PHYSIOLOGICAL ECOLOGY OF PORPHYRA SPOROPHYTES: GROWTH, PHOTOSYNTHESIS, RESPIRATION AND PIGMENTS

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PHYSIOLOGICAL ECOLOGY OF *PORPHYRA* SPOROPHYTES: GROWTH, PHOTOSYNTHESIS, RESPIRATION AND PIGMENTS

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Abstract

Growth, photosynthesis, respiration and photosynthetic pigments of the sporophytic stage for Alaskan *Porphyra* species were investigated in response to various environmental variables. Optimal conchocelis growth (7.6% volume increase d^{-1}) of *P. abbottae* (Pa) occurred at 11°C, 80 μmol photons m^{-2} s^{-1} and 30ppt salinity. *Porphyra torta* (Pt) grew best (6.5% d^{-1}) at 15°C, 80 μmol photons m^{-2} s^{-1} and 30ppt. *Porphyra pseudolinearis* (Pi) generally had higher growth rates with optimal growth (8.8% d^{-1}) occurring at 7°C, 160 μmol photons m^{-2} s^{-1} and 30ppt. Salinities between 20 and 40ppt and irradiances between 20 and 160 μmol photons m^{-2} s^{-1} generally had little effect on growth rates, but there was virtually no growth at ≤10ppt.

Plant hormones were shown to promote the growth of conchosporangia, which increased by 6.9-31.7% (for Pa), 4.7-25.7% for (Pe, *P. pseudolanceolata*) and 8.9-35.1% for (Pi). Concentrations between 0.4-1.6ppm of kinetin and indole-3-acetic acid at higher temperatures generally had higher stimulatory effects, but Pe had higher volume increase at lower temperatures.

Irradiance, temperature and salinity influenced photosynthesis of the conchocelis. P-I curves, P_{max}, I_{max} and I_c varied with temperature and species. Higher photosynthesis generally occurred at 25-35ppt salinities. Pa had maximal photosynthesis at 11°C and 60 μmol photons m^{-2} s^{-1}, whereas Pi and Pt had maximal photosynthesis at higher temperatures and irradiances. The highest photosynthesis (240 μmol O_2 production g dw^{-1} h^{-1}) of Pa occurred at 11°C, 60 μmol photons m^{-2} s^{-1} and 30ppt. Pi and Pt had optimal photosynthetic rates (200 and 210, respectively) at 15°C, 120 μmol photons m^{-2} s^{-1} and 30ppt. Conchocelis had lower respiration

rates (30-35) at 7°C than at 11 and 15°C (45-58 μmol O_2 consumption g dw^{-1} h^{-1}). All three species exhibited lowest respiration at salinities between 25-35ppt.

Phycoerythrin (PE), phycocyanin (PC), carotenoid (Ca) and chlorophyll *a* (Chl.*a*) contents were significantly affected by irradiance, nutrient concentration and culture duration. For Pa, Pi and Pt, maximal PE (63.2-95.1 mg/g.dw) and PC content (28.8-64.8 mg/g.dw) generally occurred at 10 μmol photons m^{-2} s^{-1}, f/4-f/2 nutrient concentration after 10-20 days, while Pe had highest PE (73.3 mg/g.dw) and PC content (70.2 mg/g.dw) at 10 μmol photons m^{-2} s^{-1}, f nutrient concentration after 60 days. For all four species, highest Ca (3.4 - 6.3) and Chl. *a* content (3.6-8.1 mg/g.dw) generally occurred at 0-10 μmol photons m^{-2} s^{-1}, f/2-f nutrient concentration after 20-30 days. More photosynthetic pigments were generally produced at 0-10 μmol photons m^{-2} s^{-1}, f/4-f nutrient concentration. High irradiances, low nutrients and longer culture duration generally caused a decline of pigment content.

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Chapter 1

General Introduction

Porphyra (Rhodophyta: *Bangials*) occurs universally in the intertidal areas from subtropical to temperate zones around the world and is one of the economically important seaweeds (Baker 1977, Bergdahl 1990, Chiang 1978, Conway 1964, Conway *et al.* 1975, Joshi *et al.* 1992, Kornmann & Sahling 1991, Lewmanomont *et al.* 1993, Lindstrom & Cole 1992, Miura 1975,1988, Mumford *et al.* 1976, Mumford 1980, Munda *et al.* 1978, Ogawa 1978, Piriz 1990, Waaland *et al.* 1986). With its high nutritional value, especially the large percentage of protein as well as its unique delicious flavor, *Porphyra* is very popular and it has long been used as food in many countries, especially in Japan, China and Korea (Chiang 1978, Iwasaki 1965, Li *et al.* 1992, Korringa 1976, Moreland 1979, Mumford *et al.* 1985, Mumford *et al.* 1988, Mumford 1990, Nisizawa *et al.* 1990, Noda *et al.* 1975, 1981, Tseng *et al.* 1947). *Porphyra* mariculture has become the highest valued nearshore fishery in the world, with a growing market demand worldwide for its products. It is estimated that the annual retail value on the Japanese market alone is around 2 billion US dollars. The value of US imports exceeds 25 million US dollars each year (Merrill 1993). Besides its use as food, *Porphyra* has the potential to be used as a source for chemical extracts such as biopigments and some biomedical substances (Abe *et al.* 1967, Abe *et al.* 1971, Amano & Noda 1978, Brooker & Cooper 1961, Chapman 1970, Hoppe, Levring & Tanaka 1979, Vadas 1979, Stekoll & Lindstrom personal comm.).

The life history cycle of *Porphyra* is essentially biphasic and involves an alteration between a macroscopic gametophyte phase (also called the foliose, leafy phase) and a microscopic sporophyte phase(also called the filamentous, conchocelis phase). Conchospores produced by the filamentous phase germinate to produce the haploid *Porphyra* thallus. Later, mature thalli produce spermatangia and carpogonia. Division of the carpogonium following

fertilization is mitotic, forming packets of diploid carpospores which germinate initially in a unipolar fashioned ultimately develop into the highly branched, prostrate filamentous system of the conchocelis phase. Terminal and intercalary cells of the conchocelis phase undergo meiosis and generate files of large, thick-walled cells, the conchosporangia, which release conchospores. Then, conchospores undergo meiosis, germinate and develop into the leafy phase.

Historically, *Porphyra* was harvested from wild stocks growing on natural rocky reefs or from artificial substrates (such as bundles of branches or bamboo, ropes, nets and so on) that had been placed in water. These substrates provided attachment for wild conchospores released by mature conchosporangia in natural habitats (Bardach *et al.* 1972, Chiang 1978, Korringa, 1976). *Porphyra* blades can be harvested several times during a growing season and processed as commercial nori sheets. Early *Porphyra* cultivation depended solely on conchospores naturally present in the seawater. This situation often resulted in variable spore release leading to large fluctuations of production of *Porphyra* (Baker, 1977, Chiang 1978, Chiang & Wang 1980, Hanson *et al.* 1981, Li 1991, Li *et al.* 1992). Drew's discovery of the shell-boring conchocelis stage of *Porphyra* in 1949 and subsequent investigations of *Porphyra* life history led to major breakthroughs in improving *Porphyra* cultivation (Drew 1949). It was found that conchocelis cultures grown under artificially controlled conditions could be induced to produce mature conchosporangia and release conchospores used to seed nets. These nets with "nori seeds" could then be outplanted in natural seawater (Kurogi & Hirano 1956, Kurogi 1959, Tseng & Chang 1955). This method of net seeding greatly advanced *Porphyra* aquaculture, because this method could be used to control the time, place and scale of *Porphyra* cultivation.

Currently, artificial net seeding is done from conchocelis shell cultures. Carpospores are collected from natural, mature *Porphyra* blades. These released spores are then allowed to settle on oyster or scallop shells spread over the bottom of shallow tanks or pools containing seawater.

The carpospores germinate and filaments borrow into the shells, developing into conchocelis stage. After the conchocelis produces mature conchosporangia, conchospores are released and attach to nets which can be used for outplanting (Bird *et al.* 1972, Bird 1973, Campell & Cole 1984, Chen *et al.* 1970, Chiang 1978, Conway 1964, Conway *et al.* 1977, Dawes 1982, Iima & Migita 1990, Korringa 1976, Liu *et al.* 1981, Matsuo *et al.* 1994, Melvin *et al.* 1986, Migita 1972, Migita *et al.* 1987, Notoya *et al.* 1992, 1993, Teraoto *et al.* 1969). Although this style of net seeding from conchocelis shells is used extensively, there still exist a few shortcomings in this operation. For instance, a large amount of space and equipment are needed for maintaining conchocelis shells and many man hours are needed to brush or clean the shells of undesired algae. These factors increase the cost of *Porphyra* production. In addition, because the conchocelis lives within the shells, it is not easy or convenient for growers to judge the instantaneous status of health and development of the conchocelis. It is not unusual for diseases to occur and result in failure of cultures of conchocelis shells during the conchocelis phase (Migita & Abe 1966, Fujita 1990).

There is another alternative approach that can be used in net seeding. Net seeding can be done from the cultures of free-living conchocelis. In this method, cultures of the entire filamentous stage, from carpospore through conchosporangia, are grown in enriched seawater without providing shell substrates for attachment (Chiang & Wang 1980, Dickson & Waaland 1984, Imada & Abe 1980, Li 1988, 1991). This method of producing conchospores has several advantages compared to the shell method.

1). Under artificially-controlled conditions, high densities of conchocelis can be maintained in containers with only a small volume of culture medium. In addition, a minute amount of conchosporangia material will provide plenty of spores for net seeding. Therefore, less space and equipment and fewer facilities are needed.

2). No labor-intensive routines such as brushing and cleaning shells are necessary.

3). During the growth of the filamentous stage, information about the development and health status of free cultures can be conveniently observed and monitored at any time. Remedial action can be taken in case of abnormal growth or disease (such as modification in culture conditions or addition of antibiotics).

4). The incidence of contamination can be reduced leading to high quality production.

Among the interesting and yet little-studied questions about *Porphyra* are physiological and ecological aspects of the conchocelis stage. Relatively speaking, the microscopic sporophytes of *Porphyra* are more difficult to study, especially in the field, so most of the research has focused on the macroscopic gametophytes. The sporophytic stage (*i.e.* juvenile or conchocelis stage) is very important to successful *Porphyra* aquaculture. A few studies have been conducted on the influence of environmental conditions on conchocelis growth with regard to individual factors such as temperature, irradiance and daylength (Dring 1967, Kapraun & Luster 1980, Waaland *et al.* 1987). Sidirelli (1992) reported that the optimal culture conditions are 15°C temperature, 40 μmol $m^{-2}s^{-1}$ irradiance with 16L:8D photoperiod for the conchocelis growth of *Porphyra leucosticta*. Waaland *et al.* (1987) reported that high conchocelis growth rates occur at 10-15°C, 25-100 μmol $m^{-2}s^{-1}$, 16L:8D for *Porphyra torta*. The influence of different nitrogen sources and concentrations on conchocelis growth of *Porphyra haitanensis* has been studied (Meiqin *et al.* 1979). Optimal conditions have been obtained for culture of conchocelis of *P. columbina* at a water temperature of 15°C, 10-50 μmol $m^{-2}s^{-1}$ of irradiance and seawater nitrogen concentrations above 100 μmol L^{-1} (Frager & Brown 1995).

As for research on photosynthesis and pigments of *Porphyra*, many reports have appeared. However, almost all of these experiments dealt with only the gametophyte stage of

Porphyra species. For instance, a wide variety of environmental factors have been examined to investigate their influence on leafy blades of *Porphyra*. Such environmental factors as temperature (Chang *et al.* 1983, Wu *et al.* 1984, Smith & Berry 1986, Gao & Aruga 1987), salinity (Oqata *et al.* 1971, Reed *et al.* 1980, Wiencke & Lauchi 1980, Satoh *et al.* 1983, Chang *et al.* 1983), irradiance (Herbert 1984), desiccation (Fork & Oequist 1981, Levitt & Bolton 1991, Lipkin *et al.* 1993), diurnal rhythm (Oohusa *et al.* 1978, 1980, Coutinho 1984), light wavelength (Luening & Dring 1985), nutrients and dissolved inorganic carbon (Zavodnik 1987, Kapraun *et al.* 1987), seawater pH (Gao & Zhao 1988) and seawater current speed (Gao *et al.* 1991) were shown to exert significant impact on photosynthetic or respiratory activities of *Porphyra* blades.

Similarly, many reports have been published on photosynthetic pigments of *Porphyra* blades. For example, Gao *et al.* (1991) studied the chemical properties and components of photosynthetic pigments and phycobiliproteins of *P. haitanensis* from China. Chlorophyll contents of *P. umbilicalis* have been studied in relation to temperature, irradiance and photoperiod (Fortes & Luening 1980). Subunits, chemical structure and compositions of phycocyanin from *P. tenera* have been analyzed (Fujiwara *et al.* 1985). A few comparative studies have been done on light-harvesting pigment contents of photosynthesis from different red algae or different strains of *Porphyra* species (Amano & Noda 1978, Czeczuga & Taylor 1983, Merrill *et al.* 1983, Fujita & Migita 1984). Lopez *et al.* (1991) investigated the effect of light pulses with different wavelength on chlorophyll, phycoerythrin and phycocyanin synthesis in *Porphyra umbilicalis*.

As mentioned above, although many reports can be found in the literature on various aspects of *Porphyra* research, including growth and photosynthesis in leafy thalli stage of *Porphyra* species, very few studies have examined and investigated the growth and photosynthetic features regarding free-living filamentous stage of *Porphyra* species.

Specifically, no studies have been reported on the combined effects of multiple factors on conchocelis growth and photosynthetic physiology. Only one paper has studied photosynthesis and pigment analysis of *Porphyra* conchocelis (*P. leucosticta*) and only light was taken into consideration as a variable in this experiment (Sheath *et al* 1977). Many different environmental factors may affect *Porphyra* conchocelis growth. Specific *Porphyra* species may demonstrate different responses to environmental variables. More research and experiments are needed to deepen and broaden our knowledge of *Porphyra* conchocelis physiology and ecology.

In natural habitats, the microscopic sporophytes (conchocelis stage) of *Porphyra* probably occur in intertidal areas or probably extend to subtidal areas, yet little is known about their ecological significance as a kind of particular plant style. Although conchocelis are hardly ever observed and noticed in the conventional survey of coastal vegetation, they may play a role in improving the habitat quality of some micro-environments for other benthic organisms because conchocelis have the ability to perform photosynthesis even if the environmental irradiance available is so low that other plants might not possess net photosynthesis. In addition, despite their rarely being seen and noticed the conchocelis are one of the members of the algal community where they take place and may be an important member that should be taken into consideration with respect to ecological significance. Because the gametophyte (leafy) stage of *Porphyra* comes from the conchocelis, the growth and survival of microscopic conchocelis stage will determine and affect the leafy stage of *Porphyra* in many aspects such as occurrence and existence, abundance and biomass of leafy *Porphyra* populations, which maybe extend their distribution in intertidal or subtidal areas and form densely-populated vegetation in some proper habitats.

However, in natural habitats, because *Porphyra* conchocelis penetrate into and live in calcareous shells, some practical and potential obstacles could be encountered for the

investigation of physiology and ecology of *Porphyra* conchocelis in a field study. For example, it may not be easy to obtain the necessary samples for analysis. Moreover, it may be difficult to observe and measure the growth of conchocelis or other physiological indicators. It may be also difficult to determine how environmental variables affect conchocelis or to what extent environmental variables affect conchocelis.

Because of these difficulties in a field study, it is necessary to conduct the experimental research under controlled laboratory conditions. Free-living conchocelis can be used to investigate and understand critical physiological and ecological aspects of conchocelis stage for different *Porphyra* species.

The sporophyte stage (*i.e.* conchocelis stage) is very important to successful *Porphyra* aquaculture. Environmental factors should be examined to investigate their influences on important physiological processes and biochemical composition of the *Porphyra* conchocelis stage. Such research is critically needed prior to the establishment of a nori mariculture industry. It is especially important to determine what are the optimal conditions for healthy conchocelis and conchosporangia culture of *Porphyra* species. Basic information and research are needed on growth, photosynthetic physiology and photosynthetic pigments for *Porphyra* sporophyte stage. Unfortunately, up to now, we have little understanding of this basic biological information on Alaskan *Porphyra* species. The research reported here involves both applied and basic sciences. This research relates closely to preserving, enhancing and effectively utilizing Alaskan natural resources. Information and data critical to the establishment of a *Porphyra* mariculture industry in Alaska can be obtained through these experimental research.

There is good potential for commercial scale *Porphyra* aquaculture in Alaska. All the operations and activities of *Porphyra* aquaculture industry are permitted and encouraged in Alaska through the Aquatic Permit Program. A number of local organizations and individuals

have expressed interest in *Porphyra* farming. Relatively pristine, unpolluted coastal waters provide ideal conditions for *Porphyra* cultivation in Alaska. There exist about 25 species of *Porphyra* in Alaska. Among them, some have sociological importance and potential commercial value. For example, *Porphyra abbottae* has been utilized as a traditional food by the indigenous people of British Columbia and southeast Alaska and is currently harvested, processed and sold as "black seaweed" by southeast Alaskan Natives. *Porphyra pseudolinearis* and *P. torta*, which occur naturally near Juneau and in other coastal areas of Alaska, are also potential species for successful mariculture. Not only would *Porphyra* aquaculture industry increase employment opportunities for coastal communities and strengthen the state' economy, but also be an effective way to conserve and utilize Alaskan natural resources.

Light is without doubt the most important factor affecting *Porphyra* conchocelis stage. The continuous ebb and flood of tides have a profound effect on the quantity and quality of the sun's energy reaching *Porphyra* and add greatly to the variation already present in irradiance at the seawater surface. The primary importance of light to *Porphyra* is in providing the initial energy for photosynthesis, and ultimately for all biological processes. Temperature is another fundamental factor for plants because of its effects on molecular enzyme activities and properties in plant cells, and hence on virtually all aspects of plant metabolism. The aspects of salinity that are of biological significance are ion concentration, density of seawater, and especially, osmotic pressure. Photosynthesis, respiration and growth of the plant all tend to have optimum salinities, just as they have optimum temperatures. In addition, nutrient supply is very important for the growth and development of *Porphyra* conchocelis. Because *Porphyra* conchocelis inhabit intertidal zones, they have to tolerate a constantly varying environment, including the variations in temperature, salinity, light and nutrient availability. Undoubtedly, there exist some interactions between conchocelis and their physicochemical environment. There are numerous

freshwater inputs from streams and rivers along the coastal waters of southeast Alaska. High annual rainfall, glacier and snow melting may further cause a significant decrease in surface water salinities in the summer and fall seasons (Stekoll, 1998). All of these freshwater inputs not only result in a wide salinity fluctuation in the coastal waters, but also in variations of temperature and/or light conditions of the environment for *Porphyra* conchocelis occurring in these habitats. Consequently, these environmental variables will have an important impact on the growth and survival of conchocelis stage of *Porphyra* because of potential physiological stress on conchocelis. Therefore, among the major environmental factors affecting conchocelis stage of *Porphyra* are light, temperature, salinity, and nutrient availability, naturally these factors should be considered in investigating the physiology and ecology of *Porphyra* conchocelis. Further study of interactions between these factors will throw more light on physiological and ecological characteristics of *Porphyra* conchocelis stage in response to the varying environment.

Biogeography, physiological and ecological characteristics of *Porphyra* conchocelis occurring in a given region actually represent and reflect the entire outcome of historical evolution and their interactions with environmental variables, because different geographical distributions (for different plant species or same species) will result in unique physiological and ecological characteristics in adaptation to a given region as a result of the historical evolution and the long-term influencing impact of environmental variables of that given habitat. This case is also true for *Porphyra*, a universally-distributed plant group. Since there is very little information available on the physiological ecology of *Porphyra* conchocelis, especially for the species occurring in high-latitude (subarctic) areas, it is necessary and important for us to conduct such research. With research results on the relationships between conchocelis stage and the environment, a more comprehensive picture can be demonstrated regarding physiological and ecological aspects of *Porphyra* conchocelis. Furthermore, such research will also provide

information on those species having the best potential for mariculture, based on their ability to tolerate environmental variations and on their physiological and ecological characteristics from the experimental investigations.

Objectives of Research

Growth rate, photosynthetic activity and photosynthetic pigment contents are appropriate indicators that reflect the physiological state of the *Porphyra* conchocelis stage in response to varying environmental conditions. Therefore, the overall objective of this research is to investigate the effects of environmental factors on the sporophytic stage of some Alaskan *Porphyra* species through analysis of morphological (the growth rate of conchocelis cell), physiological (photosynthesis and respiration) and biochemical (photosynthetic pigment content) characteristics of these *Porphyra* species and to determine what are the optimal conditions for the sporophyte stage culture of these *Porphyra* species.

The research had several specific objectives:

1) to investigate the effect of environmental factors (temperature, salinity, irradiance) on the growth of *Porphyra* conchocelis and to determine the optimal environmental conditions for the growth of *Porphyra* conchocelis.

2) to investigate the effect of environmental factors (temperature, plant hormone concentration and type, photoperiod) on the volume increase of *Porphyra* conchosporangia and to determine the growth-stimulating effects of different plant hormones.

3) to determine the effect of environmental factors (temperature, salinity, irradiance) on photosynthetic and respiratory activities of *Porphyra* conchocelis and to determine favorable culture conditions in term of photosynthetic activity.

4) to study the effect of environmental factors (irradiance, nutrient concentration and culture duration) on photosynthetic pigment contents of *Porphyra* conchosporangia and to determine favorable culture conditions for maximum production of phycobiliprotein.

