concentration in *P. torta*, which is not surprising since some form of reduced nitrogen is essential and growth-limiting for seaweeds (Lobban & Harrison, 1994; Reed, 1990; Hanisak, 1983). The most commonly utilized forms of nitrogen in the seaweeds are nitrate and ammonium; nitrate was used in these growth experiments since it is far more abundant in area waters. Two levels of nitrate were tested; the high level was about twice the maximum concentration of nitrate in local waters and the low level was slightly higher than the minimum concentration in local waters (Appendix, Fig. A-3). The lower concentration was insufficient to sustain growth of *P. torta*, but *P. torta* does have the capacity to use stored nitrogen reserves for growth for up to several weeks. In *Gracilaria*, the critical level for internal nitrogen is between the minimum growth requirement of 0.8% and growth saturation at 2.0% of dry weight (Hanisak, 1983). The capacity for nitrogen storage has been demonstrated in at least some members of *Gracilaria, Polysiphonia, Palmaria, Chondrus* and *Porphyra* when environmental levels

exceeded growth saturation (Reed, 1990; Kain & Norton, 1990). A limited amount of inorganic nitrogen can be stored by red algae; organic compounds, including amino acids, small peptides, proteins and phycobiliproteins, form the major reserves (Naldi & Wheeler, 1999; Reed, 1990). The phycobiliprotein phycoerythrin can comprise from a few percent up to 30% of total internal nitrogen reserves in red algae (Naldi & Wheeler, 1999; Lapoint & Duke, 1984), and may be mobilized as a reserve nitrogen source before other proteins (Bird *et al.*, 1982). Abundant phycoerythrin and other photosynthetic pigments give the cultivated *Porphyra* product, or nori, its desired black color (Kudoh, 1987). The relationship of this property to the availability of nitrogen has been recognized by Japanese mariculturists and researchers for over 40 years (Amano & Noda, 1987).

The large seasonal fluctuation of nitrate in temperate coastal waters greatly influences the seasonal cycle of growth and reproduction in the macroalgae (Gunnarsson & Ingolfsson, 1995; Thomas & Harrison, 1985). *Porphyra torta* gametophytes appear and grow in winter when nitrate is not limiting. The timing of gametophyte growth in *P. abbottae* is nearly coincident with the spring phytoplankton bloom and peaks in late spring or early summer when the nitrate level has already fallen. Perhaps *P. abbottae* can successfully compete with phytoplankton for limited nitrogen resources, or perhaps it can accumulate enough nitrogen prior to the spring bloom for the rest of the season's growth. Some kelps have endogenous circannual growth rhythms which enable them to

initiate growth before the onset of the spring phytoplankton bloom, while nitrogen is still high, and cease active growth before total nitrogen depletion in summer (Lüning, 1993).

Recovery from stress

Prolonged exposure to low nitrate impaired the ability of *Porphyra torta* to recover growth and phycoerythrin content, but when the exposure lasted 4 weeks or less, the blades could recover to normal growth rate and phycoerythrin levels. Nitrogen-deficient blades accumulated phycoerythrin at a higher level, once they were resupplied with nitrogen, than nitrogen-replete blades. In some macroalgae, an increase over normal nitrogen uptake rates has been observed in nitrogen-starved plants, with a preferential uptake of ammonium over nitrate (Thomas & Harrison, 1985; D'Elia & DeBoer, 1978). Nitrate uptake is generally rate-saturating at normal levels of external nitrate, depending on internal nitrate concentration, nitrate reductase activity and assimilation rates, but the uptake rate increases in nitrogen-starved algae (Thomas & Harrison, 1985; Hwang *et al.*, 1987; D'Elia & DeBoer, 1978). Increased rates of nitrate uptake, reduction and assimilation may have allowed the nitrogen-deficient *P. torta* blades to build up phycoerythrin to a higher than normal level when presented with sufficient nitrate. In experiments with cultivated Japanese *Porphyra yezoensis*, fertilization of nitrogen-deficient, faded fronds with amino acids, urea, ammonium or nitrate resulted in recovery of all photosynthetic pigments, normal cell size and

appearance and normal color (Amano & Noda, 1987). Ammonium was absorbed faster and resulted in higher levels of pigment than nitrate, but was toxic at higher levels. Recovery of pigmentation was possible after an N-deficient period of up to 8 days, but after 10 days cells and chloroplasts shrank and the fronds could no longer recover (Amano & Noda, 1987).