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Chapter 2

Conchocelis Growth of Three Indigenous Alaskan *Porphyra* Species: Response to Environmental Variables

Abstract

Experiments were performed to determine the range and optima of environmental parameters under which indigenous species of Alaskan *Porphyra* can grow. Growth in enriched media under varying conditions of irradiance (20, 40, 80 and 160 μmol photons m^{-2} s^{-1}), temperature (7, 11, 15 and 19°C) and salinity (5, 10, 20, 30 and 40ppt) were measured for the conchocelis phase of *Porphyra abbottae*, *P. torta* and *P. pseudolinearis* under long day conditions (16L: 8D). Optimal growth (7.6% increase in volume per day) of *P. abbottae* occurred at 11°C, 80 μmol photons m^{-2} s^{-1} and 30ppt salinity. *Porphyra torta* grew best (6.5% d^{-1}) at 15 °C, 80 μmol photons m^{-2} s^{-1} and 30ppt salinity. *Porphyra pseudolinearis* generally had higher growth rates than the other two species with optimal growth (8.8% d^{-1}) occurring at 7°C, 160 μmol photons m^{-2} s^{-1} and 30ppt. For all three species salinity had little effect on growth between 20 and 40ppt, but there was virtually no growth at salinities of 10ppt and below. Irradiances between 20 and 160 μmol photons m^{-2} s^{-1} generally had little effect on growth rates. However, growth of *P. abbottae* increased with irradiance at 7°C but was inhibited at irradiances over 40 μmol photons m^{-2} s^{-1} at 15°C and higher temperatures. *Porphyra torta* also showed growth inhibition at temperatures of 15°C at higher irradiances. *Porphyra pseudolinearis* appeared to be the most robust species with respect to tolerance to extremes of salinity and irradiance.

Introduction

Porphyra mariculture, the world's highest valued nearshore fishery, is also one of the world's major aquacultural crops. The annual retail value for nori, the major product of *Porphyra*, is about US$2 billion on the Japanese market alone, and US imports of nori, valued at over US$25 million, have increased tenfold in the last ten years (Mumford & Miura, 1988; Merrill, 1993; Steve Crawford, personal communication). There is a growing worldwide market for this and other *Porphyra* products.

Interest in *Porphyra* farming in western North America has involved both Japanese cultivars and indigenous species (Bergdahl, 1990; Mumford, 1990). Woessner and colleagues (1974, 1977), who conducted biological and economic studies on *Porphyra nereocystis* at University of California at Santa Barbara, estimated that the natural crop within a 55-mile (90 km) distance along the California coastline could be worth over half a million dollars. Waaland *et al.* (1986) identified five native North American species with commercial potential, and they established optimal conditions for maturation and release of conchospores of these species (Waaland *et al.*, 1987, 1990). They have also grown these species to harvestable size in experimental farms set up in the waters of Puget Sound. Their studies provided the basis for our own investigations into optimal conditions for growth of species occurring in southeast Alaska. One of the species farmed by the Japanese (*P. pseudolinearis*) occurs naturally near Juneau and in other parts of coastal Alaska to the west. Four of the species studied by Waaland and colleagues grow in Alaska, and an additional ten species are known to occur in Southeast Alaska (Lindstrom & Cole, 1992; Scagel *et al.*, 1989). Although the essential technology for *Porphyra* aquaculture in northwest America was established in Washington State, with modifications made in British Columbia and Maine, further modifications are required for Alaska because of the requirement to use native rather than an imported, previously domesticated species.

Mariculture of all species of *Porphyra* utilizes artificial control of the life cycle to regulate the production of spores for seeding nets. Thus, it is imperative that we understand the factors that affect growth of the conchocelis and induce conchospore production (as well as other aspects of *Porphyra* development) at both a practical level as well as at a basic biological level. Such understanding will help to avoid the "boom or bust" cycles associated with natural production. This investigation comprises the first phase of a project to domesticate Alaskan *Porphyra* species. We report here on the growth of the conchocelis phase of three species of Alaskan *Porphyra*.

Materials and Methods

Unialgal cultures of each *Porphyra* species (*Porphyra abbottae* Krishnamurthy - strain PaJB03, *P. pseudolinearis* Ueda - strain PiSC06 and *P. torta* Krishnamurthy - strain PtCH03) were obtained from carpospore release. Mature blades of the gametophyte stage of each species were collected from the field. Blades were washed and scrubbed with sterile seawater to remove surface contamination. The cleaned blades were placed in sterile seawater in petri dishes for carpospore release. After 24-36 hours the blades were removed and the dishes incubated in Provasoli's enriched seawater (PES; McLachlan, 1973) under 16L:8D photoperiod at 15°C. Conchocelis segments (around 110-250 μm) of each species were placed in cell well plates (one piece per well) and incubated at 33ppt salinity and 11°C (100-120 μmol photons m^{-2} s^{-1} irradiance) or 15°C (140-160 μmol photons m^{-2} s^{-1}) for the culture of pure genotype conchocelis. PES enriched seawater culture medium was used.

Conchocelis growth experiments were conducted in several incubators that had been set at different temperatures and illuminated with cool-white fluorescent lamps. Irradiance gradients

were obtained by wrapping the culture containers with varying layers of white paper. Autoclaved natural seawater-based PES medium, with a GeO_2 concentration of 1.25 mg L^{-1}, added to inhibit diatom growth, was used in the growth experiments. At the beginning of experiments, the pH of the culture medium was adjusted to 7.6-7.8 (the ambient pH of the seawater in the inside waters of SE Alaska) using 6 M HCl or 6 M NaOH. Experimental seawater with different salinities was obtained either by boiling natural seawater (for 40ppt salinity) or by diluting natural seawater with distilled water. Nutrients were added after salinities were adjusted. For the growth experiments different levels of three environmental factors were employed as follows:

Temperature: 7, 11, 15, 19°C

Salinity: 5, 10, 20, 30, 40ppt

(*P. torta* was tested at 15, 20, 30, 40, 50ppt)

Irradiance: 20, 40, 80, 160 μmol photons m^{-2} s^{-1}

All of the conchocelis fragments died quickly at 5ppt and therefore no data analysis was done for growth at this salinity.

Corning cell wells (24 wells with lids) were used as culture containers. About 4 ml of culture medium (PES) were placed in each cell well. A fully factored experiment was employed using all combinations of the three environmental factors. The growth of free conchocelis was observed and recorded under the experimental conditions. For each experimental combination, four replicate wells were used each with 4-7 small, spherical conchocelis tufts per well. Culture media were changed every 15 days. Long day (16L: 8D) photoperiods were used. Conchocelis tufts were measured for their diameters with a microscope and their volumes were estimated using the formula for the volume of a sphere $V = (1/6) \cdot \pi \cdot D^3$ (V and D respectively represent

the volume and the diameter of conchocelis tuft). Growth was determined from the volume increase of the filamentous tufts as calculated from the mean diameters at the beginning and the end of experiments. Conchocelis specific growth rates (μ) were calculated as the mean per cent volume increase per day (± SE) using the formula:

$$\mu = \frac{100[\ln(Vt / Vo)]}{t}$$

in which V_t and V_0 represent respectively the mean tuft volume in every well at the end and the beginning of the experiment, and t is the number of days. The equation assumes that growth was exponential (DeBoer *et al.*, 1978). The experiment lasted 31 d for *P. abbottae*, 26 d for *P. pseudolinearis*, and 31 d for *P. torta*.

Statistical analyses of the experimental data

Growth rate differences were initially analyzed by a three-way model I ANOVA (growth as a function of light, temperature and salinity) using S-Plus 3.1 for windows (Statistical Sciences, Inc. 1993). Post hoc tests were performed using the Newman-Keuls multiple comparison test (Zar, 1996) to identify which tested factors were important in controlling growth of the conchocelis. Statistical power analysis for main effect factors was conducted according to Cohen's methods (Cohen, 1988).

Results

Growth of *Porphyra abbottae*

The growth of the conchocelis of *Porphyra abbottae* was influenced by all three factors (Figure 2.1, Table 2.1). At low salinity (10ppt) growth was virtually nil, and cells became bleached after 8-10 days. Higher salinities promoted growth rates of nearly 8% d^{-1}, with best

growth rates at 20-30ppt salinity. The optimal temperature was 11°C in this salinity range. Growth was significantly greater at this temperature ($P<0.01$, Figure 2.4). However, growth was affected by the interaction of temperature and irradiance. Higher temperatures (15-19°C) combined with the higher irradiances (80-160 μmol photons m^{-2} s^{-1}) inhibited growth profoundly. This result is in contrast with light effects at lower temperatures, where growth was often greater at the higher irradiances (*e.g.* 30 and 40ppt, Figure 2.1). Growth was still reasonable at the higher temperatures if lower irradiances were employed. For example at 30ppt and 19°C, growth at 80-160 μmol photons m^{-2} s^{-1} was nearly zero, but was 5.0-5.4% d^{-1} at 20-40 μmol photons m^{-2} s^{-1}. The maximum growth (7.6% d^{-1}) was achieved at 30ppt salinity, 11°C and 80 μmol photons m^{-2} s^{-1} irradiance. *P. abbottae* became conchosporangial under all of the conditions used in this experiment.

Growth of *Porphyra pseudolinearis*

Growth of the conchocelis of *Porphyra pseudolinearis* was also near zero at 10ppt salinity, similar to that of *P. abbottae* (Figure 2.2). The ANOVA model showed significant effects only with respect to salinity (Table 2.1). Peak growth occurred at 30ppt. Unlike *P. abbottae*, *P. pseudolinearis* was not strongly inhibited by combinations of high temperatures and high irradiance (Figure 2.2). The range of tolerance was greater for *P. pseudolinearis* than for *P. abbottae*. Growth was relatively high at all temperatures and irradiances tested. Growth was independent of irradiance from 20 to 160 μmol photons m^{-2} s^{-1} ($P>0.05$, Figures 2.2 and Figure 2.4). There was a slight trend for growth to be inversely proportional to temperature at the highest irradiance tested. Optimal growth (8.8% d^{-1}) occurred at 30ppt salinity, 7°C, and 160 μmol photons m^{-2} s^{-1} irradiance. Morphologically, *P. pseudolinearis* remained in the vegetative state throughout the experiment.

Growth of *Porphyra torta*

Porphyra torta growth rates were generally lower than that of the other two species (Figure 2.3). ANOVA results showed that growth was significantly affected by salinity (Table 2.1) and temperature but not by irradiance under the conditions tested. We did not test *P. torta* at 10ppt since the other species did not grow at that salinity. But the growth of *P. torta* at 15ppt was fairly low except at 7°C, the lowest temperature tested (Figure 2.3). Growth at 30ppt was significantly greater than at the other salinities tested ($P<0.01$, Figure 4). *P. torta* had significantly greater growth rate at 7°C and 15°C than at 11°C or 19°C ($P<0.01$, Figure 2.4). There was some indication from the data that *P. torta* growth was inhibited by high light at 19°C, but the effect was not as drastic as that in *P. abbottae*. The optimal culture condition for the growth of *P. torta* was 30ppt salinity, 15°C and 80 μmol photons m^{-2} s^{-1} irradiance (6.5 % d^{-1}). *P. torta* was conchosporangial throughout the experiment.

Growth Difference Between Species

Comparison of pooled conchocelis growth rates of three species of *Porphyra* for each parameter tested (for comparison of differences between species) is shown on the right column of Figure 2.4. Overall, *P. pseudolinearis* had the best average growth rate, with *P. abbottae* having the second highest growth rate for all of factorial levels with the exception of 19°C (Figure 2.4). Salinities of 30ppt were optimal for all three species, although they were tolerant of 20ppt (Figure 2.4). The conchocelis in all treatments were killed by 5ppt salinity. Irradiance was generally not a factor, but the growth rate of each species differed from the others at each irradiance tested with the exception of 80 and 160 μmol photons m^{-2} s^{-1} for *P.abbottae* and *P. torta*. Comparison of the pooled conchocelis growth rates (grand average value) for three species of *Porphyra* indicated there was a significant difference in growth between different species. *P. pseudolinearis* had significantly higher growth rate (7.2% increase in volume per

day) than the other two species, with *P. abbottae* having the second highest growth rate (4.6% increase in volume per day) and *P. torta* the lowest growth rate (3.7% increase in volume per day, Figure 2.5).

Statistical power (1-β) analysis

The results of statistical power (1-β) analysis indicated that main effect factors (salinity, temperature, irradiance) have high power values for all species tested (>0.99, Table 2.2).

Discussion

Growth of *Porphyra* in various salinities was of interest due to the fact that the inside waters of the Alexander Archipelago in Southeast Alaska have numerous freshwater inputs from streams and rivers along the coast. The high annual rainfall and snow melt cause a significant decrease in surface water salinities in the summer and fall seasons (Stekoll, 1998). Most of the more accessible sites for the aquaculture of *Porphyra* in southeast Alaska are located in the inside waters. For economic and practical reasons it is necessary to understand the salinity tolerances of the various potential commercial species. Results from this study indicate that salinities of 20 to 35ppt would be optimal for the culture of *Porphyra* conchocelis, but that conchocelis growth begins to decline at salinities of 15ppt and less.

In these experiments, *Porphyra abbottae* conchocelis exhibited photoinhibition of growth at moderate and higher irradiances (above 40 μmol photons m^{-2} s^{-1}) when grown at relatively high temperatures (15°C and above). It is interesting to note that the growth of the blade phase of this species is also reduced at higher irradiances (140 μmol photons m^{-2} s^{-1}) and higher temperatures (12°C, Hannach & Waaland, 1989).

The higher growth rate of *Porphyra* pseudolinearis compared to the other two species may result from the conchocelis remaining mostly vegetative in this species compared to the 100% conchosporangial condition of the *P. abbottae* and *P. torta* conchocelis. More energy in *P. pseudolinearis* goes into growth in filament length, which is what we measured, rather than filament width, which is significantly greater in the conchosporangial thalli.

The tolerance of the conchocelis of *Porphyra* pseudolinearis to a wider range of salinities, irradiances and temperatures than the other two species fits with the local distribution of this species in southeast Alaska. Whereas *P. abbottae* and *P. torta* are widely and abundantly distributed on the outer coast of southeast Alaska, extending to the inside waters only up major straits with direct connection to the outer coast, *P. pseudolinearis* has to date been recorded only from a limited stretch of coastline near Juneau, Alaska, several straits removed from the outer coast (Lindstrom *et al.*. 1986). As mentioned above, this area experiences wider temperature, salinity, and irradiance (due to reduced visibility during intense spring plankton blooms and glacial run-off in summer) fluctuations than the outer coast (Stekoll, 1998). The persistence of this species in this area requires a highly tolerant cryptic phase (the conchocelis) to allow it to survive in this area year after year.

Measured growth rates for *P. torta* were similar to those of Waaland *et al.* (1987) who used strains from Puget Sound. The growth of *P. torta* from Puget Sound was significantly affected by irradiances over the range of 5-300 μmol photons m^{-2} s^{-1}, whereas I saw no significant effect of irradiance on the Alaska strain of *P. torta*. However, my tested range of irradiances was narrower. Both the Alaska and Puget Sound strains had optimal growth around 15°C at intermediate irradiances.

Porphyra mariculture offers an opportunity to develop a new industry in Alaska for seafood and seafood-based products for which markets already exist both locally and globally.

The sale and barter of locally harvested species of *Porphyra* still occur among Natives in southeast Alaska, First Nations peoples in British Columbia, and Japanese-Canadians in southern British Columbia. These existing networks could provide the first market entry for a product from indigenous species (Roberts, 1993). At the national level, the primary markets for *Porphyra* are Japanese restaurants, Oriental food stores, health food outlets, and chemical companies. The research reported here is fundamental to the successful culture of *Porphyra* in Alaska. It will be necessary to produce spores reliably and in quantity in order to seed nets for the production phase. Nets must be seeded with the species and spore density that growers demand when they need them. Nets need to be provided in the quantity needed for a commercial level of activity. Since environmental cues for reproduction are species- and possibly even population-specific, stocks selected from the wild must be manipulated in the laboratory to obtain this information.

Conclusions

Culture studies with indigenous strains of Alaskan *Porphyra* species showed the range of environmental conditions under which growth was successful. *Porphyra abbottae* because of its existing market value in Alaska is of special interest. However, this species is more difficult to culture, in part because of its sensitivity to certain combinations of irradiances and temperatures. Its sister species *Porphyra torta* may be more amenable to domestication. However, *P. torta* quickly becomes conchosporangial in free culture, making it difficult to inoculate shells for net seeding. The tolerance of *P. pseudolinearis* to a wider range of environmental conditions and its ability to grow in or tolerate relatively low salinities are useful traits for a commercial species in Southeast Alaska. Further work on domestication of this genus is necessary before selection of a candidate species for commercialization in Alaska.

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Table 2.1. ANOVA table for growth of conchocelis of three different *Porphyra* species at combinations of salinity, irradiance and temperature. [a] 10, 20, 30, 40ppt; [b]7, 11, 15, 19°C; [c]20, 40, 80, 160 μmol photons m^{-2} s^{-1} ; [d]15, 20, 30, 40, 50ppt (* P<0.05, **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Salinity[a]	3	12.211	4.070	387.38**
Temperature[b]	3	2.221	0.740	70.45**
Light[c]	3	0.916	0.305	29.06**
Sal. x Temp.	9	0.469	0.052	4.96**
Sal. x Light	9	0.099	0.011	1.05
Temp. x Light	9	2.609	0.290	27.59**
Sal. x Temp. x Light	27	0.483	0.018	1.70*
Residuals	192	2.017	0.011	
P. pseudolinearis				
Salinity[a]	3	71.216	23.739	238.74**
Temperature[b]	3	0.376	0.125	1.26
Light[c]	3	0.687	0.229	2.30
Sal. x Temp.	9	1.552	0.172	1.73
Sal. x Light	9	2.052	0.228	2.29
Temp. x Light	9	1.745	0.194	1.95*
Sal. x Temp. x Light	27	4.996	0.185	1.86**
Residuals	192	19.091	0.099	
P. torta				
Salinity[d]	4	2.751	0.688	38.53**
Temperature[b]	3	0.532	0.177	9.93**
Light[c]	3	0.124	0.041	2.32
Sal. x Temp.	12	0.683	0.057	3.20**
Sal. x Light	12	0.293	0.024	1.37
Temp. x Light	9	0.565	0.063	3.52**
Sal. x Temp. x Light	336	1.521	0.042	2.37**
Residuals	240	4.288	0.018	

Table 2. 2. Statistical power (1- ß) based on the results of variance analysis for experiments with the growth rate of *Porphyra* conchocelis. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 10%. Significant criterion α is equal to 0.05. (** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Salinity	3	387.38**	3	49	1.8143	>0.99
Temperature	3	70.45**	3	49	1.2422	>0.99
Light	3	29.06**	3	49	1.2012	>0.99
Porphyra pseudolinearis						
Salinity	3	238.74**	3	49	1.5205	>0.99
Temperature	3	1.26	3	49	0.8341	>0.99
Light	3	2.30	3	49	0.8354	>0.99
Porphyra torta						
Salinity	4	38.53**	4	39	1.8663	>0.99
Temperature	3	9.93**	3	49	1.6540	>0.99
Light	3	2.32	3	49	1.6220	>0.99

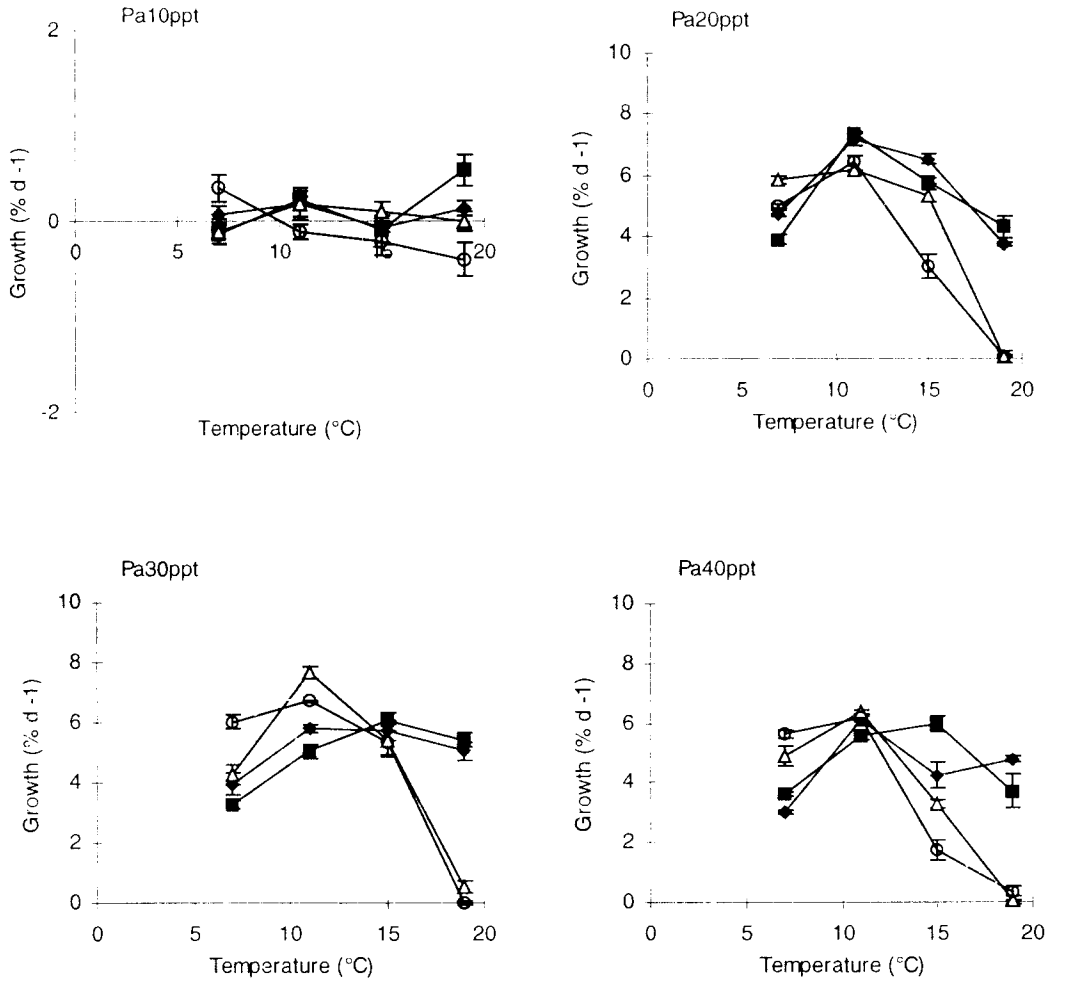

Figure 2.1. *Porphyra abbottae* (Pa). Conchocelis growth as a function of salinity (ppt), irradiance (◆, 20; ■ , 40; Δ, 80; O,160 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Growth rate is expressed as percent increase in volume per day. Note the y-axis scale for 10ppt is different from the others. Negative growth rates are a consequence of the sampling design.

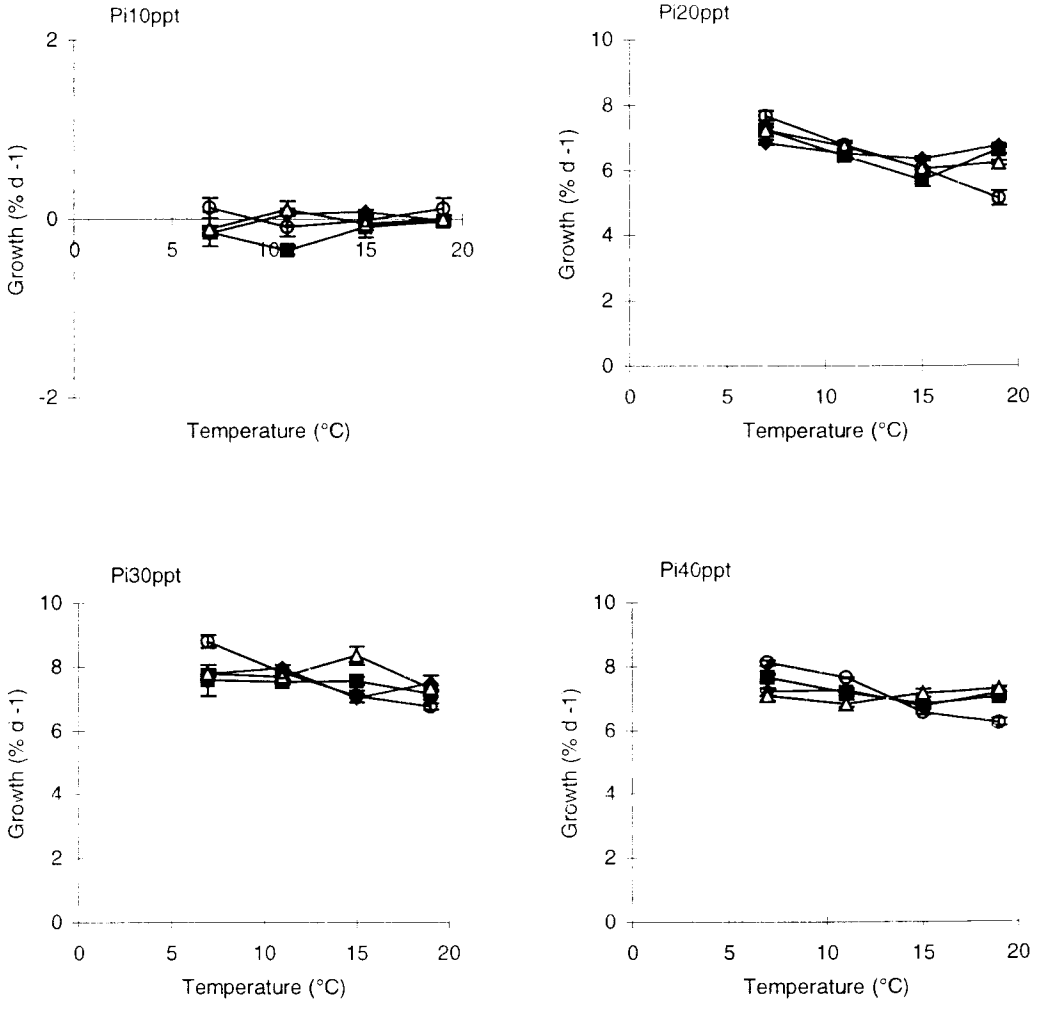

Figure 2.2. *Porphyra pseudolinearis* (Pi). Conchocelis growth as a function of salinity (ppt), irradiance (◆, 20; ■, 40; Δ, 80; O,160 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Growth rate is expressed as percent increase in volume per day. Note the y-axis scale for 10ppt is different from the others. Negative growth rates are a consequence of the sampling design.

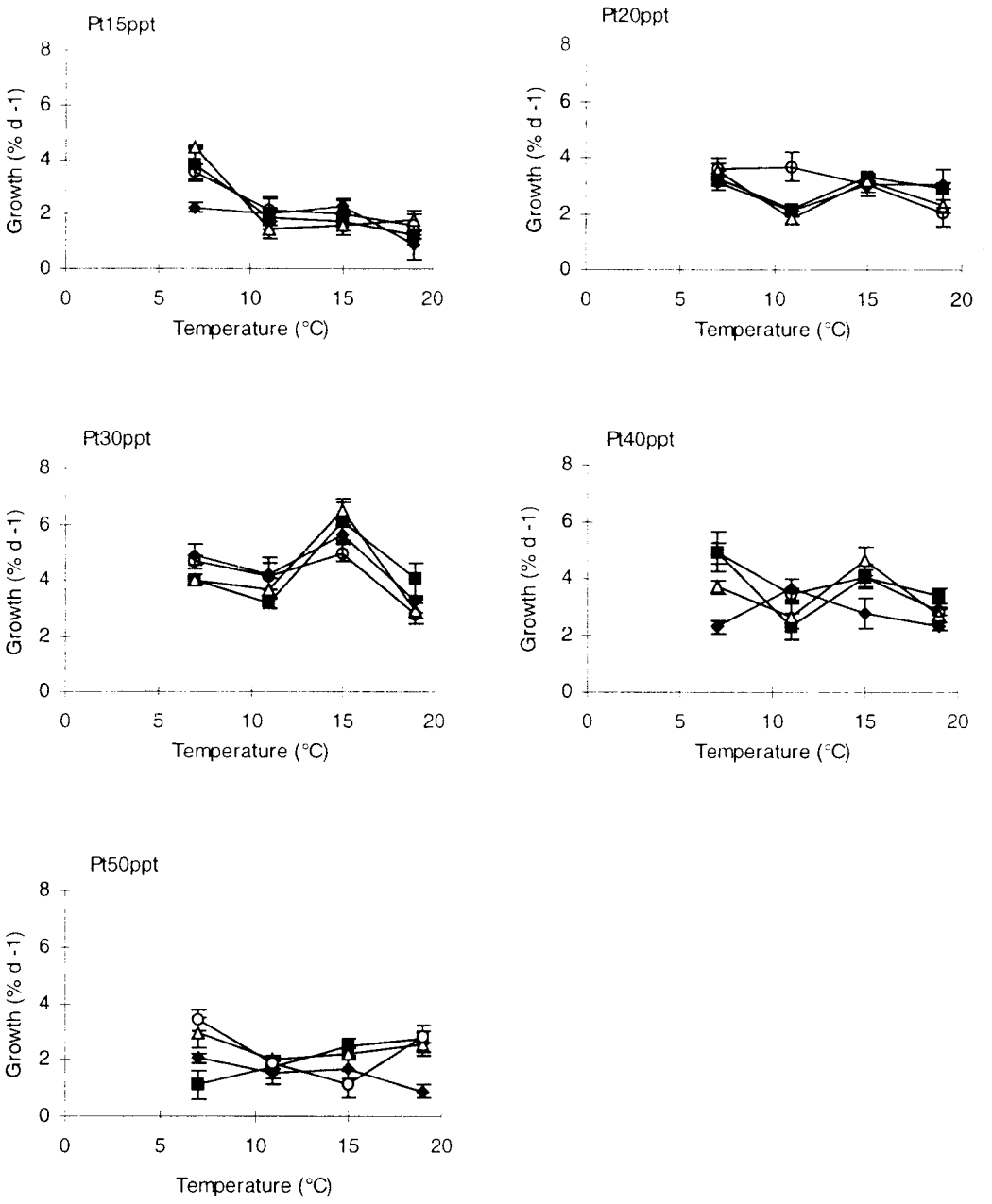

Figure 2.3. *Porphyra torta* (Pt). Conchocelis growth as a function of salinity (ppt), irradiance (◆, 20; ■, 40; Δ, 80; O,160 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Growth rate is expressed as percent increase in volume per day.

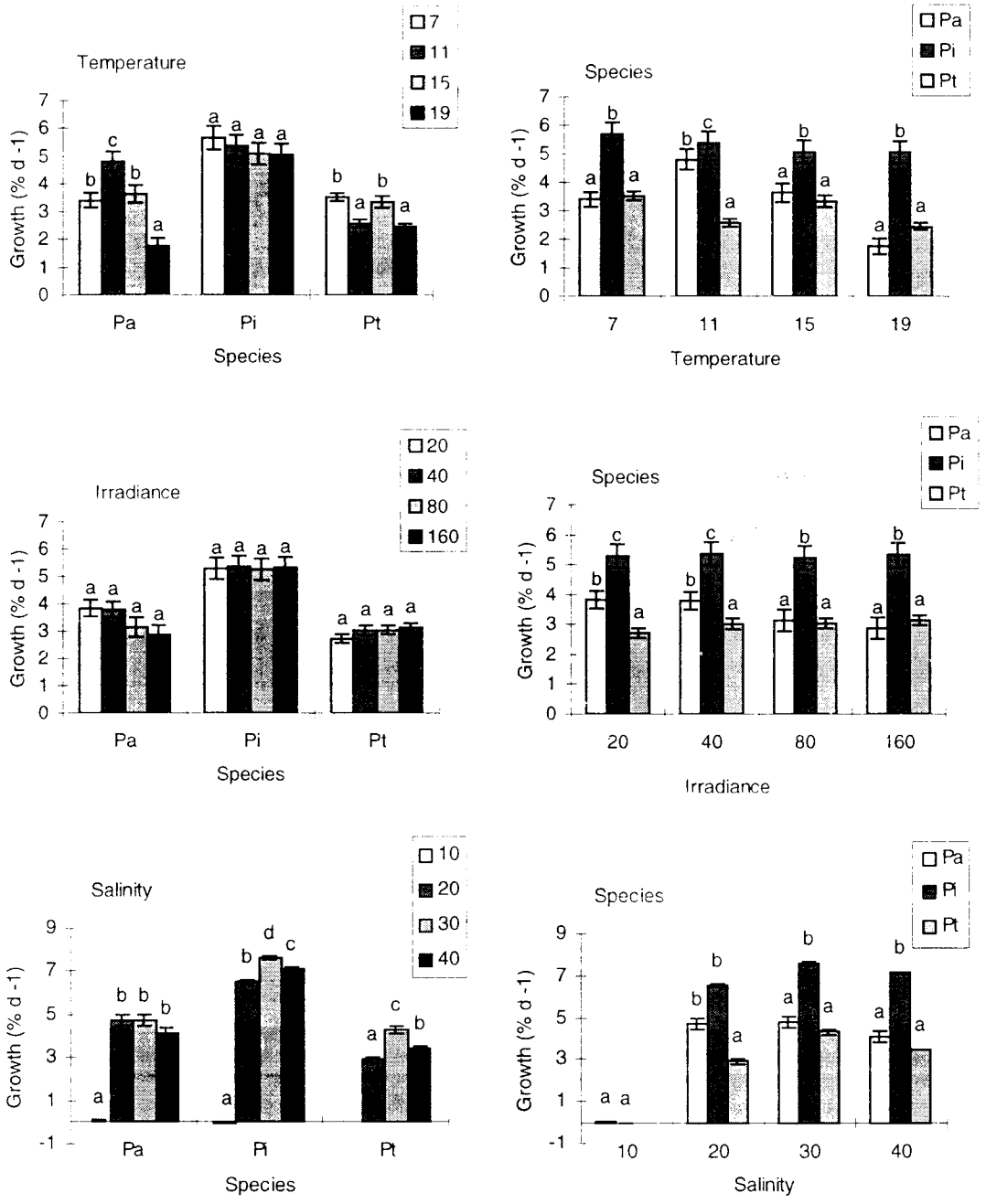

Figure 2.4. Comparison of pooled conchocelis growth rates of three species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only within a specie (for the figures on the left side) and letter comparisons are relevant only between specie (for the figures on the right side). Units of parameters tested are: temperature (°C), irradiance (μmol photons m^{-2} s^{-1}) and salinity (ppt).

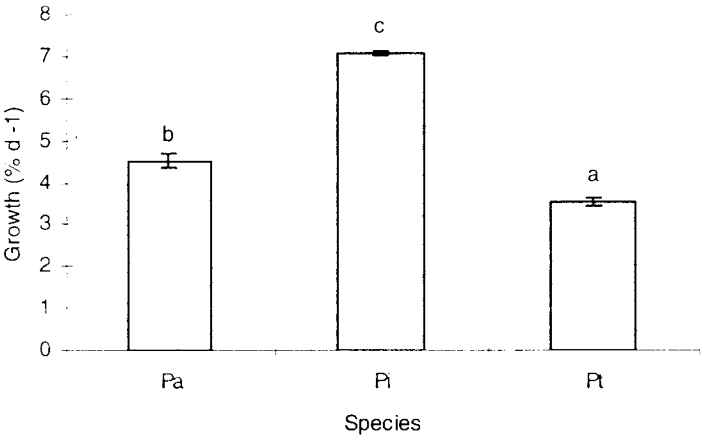

Figure 2.5. Comparison of pooled conchocelis growth rates (grand average value) for three species of *Porphyra*. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test.

Chapter 3

Effects of Plant Hormones on Conchosporangia Growth of Three Indigenous Alaskan *Porphyra* Species in Conjunction with Environmental Variables

Abstract

Experiments were conducted to investigate the effects of plant hormones on the conchsporangia growth of three indigenous species of Alaskan *Porphyra* (*P. abbottae*, *P. pseudolanceolata*, *P. pseudolinearis*). Volume increases were measured under different combinations of culture conditions of hormone concentrations (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 ppm), hormone type (gibberellic acid, kinetin and indole-3-acetic acid), temperature (7, 11 and 15°C) and photoperiod (16L:8D and 8L:16D). Experiments revealed these three plant hormones could effectively promote the growth of *Porphyra* conchosporangia. Mean volume increases amounted respectively to 6.9-31.7% (for *P. abbottae*), 4.7-25.7% (for *P. pseudolanceolata*) and 8.9-35.1% (for *P. pseudolinearis*), depending upon the type and concentration of hormones, temperature and photoperiod. Maximal mean volume increase (31.7%) of *P. abbottae* occurred at 0.8 ppm kinetin, 15°C and short day culture (8L:16D). *P. pseudolanceolata* reached the best volume increase (25.7%) under the conditions of 0.4 ppm indole-3-acetic acid, 7°C and long day culture (16L:8D). Indole-3-acetic acid stimulated most significantly the volume increase (35.1%) of *P. pseudolinearis* at 0.4 ppm concentration, 15°C and long day culture (16L:8D). For both *P. abbottae* and *P. pseudolinearis*, intermediate hormone concentrations (0.4-1.6 ppm) generally had optimal stimulating-growth effect than the lower or the higher, whereas higher volume increases principally occurred at concentrations between 0.1and 0.8 ppm for *P. pseudolanceolata*. In most cases, stimulatory effect of hormones was reflected most conspicuously in the volume increase of *P. pseudolinearis*, with *P. abbottae* being the second

rank and then *P. pseudolanceolata.* Kinetin and indole-3-acetic acid generally had more influence on the volume increase than gibberellic acid. Although hormone concentrations over 1.6 ppm continued to have a stimulatory effect on conchosporangia, volume increase demonstrated a declining trend, especially in *P. pseudolanceolata*. For both *P. abbottae* and *P. pseudolinearis*, higher temperature resulted in higher volume increase, in contrast to *P. pseudolanceolata* having higher volume increases at the lower temperatures. There appeared not to be significant differences in volume increase between long day and short day culture in terms of comparison of their grand mean values.

Introduction

There are many endogenous and exogenous factors that control and regulate the growth and development of plants. Various life stages of plants can be viewed as the comprehensive interactions with these factors. In the study of higher plants, it was found that plant hormones can trigger or initiate many complex biochemical processes, which in turn ultimately lead to the progress of growth and development of plants. They can exert different effects on plants through influencing the process of cell division, cell enlargement, cell differentiation and even exert the effect at subcellular and molecular levels (Jacobs 1979, Davies 1987). Therefore, plant hormones have often been used to study physiological effects on the growth and development of higher plants.

Although concept , function and physiological effect of the plant hormones are basically derived from research with higher plants, it has been demonstrated that some of the plant hormones that operate in higher plants could have a similar role in other plant categories. Some research has been extended to seaweeds. For example, auxins have been found in *Ulva* , *Undaria, Hizikia* and *Porphyra* (Abe *et al*, 1972, Zhang *et al*, 1993), cytokinin in species of *Laminaria, Fucus* and *Phaseolus* (Brain *et al*, 1973, Reitz *et al*, 1996), gibberellin in *Enteromorpha* and *Ecklonia* (Jennings, 1968), and abscisic acid in *Laminaria* species (Schaffelke, 1995). In addition to identification and analysis of hormone composition from seaweed extracts, some studies have been carried out on how exogenous hormones affect algal growth or development. For instance, Rao *et al* (1972) examined four plant hormones (IAA, IBA, IA and AA) for their effects on the growth of vegetative fragments of *Gelidiella acerosa*. Kathiresan *et al* (1994) studied the effects of plant hormones (IAA and GA3) on seeding performance of *Aricennia marina*. Kaczyna *et al* (1993) studied the growth and callous induction of two plant hormones (IPA and IAA) on *Gracilaria verrucosa*. Borowczak *et al*

(1977) studied the effect of gibberellin and kinetin on the regeneration ability of *Fucus vesiculosus*.

There are about 440 species of algae in Alaska. Quite a few, especially *Porphyra*, have ecological importance and potential commercial value (Stekoll, 1990). There exist more than 20 species of *Porphyra* in British Columbia and adjacent areas (Lindstrom, 1991). Some of these species have the potential to be used successfully in mariculture. In the life cycle of *Porphyra*, two distinct phases are involved: one is the leafy thalli phase known as *Porphyra* and the other is the filamentous phase called the conchocelis, which generally lives inside of molluscan shells. Although the conchocelis culture of different *Porphyra* species have been reported (Bird, 1972, Campbell *et al* , 1984, Conwey *et al*, 1977, Dring, 1967,Waaland *et al*., 1990), to date very few studies have examined the physiological response of free-living conchocelis to plant hormones and very little is known about physiological effects of plant hormones on the sporophyte stage of *Porphyra*. It is interesting to investigate the effects of plant hormones on conchospore growth of *Porphyra* species. The objective of this study is to investigate the effects of three plant hormones on the conchosporangia growth of *Porphyra* species.

Materials and Methods

Free-living conchocelis of three species of *Porphyra* were used in these experiments: *Porphyra abbottae* (Pa), *Porphyra pseudolanceolata* (Pe) and *Porphyra pseudolinearis* (Pi).

For plant hormone effect experiments different levels of three environmental factors were employed as follows (with salinity and irradiance fixed at 30ppt and 10 μmol photons m^{-2} s^{-1} respectively for the photoperiods 16L:8D and 8L:16D):

Temperature: 7, 11, 15°C.

Hormone type: kinetin (K), gibberellic acid (G), indole-3-acetic acid (IAA).

Hormone concentration: 0 (control), 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ppm.

Conversion from ppm to μM concentration for three different hormones is given in the following table:

	0.1 ppm	0.2 ppm	0.4 ppm	0.8 ppm	1.6 ppm	3.2 ppm
K (μM)	4.65×10^{-4}	9.29×10^{-4}	1.86×10^{-3}	3.72×10^{-3}	7.44×10^{-3}	1.49×10^{-2}
G (μM)	2.89×10^{-4}	5.77×10^{-4}	1.16×10^{-3}	2.31×10^{-3}	4.62×10^{-3}	9.24×10^{-3}
IAA (μM)	5.29×10^{-4}	1.06×10^{-3}	2.11×10^{-3}	4.23×10^{-3}	8.46×10^{-3}	1.69×10^{-2}

Corning cell wells (24 wells with lids) were used as culture containers. About 5 ml of incubation medium was provided in each cell well. Four replicates were run for each treatment. Experiments were conducted in several incubators which had been set at different temperatures using cool-white fluorescent lamps. Seawater was autoclaved and full strength of PES was added along with different concentrations of plant hormones. Incubation medium was changed every 10 days.

Irradiance levels were obtained by wrapping the culture containers with layers of white paper and was measured with a Li-Cor Radiation Sensor (Li-190SR Quantum Sensor). At the beginning of experiments, small conchocelis tufts (about 0.1-0.2mm in diameter) were transferred to the cell wells. After being incubated for 45 days under the experimental conditions, all tufts were measured for their diameters with a microscope and their tuft volumes were estimated using the formula for the volume of a sphere $V = (1/6) \cdot \pi \cdot D^3$ (V and D respectively represent the volume and the diameter of conchosporangia tuft).

The effects of plant hormones on the growth of *Porphyra* conchosporangia were determined by estimating percentage increase of conchosporangia volume for those cultures

treated with plant hormones in comparison with control cultures, *i.e.* the effects of plant hormones on the growth were expressed as the mean percentage of volume increase (VI, ±SE) which was estimated by using the following formula:

$$VI\,(\%) = \frac{100(V - Vc)}{Vc}$$

where V and Vc represent the mean conchosporangia volume in every well respectively for cultures treated with hormones and control cultures.

Statistical analyses of the experimental data

For each species and each photoperiod, three factors were included: temperature (7, 11, 15°C) and hormone concentration (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ppm) and hormone type (IAA, K, G). There were fifty-four complete combinations of different levels of these factors with four replicates per treatment and a total of N = 3 x 3 x 6 x 4 = 216 samples for each species. Two separate photoperiod experiments (16L:8D and 8L:16D) were conducted. Therefore, volume increase differences (*i.e.* the effects of these factors) were analyzed by using a three-way model I ANOVA and S-Plus 3.1 for windows (Statistical Sciences, Inc. 1993). The Newman-Keuls multiple comparison test (Zar, 1996) was performed to identify which tested factors were important in controlling volume increase of the conchosporangia of *Porphyra*. Statistical power analysis for main effect factors was conducted according to Cohen's methods (Cohen, 1988).

Results

Porphyra abbottae

Experimental results of combined effects of three environmental factors (hormone concentration, hormone type and temperature) indicated that the volume increase of *P. abbottae*

was significantly affected by all three factors (Table 3.1 and Table 3.2, Figure 3.1) for both short and long day photoperiod culture (16L:8D and 8L:16D). Volume increase in response to the three variables for two photoperiod cultures followed similar patterns (Figure 3.1).