There was no detectable recovery response after exposure of *P. torta* to low salinity, in part because the effect of low salinity was not statistically significant. The visible effects of low salinity on blades and cell structure disappeared in the recovery treatment after 2 weeks exposure, but deformities, disintegrating tissue and dead cells remained in the blades exposed to the lowest salinity (7.5 ppt) for 4 to 6 weeks. Blades exposed to 15 ppt salinity appeared normal during the recovery period. An increase in number or size of vacuoles has been observed in *Porphyra* blades exposed to low salinity (Kirst, 1989; Amano & Noda, 1987), and if the exposure period was long enough the change was irreversible (Amano & Noda, 1987). Adjustments to ionic and osmotic balances that are required in low salinity, while costing the cell in terms of energy, are likely to be reversible, while changes or damage to cellular structure are more likely irreversible.

Summary

Differences in the growth and abundance of *Porphyra abbottae* in the field are associated with differences in water temperature, wave action and current between sites, but better sampling designs and techniques are needed for accurate and representative estimation of growth and biomass. These methods are available and should be applied to management, mariculture, and ecological field experiments.

The physical conditions during conchospore release, as well as type of substrate and spore density affect recruitment and initial growth in *Porphyra torta*. How similar the laboratory results are to conditions in nature is unknown and difficult to study. Growth and mortality in young *P. torta* blades appear to be density-dependent, but the mechanisms underlying these relationships are not yet known.

Porphyra torta blades can tolerate low salinity to 15 ppt, but some cellular damage occurs at 7.5 ppt. Dilution or supplementation of inorganic carbon in the medium does not change the effects of low salinity on *P. torta* blades. Salinity stress is probably one factor limiting the natural ranges of *P. torta* and *P. abbottae* to the outer coast of southeast Alaska, but cultivation in reduced-salinity inside waters may still be possible since *P. torta* has demonstrated some tolerance to reduced salinity.

Nitrate strongly limits growth and reduces pigment content of *P. torta* blades at a concentration of 2.2 μmol L^{-1}, which is slightly higher than the low nitrate levels found in southeast Alaska coastal waters from May through September. Growth and pigment content can recover after up to 4 weeks exposure when the nitrate supply is increased, but after 6 weeks recovery is limited. Sufficient nitrate is available in this region from October through mid-May for healthy growth in *P. torta*. Fertilization would probably be necessary if cultivation were to take place in the summer, but would also provide ideal conditions for growth of competing, weedy algae during the period of long days and high irradiance levels. A pulsed fertilization schedule may be a partial solution since there is evidence that *P. torta* blades can store nitrogen.

Substantial progress has been made in this research on understanding some of the physical factors affecting gametophyte growth in *Porphyra torta* and *Porphyra abbottae*. Field cultivation trials are the next step in the development of these species for mariculture. In addition, this research has raised several questions of ecological interest in their own right, in particular, the problem of modeling of growth rates and estimating a complex set of environmental parameters.

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APPENDIX

Appendix

Water Chemistry and Environmental Monitoring

Methods

Water temperature, salinity and nitrogen-nutrients were measured weekly at the National Marine Fisheries Service dock in Auke Bay, Alaska from 1996 through 1998. Inorganic carbon was estimated weekly in 1998. Only part of the data is shown in the graphs. Water temperature and salinity were measured using a YSI Model S-C-T meter with a YSI 3300 conductivity cell. Water samples were taken at 1 m and 4 m for nitrogen and at 1 m and 10 m for carbon determinations. Water samples for nitrogen determination were vacuum-filtered through 2 μm filters and frozen for later analysis; concentration of nitrate + nitrite was determined using the method in Parsons *et al.* (1984). Total inorganic carbon was determined immediately after collection using the method in Parson *et al.* (1984).