In most cases, kinetin and indole-3-acetic acid promoted higher growth than gibberellic acid (mean: 17.5% vs. 13.5% volume increase, Figure 3.1, Figure 3.4 and Figure 3.5). Hormone concentrations between 0.1 and 0.8 ppm usually caused an increase in growth (volume increase ranged between 6.5-31.7%), with the peak occurring at concentrations between 0.4 and 0.8 ppm (Figure 3.1, Figure 3.4 and Figure 3.5).

The highest temperature (15°C) promoted higher growth than the other two temperatures (mean volume increase 18% vs. 15%), but there was no significant difference in volume increase between 7°C and 11°C (Figure 3.1, Figure 3.4 and Figure 3.5).

The highest volume increase (31.7%) was achieved at 0.8 ppm kinetin, 15°C and short days (8L:16D), with salinity and irradiance at 30ppt and 10 μmol photons m^{-2} s^{-1} respectively. There were no significant differences in volume increase between long day and short day cultures (Figure 3.7).

P. pseudolanceolata

The volume increase of the conchosporangia of *P. pseudolanceolata* was influenced by only hormone concentration and temperature for long day culture (Table 3.1, Figure 3.2) but by all three factors for short day culture (Table 3.2, Figure 3.2). Interactions existed between only hormone concentration and temperature (Table 3.1, Table 3.2). This result suggests that for long day culture *P. pseudolanceolata* exhibited a uniform response to plant hormones (mean volume increase 12.5-14%) no matter which type of hormone was employed (Figure 3.4). But for short day cultures, kinetin had significantly higher stimulatory effect on growth than the other two hormones (mean volume increase 14% vs. 10.5%, Figure 3.5).

The pattern of volume increase in *P. pseudolanceolata* was similar to that in *P. abbottae* at 7°C temperature under long or short day cultures. However, *P. pseudolanceolata* exhibited a different pattern in response to hormones at higher temperature levels (11°C and 15°C), where volume increase generally peaked at the lowest hormone concentration tested (0.1-0.4ppm, Figure 3.2).

In contrast to *P. abbottae*, highest volume increase was demonstrated at the lowest temperature (17% volume increase at 7°C vs. 10% at 11°C and 12.5% at 15°C for long day cultures). However, there was no significant difference in mean volume increases at temperatures between 7°C and 11°C for short day cultures (Figure 3.5). The highest volume increase (25.7%) was achieved at 0.8 ppm kinetin, 7°C and short day culture (8L:16D), with salinity and irradiance fixed at 30ppt and 10 μmol photons m^{-2} s^{-1}, respectively. Although there is an appearance of a difference in volume increase between long day and short day culture, the difference was not statistically significant (Figure 3.7, $P>0.05$).

P. pseudolinearis

All three environmental factors significantly affected the volume increase of the conchosporangia of *P. pseudolinearis* for long or short day culture (Table 3.1, Table 3.2, Figure 3.3). For all the conditions tested, *P. pseudolinearis* always reached maximal volume increase at 0.4 ppm hormone concentration (mean volume increase 21% vs. 12-17%, Figure 3.3, Figure 3.4 and Figure 3.5).

Kinetin and indole-3-acetic acid caused higher volume increases than gibberellic acid (mean volume increase 16% vs. 19% for long day culture and 14% vs. 17.5% for short day culture, Figure 3.3, Figure 3.4 and Figure 3.5). Higher temperatures (11°C and 15°C) resulted in greater volume increase (Figure 3.3, Figure 3.4 and Figure 3.5). Indole-3-acetic acid stimulated the greatest volume increase (35.1%) of *P. pseudolinearis* at 0.4 ppm concentration, 15°C and

long day culture (16L:8D), with salinity and irradiance fixed at 30ppt and 10 μmol photons m^{-2} s^{-1}, respectively.

Although there was a difference in volume increase between long day and short day culture, this difference was not statistically significant (Figure 3.7, $P>0.05$).

Effect difference between species

The pooled hormone effects (for comparison between species) on the conchosporangia growth of three species of *Porphyra* for each parameter tested are shown on the right column of Figure 3.4 and Figure 3.5. All three species exhibited similar volume increases at hormone concentrations between 0.1-0.2 ppm. However, there was a significant difference in volume increase between different species at higher hormone concentrations, *i.e.*, both *P. abbottae* and *P. pseudolinearis* had conspicuously higher volume increase ($P<0.05$) at 0.4-3.2 ppm hormone concentrations (Figure 3.4 and Figure 3.5).

There was also a significant difference in volume increase among species in relation to hormone type employed. Kinetin and indole-3-acetic acid had more effect on *P. abbottae* and *P. pseudolinearis* than on *P. pseudolanceolata*. Gibberellic acid had a significantly higher stimulatory effect on *P. pseudolinearis* than on the other two species (Figure 3.4 and Figure 3.5).

As far as temperature was concerned, volume increase was similar at the low temperature(7°C) for all three species (about 16.5% volume increase), nevertheless, *P. pseudolinearis* and *P. abbottae* had significantly greater volume increase than *P. pseudolanceolata* at higher temperatures (11-13% vs. 16-22.5% volume increase for long day culture and 9-10.5% vs. 15-19% volume increase for short day culture, Figure 3.4 and Figure 3.5).

The pooled hormone effect (for comparison of the grand mean value) also uniformly indicated that *P. pseudolinearis* and *P. abbottae* were stimulated to produce higher volume

increase by exposure to plant hormones (Figure 3.6). Hormone concentrations between 0.4-0.8 ppm caused the most conspicuous volume increase. Both kinetin and indole-3-acetic acid stimulated the conchosporangia growth of *Porphyra* more significantly than gibberellic acid for long or short day culture (Figure 3.6). Higher temperature resulted in greater volume increases for long day cultures but not for short day cultures (Figure 3.6).

There was also a significant difference in volume increase between species for different photoperiods. Both *P. abbottae* and *P. pseudolinearis* had higher volume increase than *P. pseudolanceolata* for long or short day culture (Figure 3.7). Total pooled hormone effect for comparison of grand mean values indicated that there was no significant difference in volume increase between long day and short day culture (grand mean 15% vs. 16.2% volume increase, Figure 3.7).

Statistical power (1-β) analysis

The results of statistical power (1-β) analysis indicated that main effect factors (hormone concentration, hormone type and temperature) have high power values (>0.90 when detectable difference in means is set at 15%, Table 3.3 and Table 3.4).

Discussion

Using GC-MS (gas chromatography with mass spectrometry or NMR (nuclear magnetic resonance), auxin, abscissic acid(ABA), and cytokinins have been identified in seaweeds. For instance, Jacobs *et al.* (1985) identified auxin in *Caulerpa paspaloides*, where it apparently mediates the growth of new rhizoids when rhizomes are reoriented.

Since the evidence has indicated that growth substances exist in seaweeds, the growth of seaweeds could be regulated and controlled by such growth substances. Therefore, research on

the growth response of *Porphyra* conchocelis to plant hormones possesses importance in a theoretical context (demonstrating the physiologically growth- regulatory function of plant hormones). More importantly, such research results can be effectively applied to artificial cultivation of *Porphyra*, *i.e.*, we can promote the growth and development of *Porphyra* conchocelis and further obtain an increase in the production of *Porphyra* conchocelis or conchosporangia by exposing them to some exogenous hormones. The analysis of the results indicates that hormone concentration, hormone type and temperature significantly affect the volume increase of the conchosporangia of these three *Porphyra* species.

Among these three plant hormones studied, indole-3-acetic acid and kinetin possess a stronger stimulating-growth effect than gibberellic acid. For example, maximum volume increase induced by indole-3-acetic acid, kinetin and gibberellic acid are ranked as 35.1%, 31.7% and 24.2% compared with control group. It is possible that different hormones would cause a different magnitude of physiological effect because of their specific chemical structures, which may affect the ease by which hormones enter the plant cell and action sites for hormones to play a role on. Furthermore, cellular location of binding sites and transport of plant hormones may also result in different magnitudes of physiological regulations on the growth of plants.

Rao *et al.* (1992) found that three hormones (indole-3-acetic acid, IBA and AA) used in their experiments caused different growth effects on the seaweed, *Gelidiella acerosa*. Kathisesan *et al.* (1994) reported that gibberellic acid (at 50 ppm of concentration) enhanced shoot growth of a seaweed (*Aricennia marina*) by 26% and root growth by 40%. Indole-3-acetic acid (at 10 ppm) increased shoot dry weight by 139% and root length by 30%.

In my experiments, the stimulatory effect of indole-3-acetic acid on three species of *Porphyra* caused volume increase by 6.0-35.1%. Kinetin and gibberellic acid promoted volume increase respectively by 5.4-31.7% and 4. 9-24.2%, depending upon different culture conditions

and species. For both *P. pseudolinearis* and *P. abbottae*, minimum volume increase usually occurred at the lowest hormone concentration (0.1 ppm). In most cases, hormone concentrations between 0.4-1.6 ppm resulted in the highest volume increase at lower temperatures (7°C and 11 °C). Hormone concentrations between 0.2-0.8 ppm exhibited a larger stimulatory effect at higher temperature (15°C). Similarly, for *P. pseudolanceolata*, the lowest concentration of hormones (0.1 ppm) induced minimum volume increase at the lower temperature (7°C). In most cases, 0.2-0.8 ppm of hormone concentrations promoted higher growth.

However, under higher temperatures (11°C and 15°C), in most cases, lower hormone concentrations led to higher volume increase. This phenomenon was particularly obvious in the experiments at 15°C. This result may demonstrate that *P. pseudolanceolata* is sensitive to the low concentrations of plant hormones at higher temperatures, which enhanced metabolism of plant cells. Although the maximum hormone concentration tested (3.2 ppm) still led to a reasonable volume increase, there was a declining tendency. This could be observed especially in the experiments with *P. pseudolanceolata*. Further investigations are needed to determine whether or not there exists an inhibitory effect on *Porphyra* conchocelis growth at higher hormone concentrations.

For these three species of *Porphyra*, plant hormones exhibited more significant influence on *P. pseudolinearis* and *P. abbottae* than on *P. pseudolanceolata*. Maximum volume increase was respectively 35.1% (for *P. pseudolinearis*, 16L:8D, 0.4 ppm of indole-3-acetic acid, 15°C), 31.7% (for *P. abbottae*, 8L:16D, 0.8 ppm of kinetin, 15°C) and 25.7% (for *P. pseudolanceolata*, 16L:8D, 0.4 ppm of indole-3-acetic acid, 7°C).

Although there was no obvious difference between long day and short day cultures for plant hormone effect, data suggest that plant hormones could exert higher growth-stimulating

effect on *P. abbottae* under short day culture and exerted higher growth-stimulating effect on *P. pseudolanceolata* and *P. pseudolinearis* under long day culture.

From the study of hormone effect on a Japanese species, *Porphyra tenera*, Iwasaki (1965) reported that gibberellic acid affected conchocelis growth most effectively. Dry weight of conchocelis increased by 200-380% at 0.02-0.1ppm of gibberellic acid concentrations for 135 days culture. Indole-3-acetic acid at 0.02-0.1 ppm and 0.1-0.2 ppm of kinetin concentrations led to 2-200% and 46-62% of increase in conchocelis dry weight. Compared with my experimental results, it is possible that different species have different susceptibility to specific plant hormones.

Conclusions

The following conclusions could be drawn from my experimental results:

1. Three plant hormones (indole-3-acetic acid, kinetin and gibberellic acid), can influence conchosporangia growth of three species of indigenous Alaskan *Porphyra (P. abbottae, P. pseudolanceolata and P. pseudolinearis).*

2. Physiological effects of these plant hormones on *Porphyra* conchosporangia can be observed and measured by estimating the percentage volume increase of the conchosporangia exposed to plant hormones.

3. Growth-stimulating effect of plant hormones is related to different conditions such as hormone type and concentration, culture temperature and species.

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Table 3.1. ANOVA table for the effects of plant hormones on the conchosporangia growth of three different *Porphyra* species with different combinations of hormone concentration (Hc), hormone type (Ht)) and temperature (Temp.) for long day culture(16 hour light:8 hour dark). [a] 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ppm; [b]gibberellic acid (G), kinetin (K), and indole-3-acetic acid (IAA); [c] 7, 11, 15°C (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Hc[a]	5	1500.01	300.00	23.98**
Ht[b]	2	480.88	240.44	19.22**
Temperature[C]	2	350.65	175.33	14.02**
Hc x Ht	10	430.88	43.09	3.44**
Hc x Temp.	10	467.96	46.80	3.74**
Ht x Temp.	4	93.76	23.44	1.87
Hc x Ht x Temp.	20	574.57	28.73	2.30*
Residuals	162	2026.47	12.51	
P. pseudolanceolata				
Hc[a]	5	1230.83	246.17	9.80**
Ht[b]	2	142.71	71.35	2.84
Temperature[C]	2	1210.20	605.10	24.09**
Hc x Ht	10	169.79	16.98	0.68
Hc x Temp.	10	1467.26	146.73	5.84**
Ht x Temp.	4	159.65	39.91	1.59
Hc x Ht x Temp.	20	285.13	14.26	0.56
Residuals	162	4069.61	25.12	
P. pseudolinearis				
Hc[a]	5	2778.10	555.62	37.01**
Ht[b]	2	389.36	194.68	12.97**
Temperature[C]	2	1204.85	602.42	40.12**
Hc x Ht	10	299.87	29.99	2.00
Hc x Temp.	10	1366.27	136.63	9.10**
Ht x Temp.	4	327.10	81.77	5.45**
Hc x Ht x Temp.	20	664.11	33.21	2.21**
Residuals	162	2432.31	15.01	

Table 3.2. ANOVA table for the effects of plant hormones on the conchosporangia growth of three different *Porphyra* species with different combinations of hormone concentration (Hc), hormone type (Ht)) and temperature (Temp.) for short day culture(8 hour light:16 hour dark). [a] 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ppm; [b]gibberellic acid (G), kinetin (K), and indole-3-acetic acid (IAA); [c] 7, 11, 15°C (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Hc[a]	5	2886.73	577.35	27.40**
Ht[b]	2	367.42	183.71	8.72**
Temperature[C]	2	684.38	342.19	16.24**
Hc x Ht	10	894.76	89.48	4.25**
Hc x Temp.	10	278.13	27.81	1.32
Ht x Temp.	4	104.50	26.12	1.24
Hc x Ht x Temp.	20	832.96	41.65	1.98*
Residuals	162	3413.92	21.07	
P. pseudolanceolata				
Hc[a]	5	848.53	169.71	10.18**
Ht[b]	2	383.43	191.72	11.50**
Temperature[C]	2	1651.09	825.55	49.53**
Hc x Ht	10	174.94	17.49	1.05
Hc x Temp.	10	515.06	51.51	3.09**
Ht x Temp.	4	114.43	28.61	1.72
Hc x Ht x Temp.	20	342.52	17.13	1.03
Residuals	162	2700.14	16.67	
P. pseudolinearis				
Hc[a]	5	2025.63	405.13	22.56**
Ht[b]	2	441.46	220.73	12.29**
Temperature[C]	2	292.74	146.37	8.15**
Hc x Ht	10	189.99	19.00	1.06
Hc x Temp.	10	689.05	68.91	3.84**
Ht x Temp.	4	16.16	4.04	0.22
Hc x Ht x Temp.	20	387.53	19.38	1.09
Residuals	162	2909.40	17.96	

Table 3.3. Statistical power (1- ß) based on the results of variance analysis for experiments with plant hormone effects on volume increase of *Porphyra* conchosporangia (long day culture, 16L:8D). Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05. Three main effect factors (hormone concentration, hormone type and temperature) are abbreviated as hc, ht and t in the table.
(** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
hc	5	23.98**	5	28	0.5291	>0.99
ht	2	19.22**	2	55	0.4804	>0.99
t	2	14.02**	2	55	0.4748	>0.99
Porphyra pseudolanceolata						
hc	5	9.80**	5	28	0.3339	0.92
ht	2	2.84	2	55	0.3143	0.95
t	2	24.09**	2	55	0.3359	0.97
Porphyra pseudolinearis						
hc	5	4.45**	5	28	0.4849	>0.99
ht	2	12.97**	2	55	0.4192	>0.99
t	2	40.12**	2	55	0.4394	>0.99

Table 3.4. Statistical power (1- ß) based on the results of variance analysis for experiments with plant hormone effects on volume increase of *Porphyra* conchosporangia (short day culture, 8L:16D). Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05. Three main effect factors (hormone concentration, hormone type and temperature) are abbreviated as hc, ht and t in the table.
(** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
hc	5	27.40**	5	28	0.4450	>0.99
ht	2	8.72**	2	55	0.3811	>0.99
t	2	16.24**	2	55	0.3879	>0.99
Porphyra pseudolanceolata						
hc	5	10.18**	5	28	0.3373	0.93
ht	2	11.50**	2	55	0.3271	0.96
t	2	49.53**	2	55	0.3656	0.99
Porphyra pseudolinearis						
hc	5	22.56**	5	28	0.5178	>0.99
ht	2	12.29**	2	55	0.4537	>0.99
t	2	8.15**	2	55	0.4486	>0.99

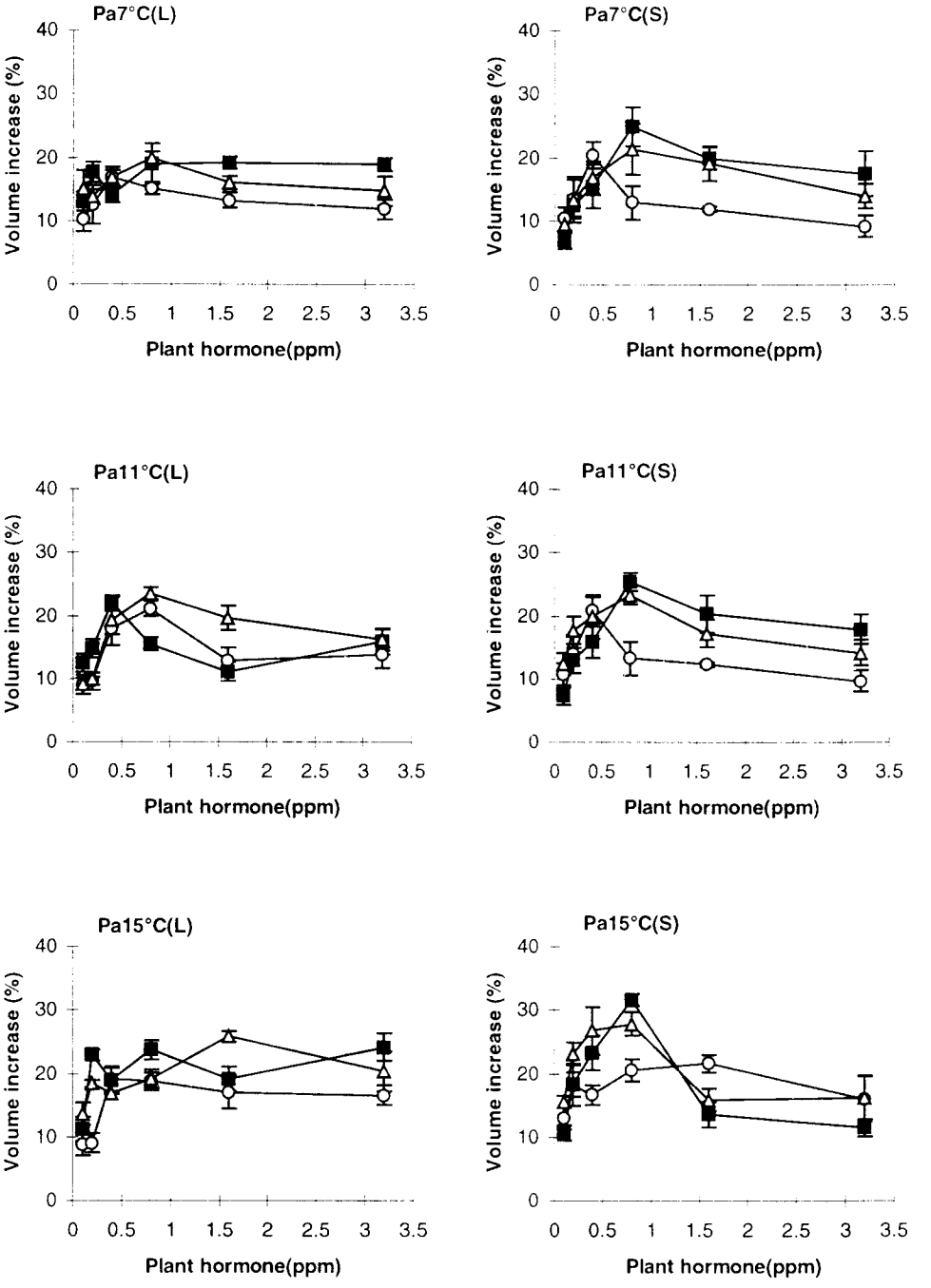


Figure 3.1. *Porphyra abbottae* (Pa). The effects of plant hormone (O, G; ■, K; Δ, IAA) on conchosporangia growth under conditions of different temperatures (7, 11, 15 °C) and photoperiods (long day: L and short day: S). Error bars are ± S.E. Volume increase is expressed as percent increase in volume compared with control.

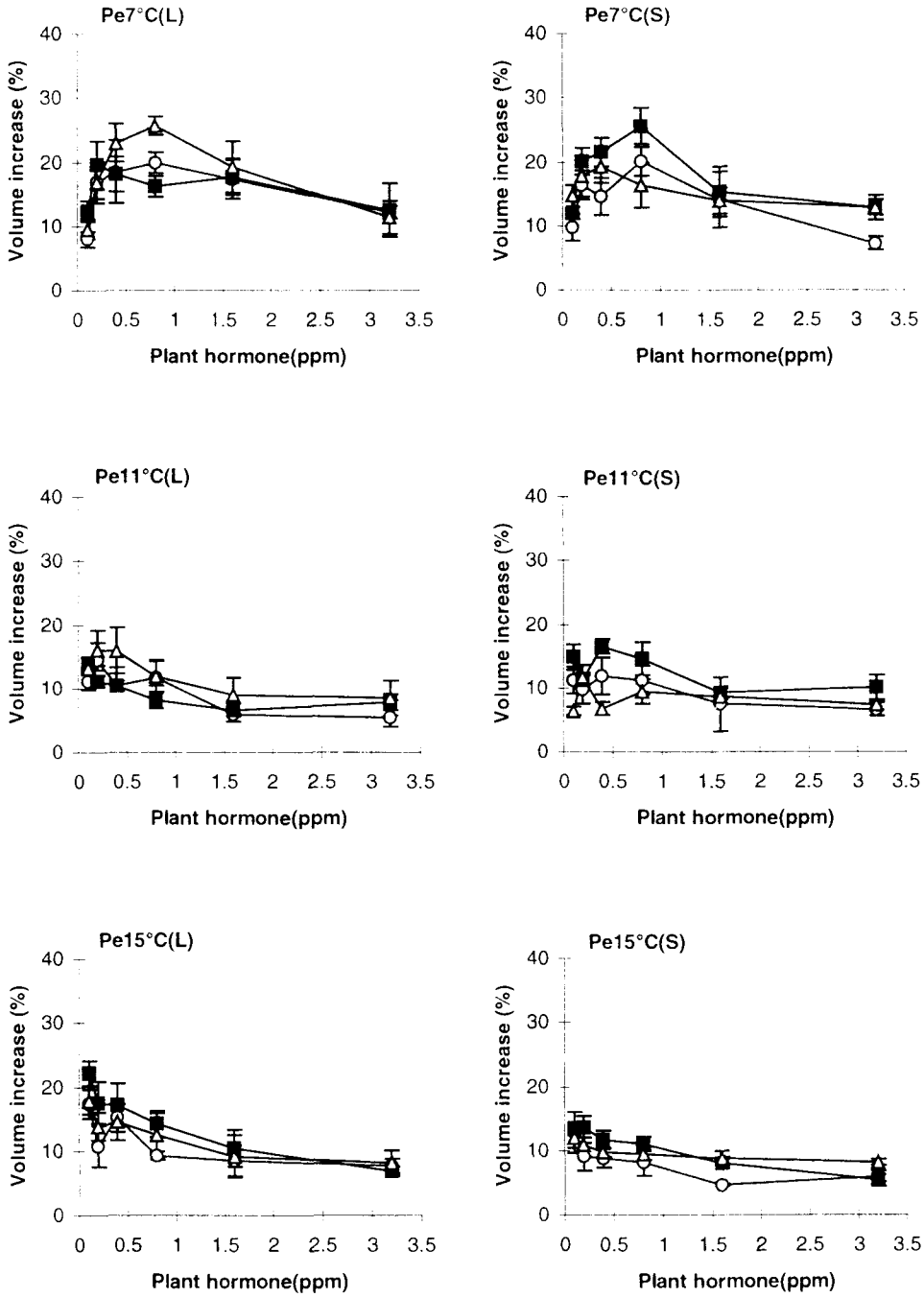


Figure 3.2. *Porphyra pseudolanceolata* (Pe). The effects of plant hormone (O, G; ■, K; Δ, IAA) on conchosporangia growth under conditions of different temperatures (7, 11, 15 °C) and photoperiods (long day: L and short day: S). Error bars are ± S.E. Volume increase is expressed as percent increase in volume compared with control.

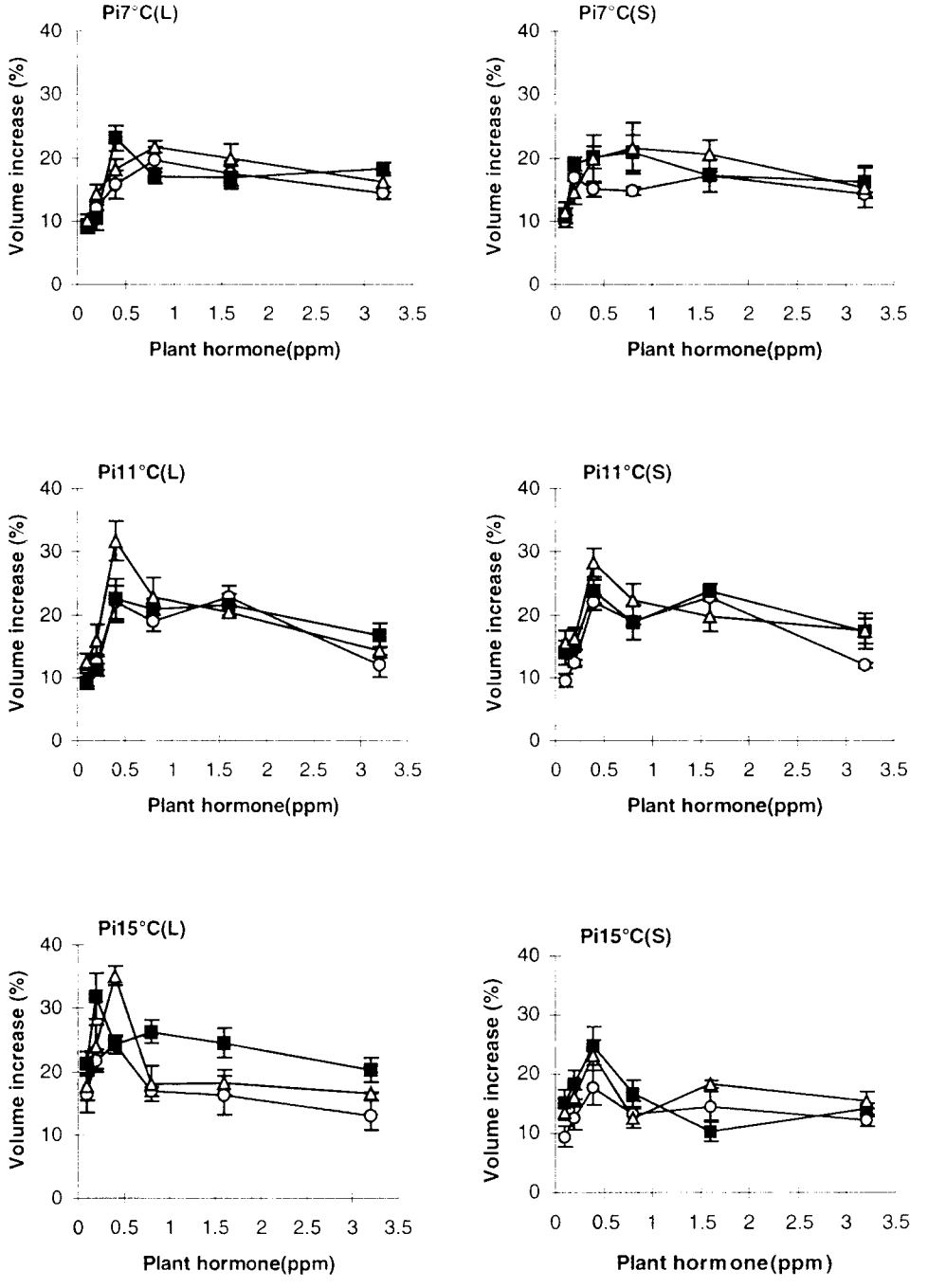


Figure 3.3. *Porphyra pseudolinearis* (Pi). The effects of plant hormone (O, G; ■, K; Δ, IAA) on conchosporangia growth under conditions of different temperatures (7, 11, 15 °C) and photoperiods (long day: L and short day: S). Error bars are ± S.E. Volume increase is expressed as percent increase in volume compared with control.

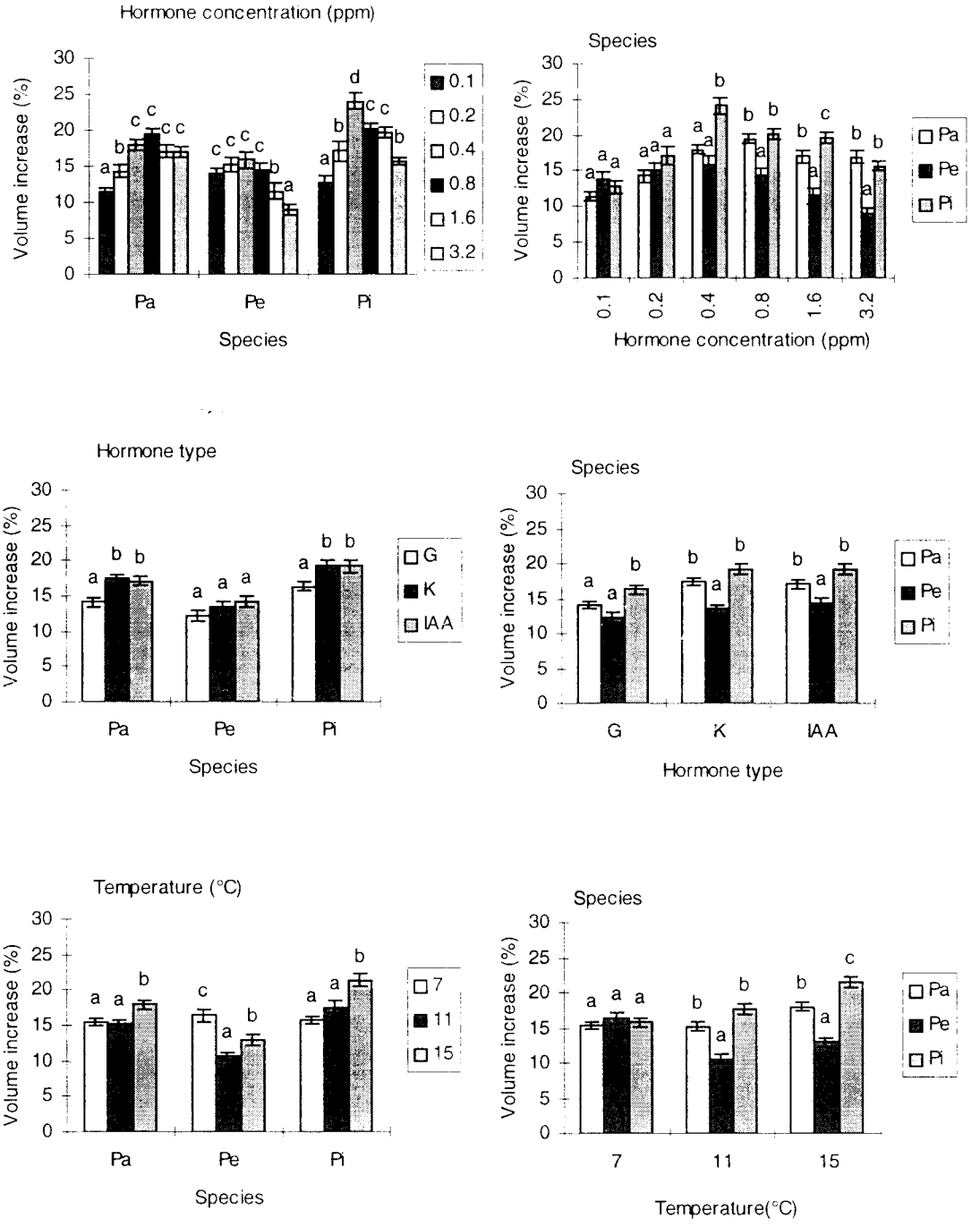


Figure 3.4. Comparison of pooled hormone effect on conchosporangia growth of three species of *Porphyra* for each parameter tested (photoperiod 16L:8D). Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only within a specie (for the figures on the left) and letter comparisons are relevant only between specie (for the figures on the right).

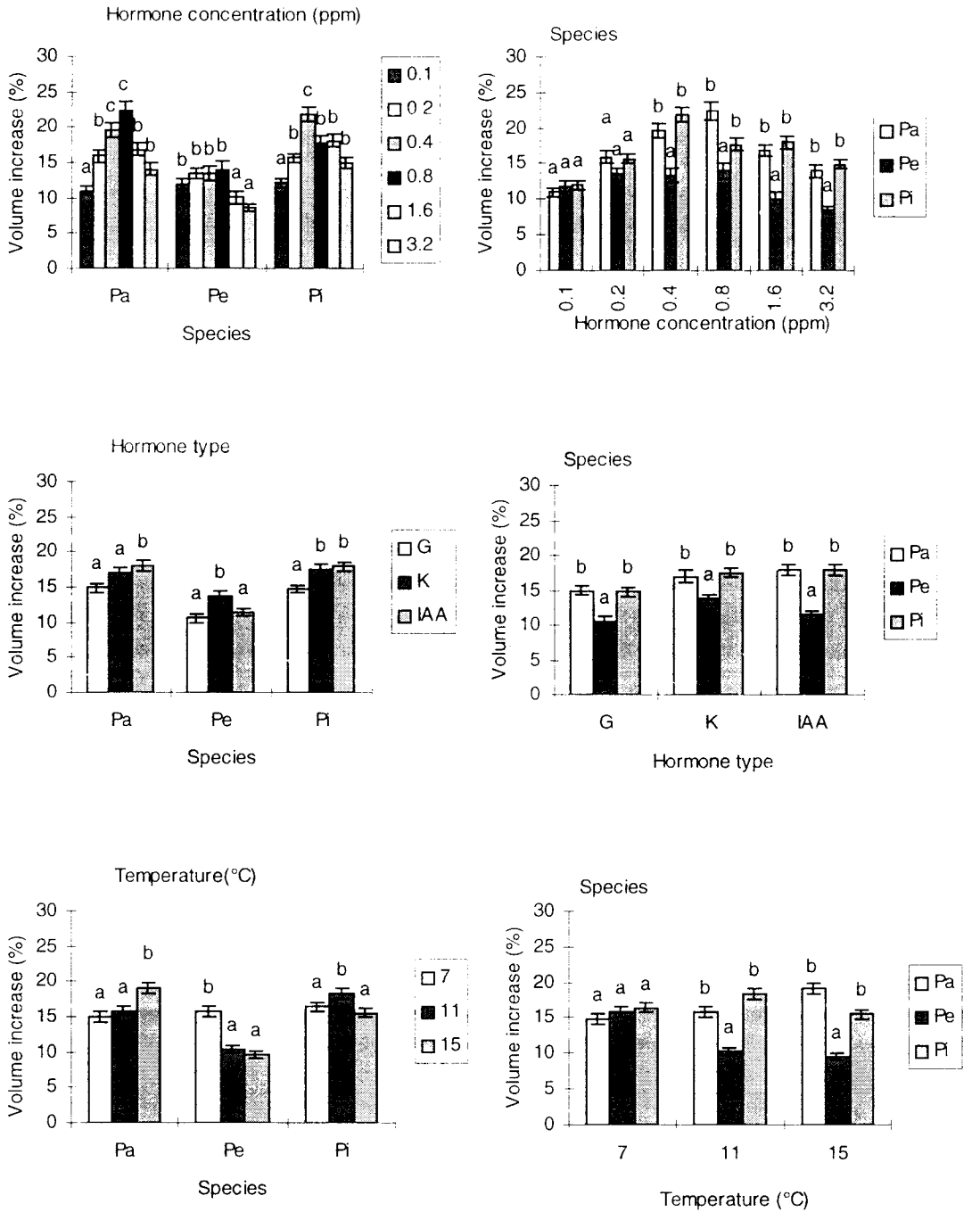


Figure 3.5. Comparison of pooled hormone effect on conchosporangia growth of three species of *Porphyra* for each parameter tested (photoperiod 8L:16D). Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only within a specie (for the figures on the left) and letter comparisons are relevant only between specie (for the figures on the right).

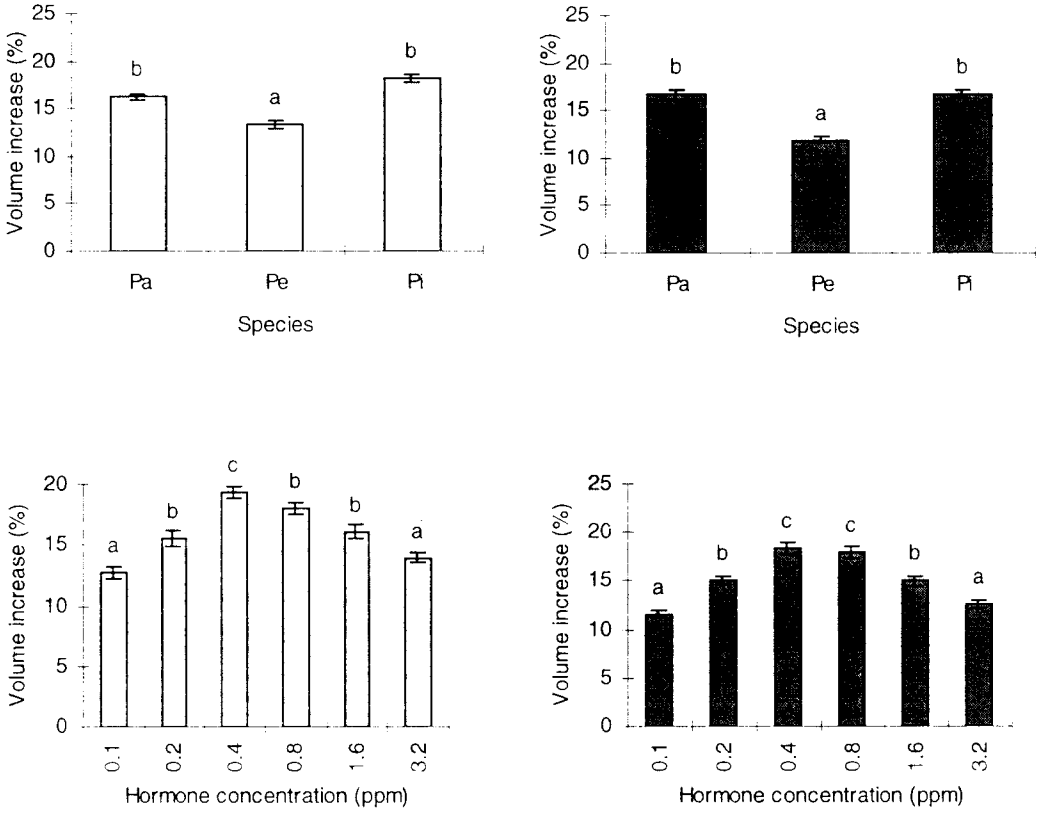
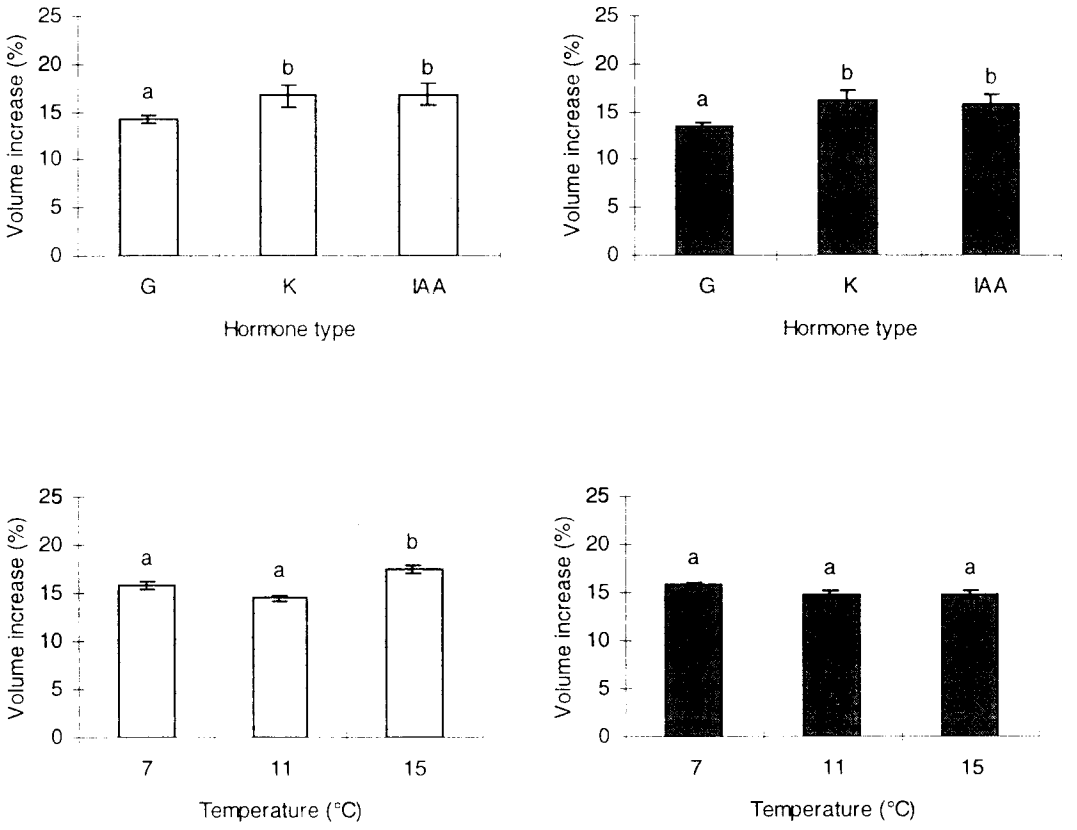


Figure 3.6. Comparison of pooled hormone effect on conchosporangia growth of three species of *Porphyra* for each parameter test (for comparison of the grand average value). Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. The figures on the left represent long day culture (16L:8D) and the figures on the right represent short day culture (8L:16D).



(Continued):

Figure 3.6. Comparison of pooled hormone effect on conchosporangia growth of three species of *Porphyra* for each parameter test (for comparison of the grand average value). Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. The figures on the left represent long day culture (16L:8D) and the figures on the right represent short day culture (8L:16D).