Reference

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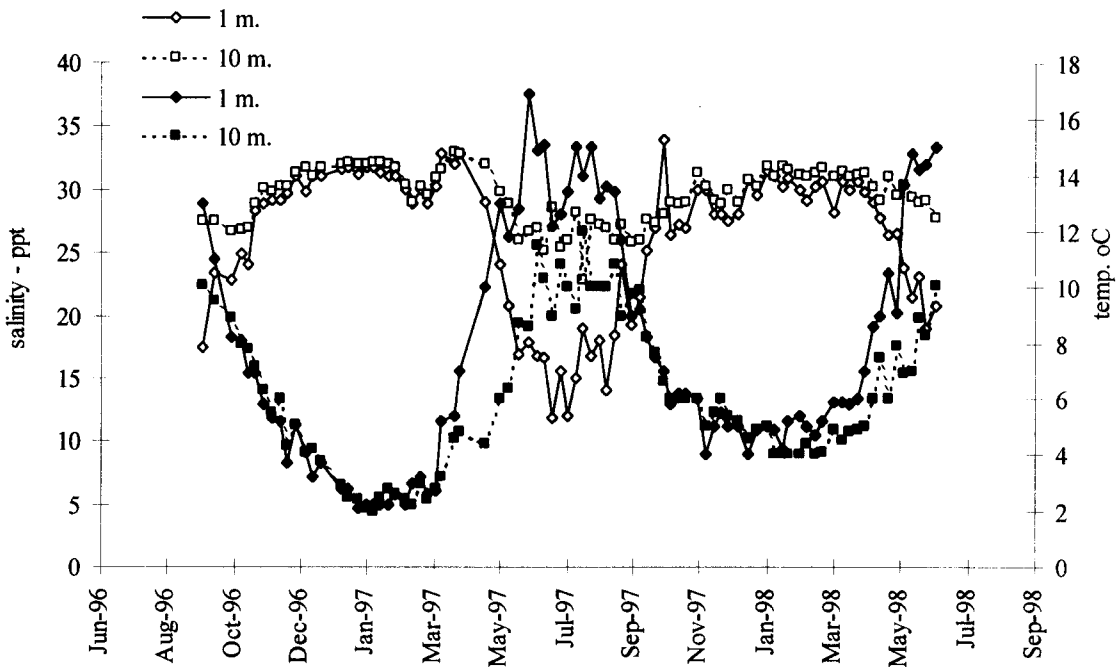

FIG. A-1. Salinity and temperature of seawater, June 1996 – Sept. 1998. Measured weekly at the National Marine Fisheries Service dock in Auke Bay, Alaska using a YSI Model 33 S-C-T meter and YSI 3300 conductivity cell. Salinity at 1m – open diamonds, solid line; salinity at 10m – open squares, dashed line; temperature at 1m – solid diamonds, solid line; temperature at 10m – solid squares – dashed line.