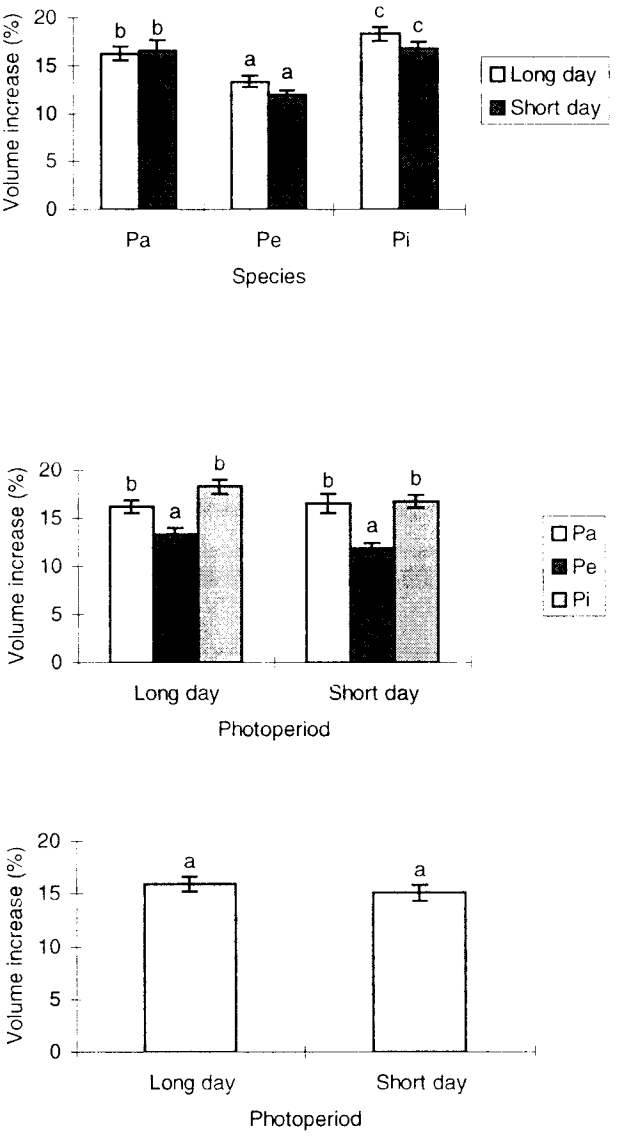


Figure 3.7. Comparison of pooled hormone effect on conchosporangia growth of three species of *Porphyra* for different photoperiod culture. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. Long day (16L:8D) and short day culture (8L:16D).

Chapter 4

Photosynthesis and Respiration of Three Indigenous Alaskan *Porphyra* Species: Response to Environmental Variables

Abstract

Experiments were carried out to investigate the physiological responses of conchocelis to environmental variables in terms of their photosynthesis and respiration for indigenous species of Alaskan *Porphyra* (*P. abbottae*, *P. pseudolinearis* and *P. torta*), and determine the range and optima of environmental parameters under which conchocelis can photosynthesize and respire.

P-I (photosynthesis vs. irradiance) curves revealed that photosynthesis varied with irradiance, however, patterns of a P-I curve, P_{max}, I_{max} and I_c depended on temperature and species. *P. abbottae* had typical features of P-I curve that showed that P_{max} (about 83-146 μmol O_2 production g dw^{-1} h^{-1}) occurred at 20-140 μmol photons m^{-2} s^{-1} (I_{max}) depending on temperature. Higher irradiances resulted in a decline in photosynthesis. Both *P.pseudolinearis* and *P. torta* exhibited higher P_{max} and I_{max} values, compared with *P. abbottae*. The P_{max} of *P. pseudolinearis* was about 200-240 μmol O_2 g dw^{-1} h^{-1} and 90-240 μmol O_2 g dw^{-1} h^{-1} for *P. torta* , with the I_{max} being 135-250 and 200-250 μmol photons m^{-2} s^{-1} respectively. Compensation irradiances (I_c) were estimated from the photosynthetic values intercepted on the x-axis by the points of 0 and the lowest irradiances for the P-I curves. Results showed that these species generally had very low I_c (about 3-5 μmol photons m^{-2} s^{-1}).

Photosynthetic activity of conchocelis was significantly influenced by irradiance, temperature and salinity. For all three species salinities between 25 and 35ppt caused higher photosynthesis, with the highest occurring at 30ppt in most cases. Photosynthesis markedly

declined at salinities lower than 25ppt or higher than 35ppt. *P. abbottae* had higher photosynthesis at 11°C and 60 μmol photons m^{-2} s^{-1}, whereas *P. pseudolinearis* and *P. torta* had higher photosynthesis at higher temperature and irradiance. The highest photosynthesis of *P. abbottae* occurred at 11°C, 60 μmol photons m^{-2} s^{-1} and 30ppt. *P. pseudolinearis* and *P. torta* had the highest photosynthesis at 15°C, 120 μmol photons m^{-2} s^{-1} and 30ppt.

Conchocelis had the lower respiratory rates at 7°C than at 11 and 15°C. All three species significantly exhibited minimal respiratory activity at salinities between 25-35ppt.

Introduction

Among the interesting and yet little-studied questions about *Porphyra* are physiological and ecological aspects of the conchocelis stage. Many research reports on photosynthesis of *Porphyra* dealt with only the gametophyte stage of *Porphyra*. For instance, a variety of environmental factors have been examined to investigate their influences on leafy blades of *Porphyra* such as temperature (Chang *et al.* 1983, Wu *et al.* 1984, Smith & Berry 1986, Gao & Aruga 1987), salinity (Oqata *et al.* 1971, Reed *et al.* 1980, Wiencke & Lauchi 1980, Satoh *et al.* 1983, Chang *et al.* 1983), irradiance (Herbert 1984), desiccation (Fork & Oequist 1981, Levitt & Bolton 1991, Lipkin *et al.* 1993), diurnal rhythm (Oohusa *et al.* 1978, 1980, Coutinho 1984), light wavelength (Luening & Dring 1985), nutrients and dissolved inorganic carbon (Zavodnik 1987, Kapraun *et al.* 1987), seawater pH (Gao & Zhao 1988) and seawater current speed (Gao *et al.* 1991). Very few studies have investigated photosynthetic and respiratory responses to environmental variables by the conchocelis stage of *Porphyra* species. Specifically, no studies have been reported on the combined effects of multiple factors on photosynthetic physiology of the conchocelis. Only one paper has briefly studied photosynthesis of *Porphyra* conchocelis (*P. leucosticta*) and light was the only variable (Sheath *et al.* 1977). Several environmental factors may affect the photosynthesis and respiration processes of *Porphyra* conchocelis.

In natural habitats, the microscopic sporophytes (conchocelis stage) of *Porphyra* generally occur in intertidal areas or probably extend to subtidal areas, yet little is known about their ecological significance. Although conchocelis are hardly ever observed and noticed in the conventional survey of coastal vegetation, they may play a role in improving the habitat quality of some micro-environments for other benthic organisms because conchocelis have the ability to perform photosynthesis even if the environmental irradiance available is so low that other plants might not possess net photosynthesis. The microscopic sporophytes of *Porphyra* are difficult to

study, especially in the field, because they live in calcareous shells. Because of these difficulties in a field study, it is necessary to conduct the study under controlled laboratory conditions. Free-living conchocelis can be used to investigate and understand physiological and ecological aspects of conchocelis stage for different *Porphyra* species.

The sporophytic stage(*i.e.* conchocelis stage) is very important to successful *Porphyra* aquaculture. Environmental factors should be examined to investigate their influences on important physiological processes of the *Porphyra* conchocelis stage. Such research is needed prior to the establishment of a nori mariculture industry in Alaska. It is especially important to determine the optimal conditions for healthy conchocelis of *Porphyra* species. Basic information and research are needed on photosynthetic and respiratory reactions of *Porphyra* sporophyte stage.

Photosynthesis and respiration are basic and important processes in the conchocelis stage. Photosynthetic and respiratory rates can reflect the metabolic conditions of the conchocelis responding to the environmental change. Therefore, such indicators can be used to mirror the physiological reactions to the environment. This research aimed to investigate the effect of environmental factors (temperature, salinity, irradiance) on photosynthetic and respiratory activities of *Porphyra* conchocelis and to determine favorable culture conditions in term of photosynthetic and respiratory activities. These experiments addressed the following questions:

(1). How does the photosynthetic rate of conchocelis vary with species, light, temperature and salinity?

(2). For these species of *Porphyra* conchocelis, what are the shapes of the P-I curves under the conditions above? What are the compensation points at which no net oxygen production occurs?

Do species show photoinhibition at high light? How do these parameters vary with different incubation temperatures or salinities?

(3). How do dark respiration rates of *Porphyra* conchocelis vary with species temperature and salinity?

Materials and Methods

Unialgal cultures of each *Porphyra* species *(Porphyra abbottae* Krishnamurthy - strain PaSGS01, *P. pseudolinearis* Ueda - strain PiSC14 and *P. torta* Krishnamurthy - strain PtCH01) were obtained from carpospore release. Mature blades of the gametophyte stage of each species were collected from the field. Blades were washed and scrubbed with sterile seawater to remove surface contamination. The cleaned blades were placed in sterile seawater in petri dishes for carpospore release. After 24-36 hours the blades were removed and the dishes incubated in Provasoli's enriched seawater (PES; McLachlan, 1973) under 16L:8D photoperiod at 11°C. Conchocelis segments (around 110-250 μm) of each species were placed in cell well plates (one piece per well) and incubated at 30ppt salinity and 11°C (100-120 μmol photons m^{-2} s^{-1} irradiance) for the culture of pure genotype conchocelis, which were used for expanding bulk free-living conchocelis for experiments. PES enriched seawater culture medium was used.

Photosynthetic experiments of the conchocelis were conducted in several incubators which had been set at different temperatures and illuminated with cool-white fluorescent lamps. Irradiance gradients were obtained by wrapping the culture containers with varying layers of white paper and determined using a Li-Cor Radiation Sensor (Li-190SB Quantum Sensor). Autoclaved natural seawater-based PES medium was used in the experiments. At the beginning of experiments, the pH of the culture medium was adjusted to 7.8 - 8.0 (the ambient pH of the seawater in the inside waters of SE Alaska) using 6M HCl or 6M NaOH. Experimental seawater

with different salinities was obtained either by boiling natural seawater (for 40ppt salinity) or by diluting natural seawater with distilled water. Nutrients were added after salinities were adjusted. Conchocelis were allowed to adapt to the specific experimental conditions for at least 5 hours before each assay. Vials of 25 ml of volume were used as experimental containers. For all the photosynthetic experiments, in order to ensure sufficient inorganic carbon source available to the conchocelis, culture media were supplemented with 5 mM $NaHCO_3$.

For the P-I curve determination different levels of two environmental factors were employed as follows (with salinity being fixed at 30ppt):

Temperature: 7, 11, 15°C.

Irradiance: 0-280 μmol photons m^{-2} s^{-1} for the experiments at 7°C and 11°C temperatures; 0-200 μmol photons m^{-2} s^{-1} for the experiments at 15°C temperature.

For multi-factorial photosynthetic experiments different levels of three environmental factors were employed as follows:

Temperature: 7, 11, 15°C.

Irradiance: 30, 60, 120 μmol photons m^{-2} s^{-1}.

Salinity: 10, 15, 20, 25, 30, 35, 40ppt.

For respiratory rate determination different levels of two environmental factors were employed as follows:

Temperature: 7, 11, 15°C.

Salinity: 10, 15, 20, 25, 30, 35, 40ppt.

Photosynthetic rates were determined by measuring differences in dissolved oxygen between vials with conchocelis and blank vials (with four replicates for per treatment condition of the experiments). Free-living conchocelis were rinsed with sterile seawater 5-6 times through

a standard sieve (100μm) and about 2-4 mg f.w. of conchocelis (there is a linear relationship between oxygen production and conchocelis amount below 7 mg.f.w., see Figure 4.1) was put in vials and gently filled with seawater. At the end of incubation period, oxygen concentrations of culture media in each vial were determined with a Check-Mate 90 meter (with a dissolved oxygen sensor). Duration period for photosynthesis was about 6hr (there is a linear relationship within 8hr, see Figure 4.1). For dark respiration experiments, about 5-8 mg f.w. of conchocelis (there is a linear relationship between oxygen consumption and conchocelis amount below 12 mg f.w., see Figure 4.1) were used in each vial. Complete darkness was formed by wrapping vials with three layers of thick black plastic sheet. The duration time of dark respiration experiment was 10-12hr (there is a linear relationship within 15hr, see Figure 4.1). Dry weights of conchocelis samples were obtained by drying in an oven at 70 °C to constant weight. Photosynthesis and dark respiration rates were expressed as μmoles O_2 g dw^{-1} h^{-1}.

Statistical analyses of the experimental data

In photosynthetic experiments, three species were used (*P. abbottae, P. pseudolinearis, P. torta*). For each species, the experiment included temperature (7, 11, 15°C), irradiance (30, 60, 120 μmol photons m^{-2} s^{-1}) and salinity (10, 15, 20, 25, 30, 35, 40ppt). There were sixty-three complete combinations of different levels of these factors with four replicates per treatment and a total of N = 3 x 3 x 7 x 4 = 252 data in the photosynthetic experiments for each species. A three-way model I ANOVA was performed to analyze the influences of these factors on oxygen production rate of conchocelis for each species of *Porphyra* by using S-Plus 3.1 for windows (Statistical Sciences, Inc. 1993).

Respiratory experiment included three species (*P. abbottae, P. pseudolinearis, P. torta*), temperatures (7, 11, 15°C), salinities (10, 15, 20, 25, 30, 35, 40ppt). There were sixty-three complete combinations of different levels of these factors with four replicates for each treatment

and a total of N = 3 x 3 x 7 x 4 = 252 data for statistical analysis of this experiment. The analysis of the effects of these three factors on respiratory rate of conchocelis were done by using a three-way model I ANOVA and S-Plus 3.1 for windows (Statistical Sciences, Inc. 1993).

The Newman-Keuls multiple comparison test (Zar, 1996) was performed to identify which tested factors were important in controlling photosynthesis and respiration of the conchocelis of *Porphyra*. Furthermore, statistical power analysis for main effect factors was conducted according to Cohen's methods (Cohen, 1988).

Results

Responses of *Porphyra* conchocelis to irradiance (P-I curve)

Characteristics of P-I curves

All of the P-I curves revealed that net photosynthesis varied with different levels of irradiance. However, the patterns of P-I curves depended considerably on different temperatures and species (Figure 4.2, Figure 4.3 and Figure 4.4).

For instance, the photosynthetic activity of *P. abbottae* conchocelis exhibited a unique P-I curve pattern under all three temperatures (Figure 4.2). That is, the photosynthetic rate increased steadily with increasing irradiance up to the maximum value for photosynthesis. Beyond this point, photosynthesis declined (photoinhibition).

Both *P. pseudolinearis* (at 11°C and 15°C) and *P. torta*(at 7°C and 11°C) showed a similar photoinhibition but under higher light levels (Figure 4.3 and Figure 4.4). It should be noted that photosynthesis of *P. abbottae* reached a peak and displayed photoinhibition at much lower light intensities, compared to those of *P. pseudolinearis* or *P. torta*. There were no photoinhibition effects occurring at 7°C for *P. pseudolinearis* and at 15°C for *P. torta*. Virtually, *P. pseudolinearis* displayed a nearly linear increase in photosynthetic rate with an

increase in light intensity up to 250 μmol photons m^{-2} s^{-1}. But further increase in light intensity did not result in the rise of the photosynthetic rate (Figure 4.3). *P. torta* exhibited increasing photosynthetic activity with an increase in light intensity up to 135 μmol photons m^{-2} s^{-1} at 15°C. Higher light intensities did not result in higher photosynthetic rates (Figure 4.4). These results implied that saturation irradiances would be about 250 μmol photons m^{-2} s^{-1} at 7°C for *P. pseudolinearis* and 135 μmol photons m^{-2} s^{-1} at 15°C for *P.* torta, respectively.

The effect of temperature

In general, with lower irradiances (at intensities less than P_{max}), the rate of photosynthesis was positively correlated with temperature for all three species of *Porphyra.*

For example, the differences in photosynthesis at 7°C and 11°C for *P. pseudolinearis* and *P. torta* conchocelis were small under the same irradiances (Figure 4.3 and Figure 4.4), but as temperature was raised to 15°C, there was a conspicuous increase in photosynthesis (Figure 4.3 and Figure 4.4). For *P. abbottae* conchocelis, the photosynthetic rate increased in a proportion with an increase in temperature (Figure 4.2), except for those photosynthetic rates above the I_{max} at 7°C and 11°C.

P-I curve parameters and the trend lines:

Compensation irradiance (I_c, the irradiance at which no net photosynthesis occurred), maximum saturation photosynthesis (P_{max}) and maximum saturation irradiance (I_{max}, the irradiance at which maximum photosynthesis was reached) are summarized in Table 4.1 for three species of *Porphyra* and different temperatures. In all conditions tested, the values of compensation points were very low (about 3-5 μmol photons m^{-2} s^{-1}, a level of light barely detectable with the sensor) for these three species (Table 4.1). The results also showed that light saturation was a function of different temperatures and species. For instance, for *P. abbottae*

conchocelis, the photosynthetic maxima (P_{max}) obviously increased with an increasing temperature. Photosynthesis of *P. torta* remained approximately the same at 7°C and 11°C. However, there was a great increase (about two times) with temperature increasing to 15°C. But, for *P. pseudolinearis* conchocelis, P_{max} remained more or less the same regardless of temperature variation (Table 4.1).

The I_{max} values also varied with species and temperatures. For example, the I_{max} of *P. abbottae* decreased with an increase in the temperature, although the P_{max} increased with the temperature. Both *P. pseudolinearis* and *P. torta* maintained more or less steady P_{max} and I_{max} values I_{max} at 7°C and 11°C, but there was an obvious increase in the P_{max} and an accompanying decrease in the I_{max} at 15°C.

The effects of environmental variables on photosynthesis:

The results of multi-factorial experiments

P. abbottae

The photosynthesis of the conchocelis of *Porphyra abbottae* was influenced by all three factors (temperature, salinity and irradiance) and the majorities of interactions between these factors (Figure 4.5, Table 4.2). Salinity had a significant effect on the photosynthesis of *P. abbottae* conchocelis. Maximum photosynthetic rates always occurred at 30ppt salinity under all temperature and light combinations (Figure 4.5 and Figure 4.8). Salinities above or below 30ppt resulted in a marked decline in photosynthesis (Figure 4.5 and Figure 4.8).

When the temperature was lower, photosynthesis was greater at the higher irradiances than at the lower irradiance for all species. However, at 15°C, increasing irradiance did not cause an increase in photosynthetic activity of *P. abbottae*, but rather, *P. abbottae* conchocelis showed an inverse relationship between irradiance and photosynthesis. *P. abbottae* conchocelis

had significantly higher photosynthesis at 60 μmol photons m^{-2} s^{-1} than at 30 or 120 μmol photons m^{-2} s^{-1} irradiance (Figure 4.8).

An overall conclusion was that less photosynthesis occurred at the lowest temperature and irradiance than at the higher temperature and irradiance (Figure 4.8). Maximum photosynthetic performance occurred at 30ppt, 11°C and 60 μmol photons m^{-2} s^{-1} irradiance (225 μmoles O_2 g dw^{-1} h^{-1}).

P. pseudolinearis* and *P. torta

The photosynthetic rates of the conchocelis of ***P. pseudolinearis*** and ***P. torta*** were also influenced by all three factors (temperature, salinity and irradiance) and the interactions between these factors (Figure 4.6, Figure 4.7, Table 4.2). The responses of both *P. pseudolinearis* and *P. torta* to the variation in salinity were similar to that of *P. abbottae*. Compared to *P. abbottae*, they seemed to have an extended range of the suitable salinity (25-35ppt) for photosynthetic activity. For instance, mean photosynthetic rates for *P. pseudolinearis* and *P. torta* were much higher at 25-35ppt than at 15ppt or at 40ppt (Figure 4.8).

Although in most cases, these two species still had the highest photosynthesis at 30ppt, the differences in photosynthesis between 25 and 35ppt salinities were not statistically significant (Figure 4.6, Figure 4.7 and Figure 4.8). Both species had low rates of photosynthesis at salinities above 35ppt and less than 20ppt. The more deviation there was from the suitable salinity range (25-35ppt), the more photosynthesis declined. For instance, 10ppt resulted in the lowest photosynthetic rate, especially at 7°C where the photosynthesis of *P. torta* fell to nearly zero at 10ppt and low light.

Photosynthesis generally exhibited a positive correlation with temperature or irradiance. The mean photosynthetic rate increased with increasing temperature and irradiance. The mean

photosynthetic rate of *P. pseudolinearis* increased with increasing temperature and irradiance, and followed a positive correlation relationship (Figure 4.8).

The mean photosynthetic rate of *P. pseudolinearis* at 120 μmol photons m^{-2} s^{-1} was significantly higher than the other two irradiances, with the lowest occurring at 30 μmol photons m^{-2} s^{-1} (Figure 4.8). For *P. torta* conchocelis, similar responses to temperature and irradiance were observed. The mean photosynthetic rates at three temperatures were significantly different from one another (Figure 4.8). However, although the mean photosynthetic rate at 30 μmol photons m^{-2} s^{-1} was significantly lower than at 60 μmol m^{-2} s^{-1} or at 120 μmol photons m^{-2} s^{-1}, no significant difference occurred at irradiances between 60 and 120 μmol photons m^{-2} s^{-1} (Figure 4.8).

For both *P. pseudolinearis* and *P. torta* conchocelis, the combined condition for maximum photosynthesis (respectively 195 and 205 µmoles O_2 g dw^{-1} h^{-1}) occurred at 30ppt salinity, 15°C temperature and 120 μmol photons m^{-2} s^{-1} irradiance.

Differences between species

Comparison of pooled photosynthetic rates of the conchocelis of three species of *Porphyra* for each parameter tested (for comparison of the effect difference between species) are shown in Figure 4.9. There were differences in photosynthesis between different species at all three temperature levels.

For instance, *P. pseudolinearis* had significantly lower photosynthesis than *P. abbottae* or *P. torta* at 7°C temperature, with no difference in photosynthesis between *P. abbottae* and *P. torta*, whereas *P. abbottae* conchocelis exhibited higher photosynthesis than *P. pseudolinearis* and *P. torta* at 11°C, with no difference in photosynthesis between *P. pseudolinearis* and *P.*

torta. However, at 15°C *P. abbottae* had significantly lower photosynthesis than the other two species, with no difference in photosynthesis between *P. pseudolinearis P. torta*.

Overall, *P. abbottae* had significantly higher photosynthetic activity at lower temperatures (7 and 11°C), whereas *P. pseudolinearis* and *P. torta* had a higher photosynthesis at higher temperatures (15°C, Figure 4.9).

As far as irradiance was concerned, there were also differences in photosynthesis between different species (Figure 4.9). Under low irradiance (30 μmol photons m^{-2} s^{-1}), *P. abbottae* had significantly higher photosynthesis than the other two species (Figure 4.9). At 60 μmol photons m^{-2} s^{-1} *P. pseudolinearis* exhibited lower photosynthesis than *P. abbottae* and *P. torta* (Figure 4.9). At 120 μmol photons m^{-2} s^{-1}, there were no differences in photosynthesis between three species (Figure 4.9).

On the whole, *P. abbottae* had higher photosynthesis at a moderate irradiances (60 μmol photons m^{-2} s^{-1}), in contrast to the other two species having higher photosynthesis at higher irradiance (120 μmol photons m^{-2} s^{-1}). Differences between species for salinity were also observed as shown in Figure 4.9. At the lowest salinity (10ppt), *P. torta* had much higher photosynthesis than *P. abbottae* and *P. pseudolinearis*. This implied that *P. torta* conchocelis possessed a higher tolerance to an environment with a low salinity.

Both *P. abbottae* and *P. torta* exhibited significantly higher photosynthesis than *P. pseudolinearis* at 15ppt salinity. Whereas at 30ppt salinity, *P. abbottae* had the highest photosynthesis which was significantly different from that of the other two species. Under the other salinities, although three species exhibited different photosynthesis, none of these differences was statistically significant (Figure 4.9). Higher photosynthetic rates at salinities between 25 and 35ppt have been demonstrated generally in *P. abbottae*, *P. pseudolinearis* and *P.*

torta. Therefore, it seemed that salinity range between 25 and 35ppt was uniformly suitable for photosynthesis of all three species of *Porphyra* (Figure 4.8, Figure 4.9).

Furthermore, comparison of pooled photosynthetic rates (grand mean value) also indicated that there was a significant difference between different species, with *P. pseudolinearis* having the lowest photosynthetic rate. But there was no significant difference in photosynthetic rate between *P. abbottae* and *P. torta* for comparison of grand mean value (Figure 4.10).

Respiration

Respiratory rates of the conchocelis of three species of ***Porphyra*** (*P. abbottae P. pseudolinearis* and *P. torta*) were influenced only by temperature and salinity factors and the interactions only occurred between temperature and species (Figure 4.13 and Figure 4.14, Table 4.3).

The variation of respiration for all three species of conchocelis had a rather uniform pattern, namely, *Porphyra* conchocelis demonstrated the lowest respiration within the range of salinity of 25-35ppt and an obvious increase in respiratory activity under the other higher or lower salinities, particularly at the lowest salinity (10ppt) where there was generally a maximum respiratory rate (Figure 4.13 and Figure 4.14).

Basically, respiration rate increased with increasing temperature for all three species, with the lowest respiration occurring at the lowest temperature (7°C, 31-37 μmoles O_2g dw^{-1} h^{-1}) and the highest respiration occurring at the highest temperature (15°C, 52-57 μmoles O_2 g dw^{-1} h^{-1}, Figure 4.13 and Figure 4.14). The effect of temperature on respiration was revealed more typically in *P. pseudolinearis* and *P. torta* than in *P. abbottae* (Figure 4.14).

Differences between species

Comparison of pooled respiratory rates of the conchocelis of three species of *Porphyra* for each parameter tested (for comparison of the effect difference between species) were shown in Figure 4.15. Although the different species exhibited different respiration activities at all three temperature levels, they were not statistically significant.

There were also no significant differences in respiration between species for all salinity levels (Figure 4.15).

Statistical power (1-β) analysis for photosynthetic and respiratory experiments

The results of statistical power (1-β) analysis indicated that main effect factors(salinity, temperature, light) have high power values (>0.80) when the minimum detectable difference in means for photosynthesis of conchocelis is set at 20% (Table 4.4). Similarly, main effect factors (salinity, temperature, species) for respiratory experiments of conchocelis have high power values (>0.90) when the minimum detectable difference in means for respiration of conchocelis is set at 10% (Table 4.5).

Discussion

The evidence that there is a positive correlation between available light and oxygen evolution for a variety of marine algae has been provided by many studies including laboratory and field investigations (Wassman 1973, Anderson & North 1969, Arnold & Murray 1980, Fork 1963, Gao & Aruga 1987). Nevertheless, the positive correlation relationship between available light and oxygen evolution of marine algae could possibly take place under some conditions within a specific range of the light intensity. In most cases, due to complicated interactions between various environmental variables, this simple positive correlation relationship would not

exist (e.g. the occurrences of photosaturation and photoinhibition, Fain & Murray 1982, Geider & Osborne 1992, Wheeler 1980).

In my experiments, different species of *Porphyra* conchocelis clearly exhibited different P-I curves, including photosynthetic increases with the light intensity under some incubation conditions, photosynthetic saturation and photoinhibition under some other conditions. *P. abbottae* exhibited photoinhibition at all three temperatures tested within a relatively narrow range of light levels. *P. abbottae* did not respond with increased photosynthesis with increasing temperature. Maximum photosynthesis occurred at 11°C rather than 15°C at a light intensity of 60 μmol photons m^{-2} s^{-1}. Increasing light intensity did not cause an increase in *P. abbottae* photosynthetic activity especially at higher temperatures. In fact *P. abbottae* showed an inverse relationship between light intensity and photosynthesis. At 15°C, 30 μmol photons m^{-2} s^{-1} light gave maximal photosynthesis. These results suggest that *P. abbottae* responds uniquely to environmental change. These results are similar to those for *P. abbottae* from earlier reported experiments which were based on growth of the conchocelis.

From a comparative study on the effects of irradiance on photosynthesis of *Porphyra yezoensis*, Zhang *et al.* (1997) reported that *Porphyra* could utilize white light for photosynthesis effectively. However, their results only represented instant effects, because plant materials were exposed to light sources for a very limited short time (less than 30 minutes at each irradiance). They found the conchocelis of *Porphyra* are very sensitive to light environments and oxygen evolution of conchocelis precisely varied with an instant increase or decrease in the light intensity, with oxygen evolution rate being between 0-2 μmol g dw^{-1} min^{-1} responding to the variation range of light pulse of 0-250 μmol photons m^{-2} s^{-1}. They also found *Porphyra yezoensis* had low I_c values (compensation point, about 6.5-10 μmol photons m^{-2} s^{-1}). Their

results was similar to those in my P-I curve experiments. It appeared that different species of *Porphyra* conchocelis share uniformly low I_c values, despite their dissimilarity in geographical distributions.

Guo *et al.* (1992) reported that physiological responses in photosynthesis and tolerance to varying environments by several *Ulvoid* green algae from coastal and estuarine habitats were closely related to their patterns of local distributions and seasonal occurrences. For instance, the photosynthetic light responses varied with the species inhabiting different coastal environments, with I_c ranging between 3 to 40 μmol photons m^{-2} s^{-1} and I_{max} ranging between 40 to 564 μmol photons m^{-2} s^{-1} depending on ecological distribution. That *Ulvaria obscura* living in the subtidal zone had low I_c (3-8 μmol photons m^{-2} s^{-1}) and *Monostroma grevillei* occurring typically in upper-mid intertidal zone exhibited relatively high irradiances for achieving saturation points (I_{max} from 270 to > 564 μmol photons m^{-2} s^{-1}) reflected the fact that photosynthetic responses were associated with the ecological features of marine algae. Several other investigators have found I_c values between 6.1-11.4 μmol photons m^{-2} s^{-1} and I_{max} between 60-200 μmol photons m^{-2} s^{-1} for some other species of coastal algae (Arnold & Murray 1980, Ohno & Nozawa 1972). In a detailed review of light responses in seaweeds, Luning *et al.*, (1975, 1978, 1981) reached a conclusion that saturation irradiances for intertidal species are typically 400-600 μmol photons m^{-2} s^{-1} versus approximately 200 μmol photons m^{-2} s^{-1} for shallow subtidal species. He also noted that compensation points for most intertidal and shallow subtidal seaweeds are generally less than 20 μmol photons m^{-2} s^{-1}. The results from my experimental studies were comparable to those reported by other researchers. However, variation differences in physiological responses could be different for different species, or for different generation stages and different developmental stages of the same species.

Many physical factors can influence species composition, phenology and distributional patterns of seaweeds (Lobban *et al.*, 1985). Temperature, salinity and irradiance are often considered the primary factors determining the growth, reproduction and distribution of seaweeds, particularly in the coastal intertidal zones or estuaries, where extreme variations in these environmental factors could lead to the failure in the growth and survival of some species or in the existence of alternative life generations (Druehl 1981, Emery & Stevenson 1957, Hoek 1982, Mathieson 1971, 1975a, 1975b, Wilkinson 1980). Therefore, variations in physiological responses of seaweeds generally mirror the ecological characteristics of different species. It should be pointed out that the conchocelis filaments of *Porphyra* had relatively lower I_c and I_{max} and photoinhibition occurred at the lower irradiances compared with other coastal marine algae or the gametophytic (leafy) stage of *Porphyra*(Luning 1979, Arnold & Murray 1980, Ohno & Nozawa 1972, Markager, 1993). These lower levels may be due to their residing in benthic environments, especially boring into shells.

Guo(1992) found that in terms of photosynthetic activity, the temperature optima and tolerances of the four Ulvoid algae were closely related to their seasonal occurrence. The winter and spring annuals *Monostroma grevillei* and *M. pulchrum* had lower temperature optima between 5 to 10°C, with limited tolerances to higher temperatures. They could resist higher lethal temperatures and exhibited much higher photosynthetic activity when a favorable salinity (30ppt) was provided. The summer annual *Ulvaria oxysperma* had a much higher temperature optimum of approximately 20°C, with the tolerance to 30 to 35°C. By contrast, the seasonal annual *U. obscura* exhibited broad and variable temperature optima (10-25°C) throughout the year, with a consistent lethal temperature of 30°C. The rates of net photosynthesis for *U. obscura* also varied seasonally; *i.e.*, they were higher at 5-20°C during the winter than in the summer. Seasonal changes of temperature optima and tolerances also have been observed in

some other species of algae (Mathieson & Norall 1975a, b). As an alternative generation of *Porphyra*, conchocelis generally occurs during summer time in natural habitats. The conchocelis of both *P. pseudolinearis* and *P. torta* have maximal photosynthetic activity at 15°C, which is a seawater temperature generally occurring in the SE Alaska in summer season. This may reflect their ecological adaptation abilities to a higher temperature.

My experimental findings showed that controlled lab experiments could be used as an effective way to evaluate physiological and ecological aspects of sporophytic stage (microscopic life stage) of *Porphyra* species. Variations in photosynthetic and respiratory activities reflected physiological characteristics of the conchocelis of these indigenous *Porphyra* species in response to various environmental conditions.

For photosynthetic responses, all three species (*P. abbottae, P. pseudolinearis* and *P. torta*) were significantly affected by the tested environmental variables: salinity, temperature and irradiance, including interactions between these factors. As the result of a long historical adaptation to unique high-latitude environments and the benthic life, the conchocelis of these indigenous *Porphyra* species could utilize the limited light to perform photosynthesis and sustain life process. It seems that these indigenous *Porphyra* species have relatively low I_c and I_{max} values, compared with the corresponding values assessed from other sun plants or the gametophytes of *Porphyra*. Rapid light-attenuation is characteristic of coastal waters, in which suspended material absorbs and scatters sunlight. On some coastal sites with freshwater runoffs of Alaska, glacier-melting process may cause high turbidity in these waters, thus low water transparency could in turn result in less light penetration and little light available to the benthic plants. Therefore, low Ic value probably has biological importance in determining compensation depths, hence vertical distributions.

On the other hand, as the results of tidal cycle, snow/glacier melting and frequent rainfalls, the sporophytic stage of these indigenous *Porphyra* may encounter extreme variations in temperature and salinity during the period of their occurrence in the natural habitats. My experimental results indicate the conchocelis of these indigenous *Porphyra* species could adapt to a range of the temperature and salinity and the net photosynthetic activity can persist at the varying levels of the factors tested. In intertidal or subtidal zones, there are numerous environmental variables influencing sporophytic stage of *Porphyra*. These factors and their interactions could exert effects on the growth, physiological state and various life processes of the sporophytic stage. Existence of main effect and interactions in photosynthetic activity for these *Porphyra* species reflects the fact that microscopic life stage of *Porphyra* is quite responsive to variations of environmental parameters in terms of photosynthetic physiology. In other words, *Porphyra* conchocelis could adjust their physiological responses (change in photosynthetic activity) to different environmental conditions.

For respiratory responses, all three species were influenced by salinity and temperature factors, but there were no interactions between these factors and there were also no differences in respiratory response among these species. Optimal salinities of 25-35ppt , where significantly higher net photosynthesis was accompanied by lowest respiratory activity, have obvious important biological implications in maintaining the healthy physiological state of *Porphyra* conchocelis. Favorable environmental conditions (such as salinity) enable the conchocelis to maintain a relatively stable low respiration rate. Whereas unfavorable conditions would result in higher oxygen consumption leading to low net photosynthetic rates.

In artificial culture of sporophytic stage, control and regulation of various factors are crucial to inducing the development and maturation of conchocelis and to successful *Porphyra* cultivation. This series of experiments have revealed the optimal condition combinations for the

conchocelis of each species to perform photosynthesis and respiration. This basic information could be used for the implementing of the culture of sporophytic stage of these indigenous *Porphyra* species. All three species are potential candidate species for commercial mariculture in Alaska. This conclusion is based on the existing local market trade, flavor, quality and adaptation to the environment.

Conclusions

Photosynthetic and respiratory activities can be used for evaluation of physiological and ecological characteristics of the sporophytic stage (microscopic life stage) of *Porphyra* species. Variations in photosynthetic and respiratory activities are the physiological responses of the conchocelis of these indigenous *Porphyra* species to various environmental conditions. Existence of main effect and factorial interactions in photosynthetic and respiratory activities for these *Porphyra* species illustrate that physiological reactions of microscopic life stage of *Porphyra* are responsive to variations of environmental parameters.

Porphyra conchocelis can effectively utilize limited light due to low I_c (compensation point of photosynthesis) and low I_{max} (irradiance at which photosynthesis reached the maximum). The conchocelis of these indigenous *Porphyra* species could also adapt to a considerable variation in temperature and salinity. Such physiological characteristics may relate to adaptation to their unique natural habitat environments. Patterns in photosynthesis in response to the conditions varied with the different species. That means there are differences in photosynthetic adaptation among these species.

For respiratory responses, all three species were influenced by salinity and temperature factors, but basically there were no interactions between these factors and there were also no differences in respiratory response between these species.

The optimal condition combinations for the conchocelis of each species to perform photosynthesis and respiration from my multiple-factor experimental study could be used as the useful basis for the culture of sporophytic stage of these indigenous *Porphyra* species.

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Table 4.1. Irradiances of compensation point (I_c), photosynthetic maxima (P_{max}) and irradiance maxima (I_{max}) for the conchocelis of three species of *Porphyra* at three different temperatures.

Temp.	*P. abbottae*			*P. pseudolinearis*			*P. torta*		
°C	Ic	Imax	Pmax	Ic	Imax	Pmax	Ic	Imax	Pmax
7°C	5.0	140	83	4.5	250	239	3.3	250	100
11°C	4.3	50	134	3.5	250	192	5.0	225	93
15°C	3.3	20	146	3.1	135	221	4.8	200	240

Note: I_{max}, I_c = μmol photons m^{-2} s^{-1}, P_{max} = µmoles O_2 g dw^{-1} h^{-1}. I_c values were estimated from the photosynthetic values intercepted on the x-axis by the points of 0 and the lowest irradiances for the P-I curves.

Table 4.2. ANOVA table for photosynthesis of the conchocelis of three different *Porphyra* species at combinations of salinity, irradiance and temperature. [a]10, 15, 20, 25, 30, 35, 40ppt; [b]7, 11, 15°C; [c]30, 60, 120 μmol photons m^{-2} s^{-1}; [d]*P. abbottae*, *P. pseudolinearis*, *P. torta* (*$P<0.05$; **$P<0.01$).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Salinity[a]	6	300408.6	50068.1	57.00**
Temperature[b]	2	59563.9	29781.9	33.90**
Light[c]	2	29330.2	14665.1	16.69**
Sal. x Temp.	12	17921.2	1493.4	1.70
Sal. x Light	12	28169.5	2347.5	2.67*
Temp. x Light	4	159544.9	39886.2	45.41**
Sal. x Temp. x Light	24	72261.9	3010.9	3.43**
Residuals	189	166024.6	878.4	
P. pseudolinearis				
Salinity[a]	6	178129.4	29688.2	40.44**
Temperature[b]	2	255792.9	127896.5	174.20**
Light[c]	2	67343.8	33671.9	45.86**
Sal. x Temp.	12	45222	3768.5	5.13**
Sal. x Light	12	20179.3	1681.6	2.29*
Temp. x Light	4	14271.2	3567.8	4.86**
Sal. x Temp. x Light	24	7185.9	299.4	0.41
Residuals	189	138762.3	734.2	
P.torta				
Salinity[a]	6	143226.1	23871.0	50.83**
Temperature[b]	2	180406.2	90203.1	192.09**
Light[c]	2	119321.4	59660.7	127.05**
Sal. x Temp.	12	57812.7	4817.7	10.26**
Sal. x Light	12	37097.9	3091.5	6.58**
Temp. x Light	4	15188.5	3797.1	8.09**
Sal. x Temp. x Light	24	39931.3	1663.8	3.54**
Residuals	189	88753.4	469.6	

Table 4.3. ANOVA table for respiration of the conchocelis of three different *Porphyra* species at combinations of salinity and temperature. [a]10, 15, 20, 25, 30, 35, 40ppt; [b]7, 11, 15°C; [c]*P. abbottae*, *P. pseudolinearis*, *P. torta* (*$P<0.05$; **$P<0.01$).

Source of variation	df	Sum of squares	Mean square	F
Salinity[a]	6	14074.46	2345.7	17.16**
Temperature[b]	2	16393.7	8196.9	59.95**
Species[c]	2	540.6	270.3	1.98
Sal. x Temp.	12	417.7	34.8	0.25
Sal. x Sp.	12	1167.5	97.3	0.71
Temp. x Sp.	4	1995.6	498.9	3.65*
Sal. x Temp. x Sp.	24	1697.9	70.7	0.52
Residuals	189	25840.5	136.7	

Table 4. 4. Statistical power (1- ß) based on the results of variance analysis for experiments with the photosynthetic activity of *Porphyra* conchocelis. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 20%. Significant criterion α is equal to 0.05.
(** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Salinity	6	57.00**	6	28	0.3443	0.96
Temperature	2	33.90**	2	64	0.2826	0.94
Light	2	16.69**	2	64	0.2880	0.95
Porphyra pseudolinearis						
Salinity	6	40.44**	6	28	0.2973	0.88
Temperature	2	174.20**	2	64	0.2734	0.92
Light	2	45.86**	2	64	0.3235	0.98
Porphyra torta						
Salinity	6	50.83**	6	28	0.3451	0.96
Temperature	2	192.09**	2	64	0.3404	0.98
Light	2	127.05**	2	64	0.3606	>0.99

Table 4. 5. Statistical power (1- ß) based on the results of variance analysis for experiments with the respiratory activity of *Porphyra* conchocelis. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 10%. Significant criterion α is equal to 0.05. (** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Salinity	6	17.16**	6	28	0.3259	0.93
Temperature	2	59.95**	2	64	0.3368	0.98
Species	2	1.98	2	64	0.2903	0.95

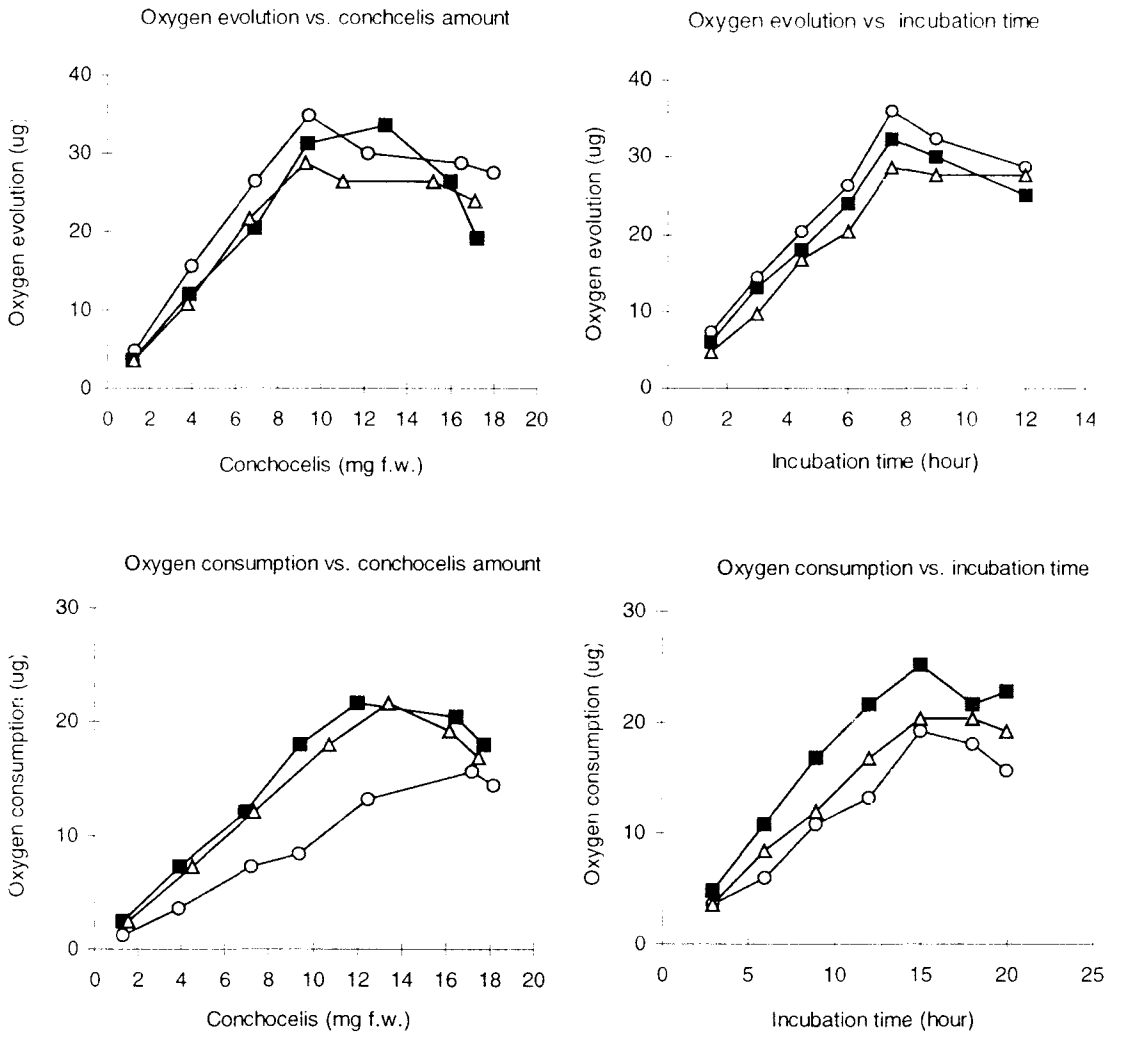

Figure 4.1. Relationships between oxygen evolution (or oxygen consumption) and conchocelis amount, incubation time for three species of *Porphyra* (○, Pa; ■, Pi; Δ, Pt). Oxygen evolution experiments were conducted at 11°C, 60 μmol photons m^{-2} s^{-1} and 30ppt for Pa; at 15°C, 120 μmol photons m^{-2} s^{-1} and 30ppt for Pi and Pt. Oxygen consumption experiments were conducted at 15°C and 10ppt in darkness.