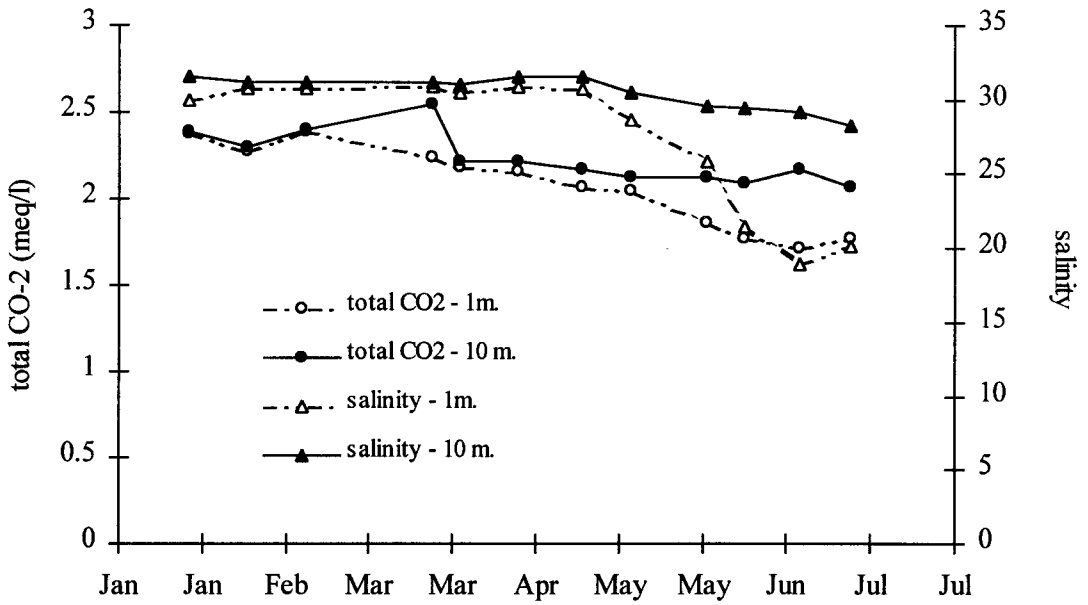

FIG. A-2. Total inorganic carbon and salinity in seawater, Jan. – June 1998. Water samples were collected weekly at the National Marine Fisheries Service dock in Auke Bay, Alaska at 1 m and 10 m. Total inorganic carbon concentration was determined according to the method in Parson *et al.* (1984); salinity was measured using a YSI Model 33 S-C-T meter and YSI 3300 conductivity cell. Total inorganic carbon at 1 m – open circles, dashed line; total inorganic carbon at 10 m – solid circles, solid line; salinity at 1 m – open triangles, dashed line; salinity at 10 m – solid triangles, solid line.

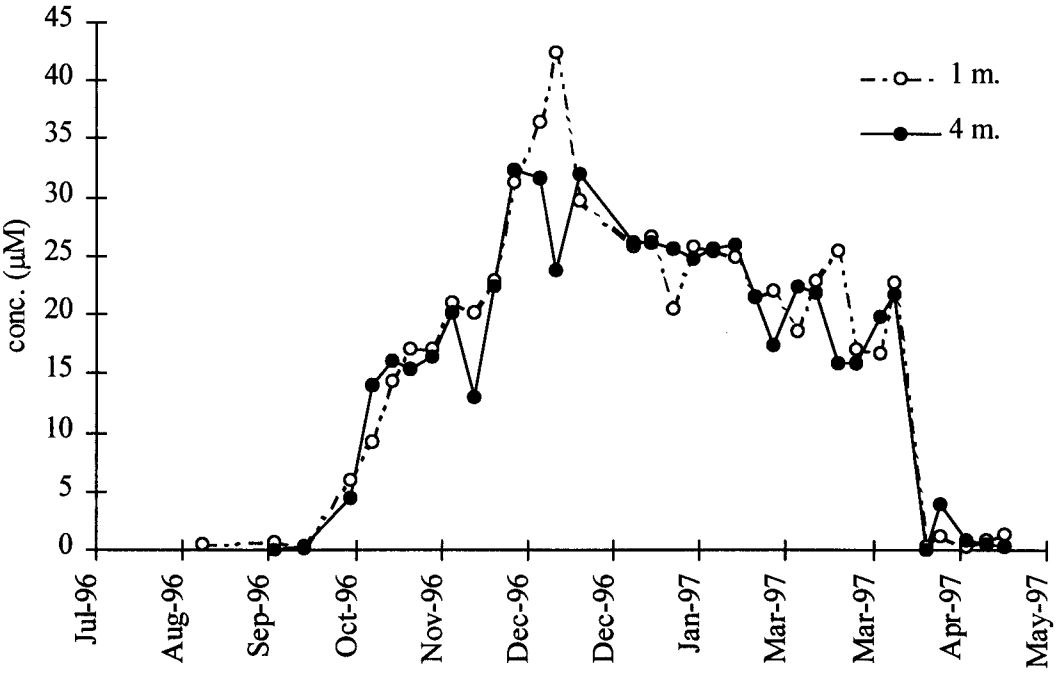

FIG. A-3. Concentration of nitrate + nitrite in seawater, July 1996 – May 1997. Water samples were collected weekly at the National Marine Fisheries Service dock in Auke Bay, Alaska at 1m (open circles, dashed line) and 4 m (solid circles, solid line); total concentration of nitrate + nitrite was determined according to the method in Parson *et al.* (1984). When nitrite was determined separately, concentration was negligible.