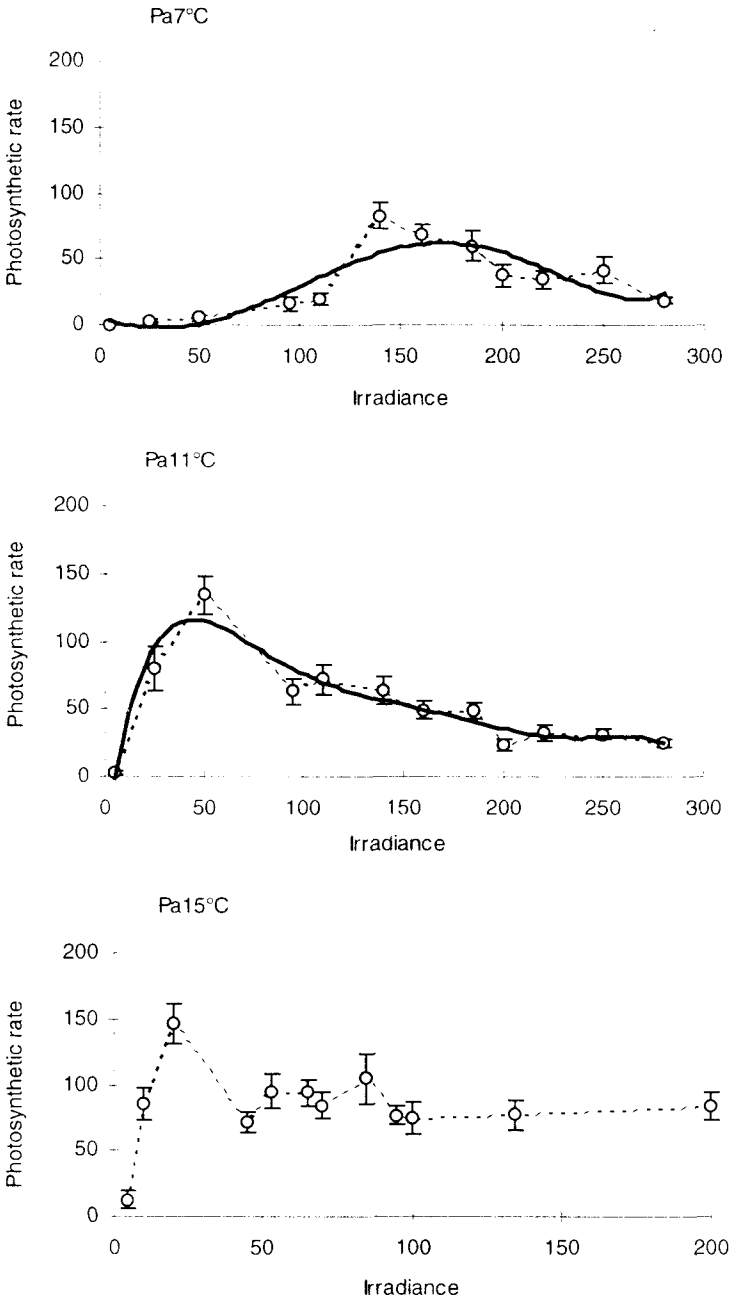

Figure 4.2. *Porphyra abbottae* (Pa). The photosynthetic rate of conchocelis versus irradiance (μmol photons m^{-2} s^{-1}) at three different temperature conditions (7, 11, 15°C). Salinity (30ppt). The dotted lines were drawn from the means of data points. Solid lines represent the fitted trend lines. Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

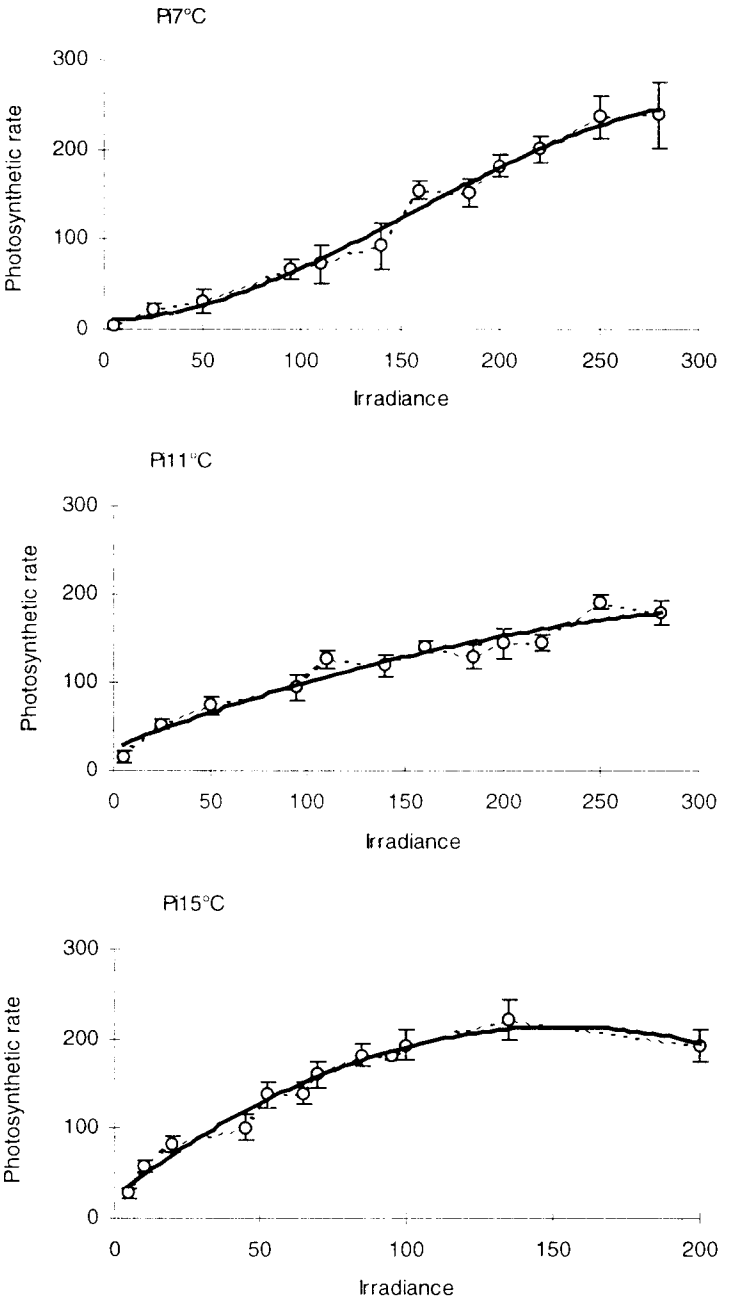

Figure 4.3. *Porphyra pseudolinearis* (Pi). The photosynthetic rate of conchocelis versus irradiance (μmol photons m^{-2} s^{-1}) at three different temperature conditions (7, 11, 15°C). Salinity (30ppt). The dotted lines were drawn from the means of data points. Solid lines represent the fitted trend lines. Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

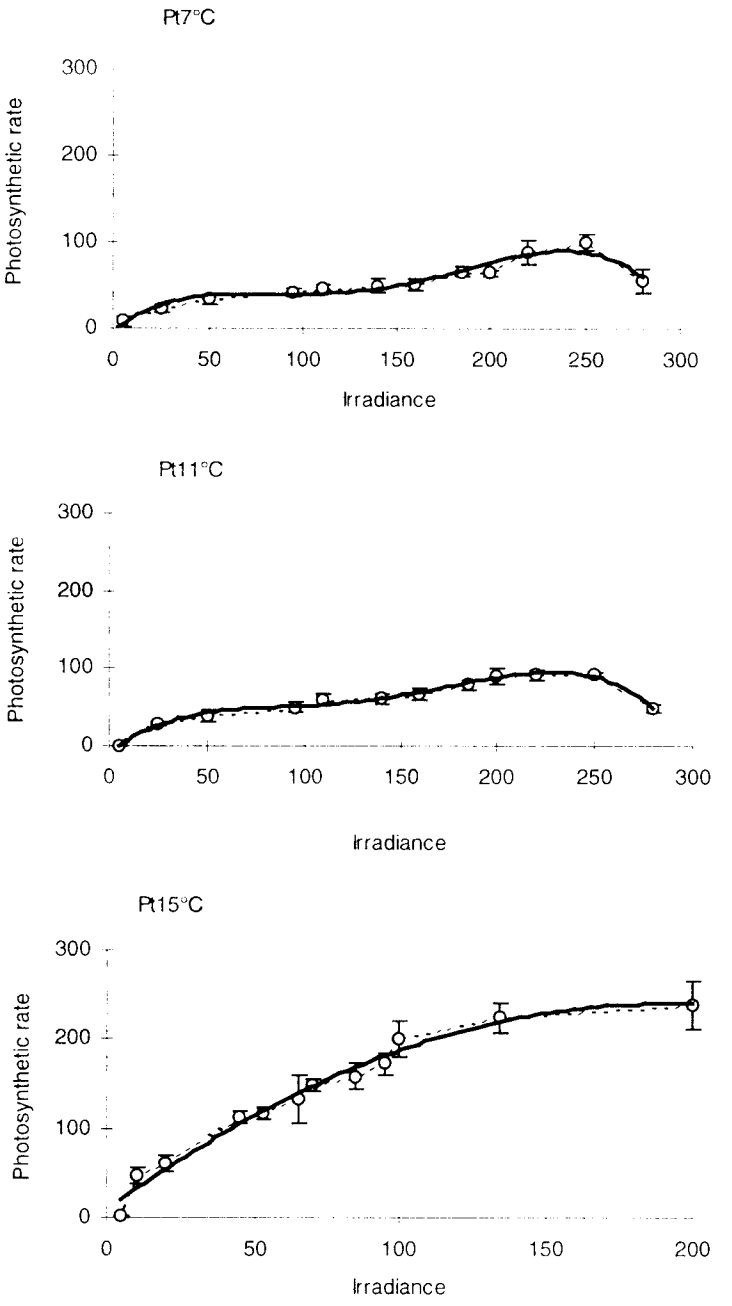

Figure 4.4. *Porphyra torta* (Pt). The photosynthetic rate of conchocelis versus irradiance (μmol photons m^{-2} s^{-1}) at three different temperature conditions (7, 11, 15°C). Salinity (30ppt). The dotted lines were drawn from the means of data points. Solid lines represent the fitted trend lines. Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

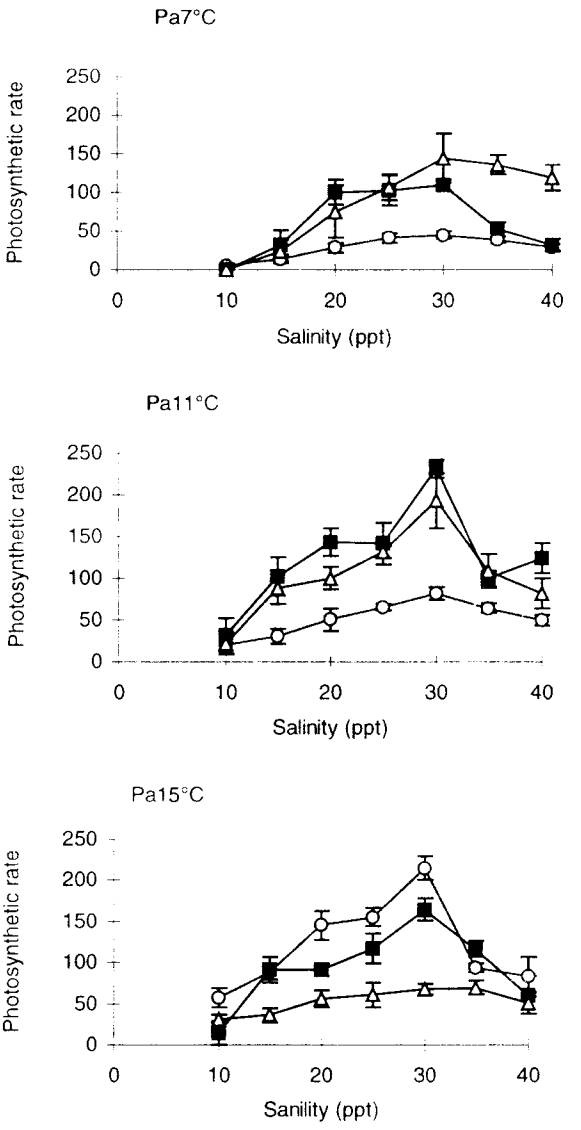

Figure 4.5. *Porphyra abbottae* (Pa). Conchocelis photosynthesis as a function of salinity (ppt), irradiance (O, 30; ■, 60; Δ, 120 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

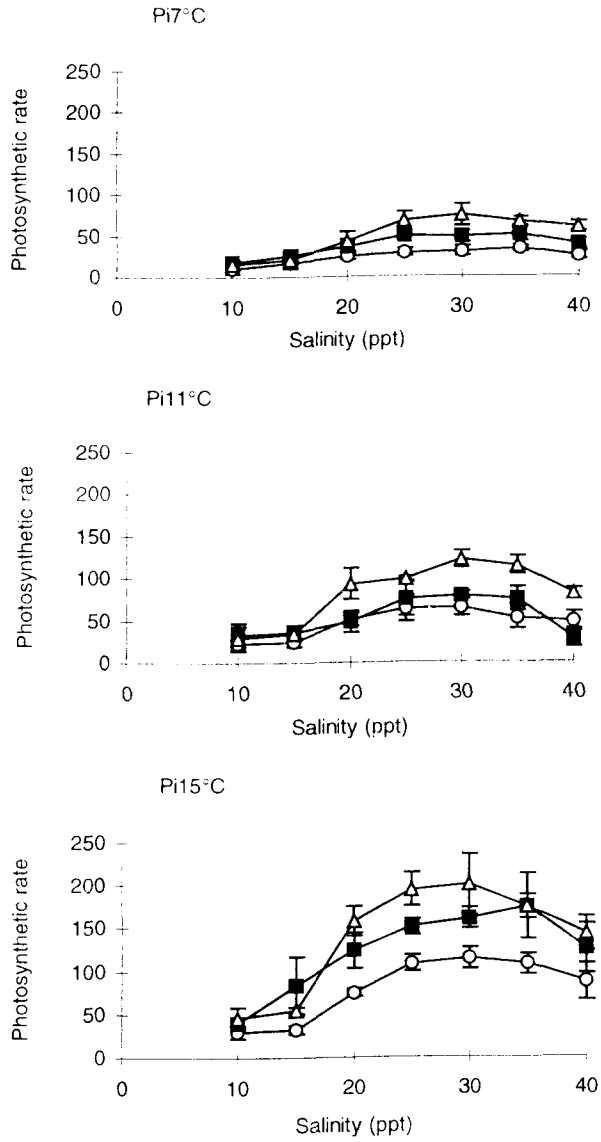

Figure 4.6. *Porphyra pseudolinearis* (Pi). Conchocelis photosynthesis as a function of salinity (ppt), irradiance (O, 30; ■, 60; Δ, 120 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

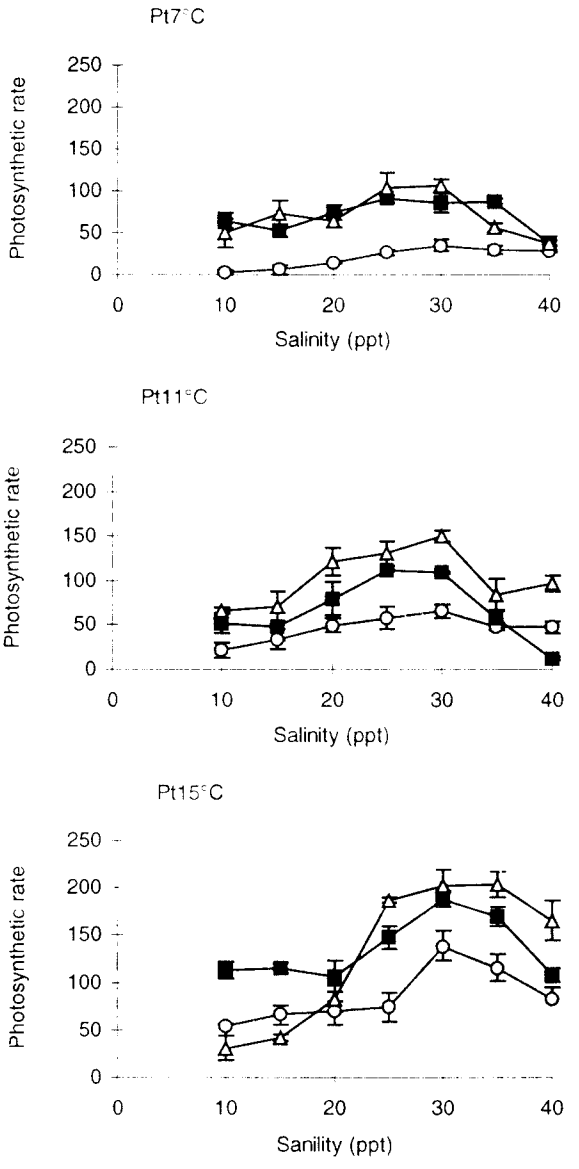

Figure 4.7. *Porphyra torta* (Pt). Conchocelis photosynthesis as a function of salinity (ppt), irradiance (O, 30; ■, 60; Δ, 120 μmol photons m^{-2} s^{-1}) and temperature (°C). Error bars are ± S.E. Photosynthesis is expressed as μmol O_2 g dw^{-1} h^{-1}.

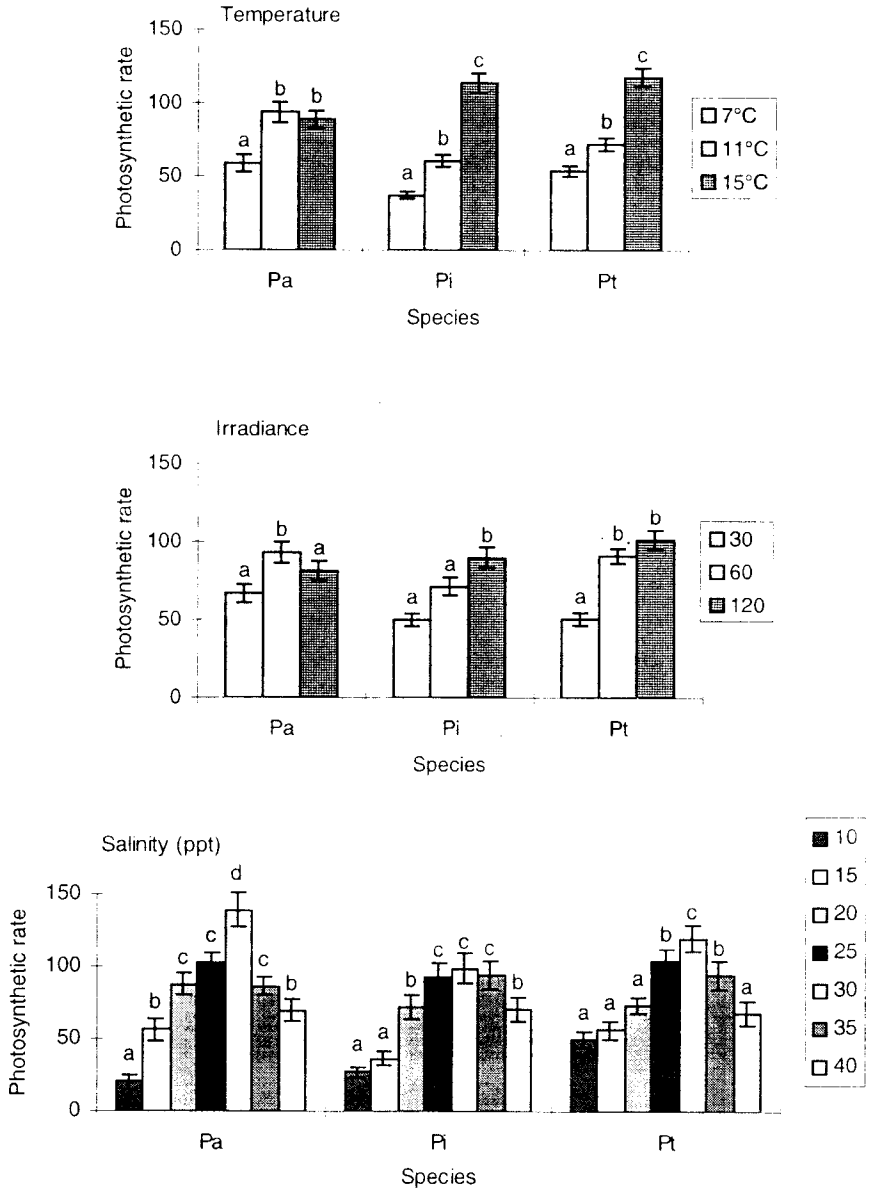

Figure 4.8. Comparison of pooled photosynthetic rates of conchocelis of three species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only within a species. Units of parameters tested are: temperature (°C), irradiance (μmol photons m^{-2} s^{-1}) and salinity (ppt).

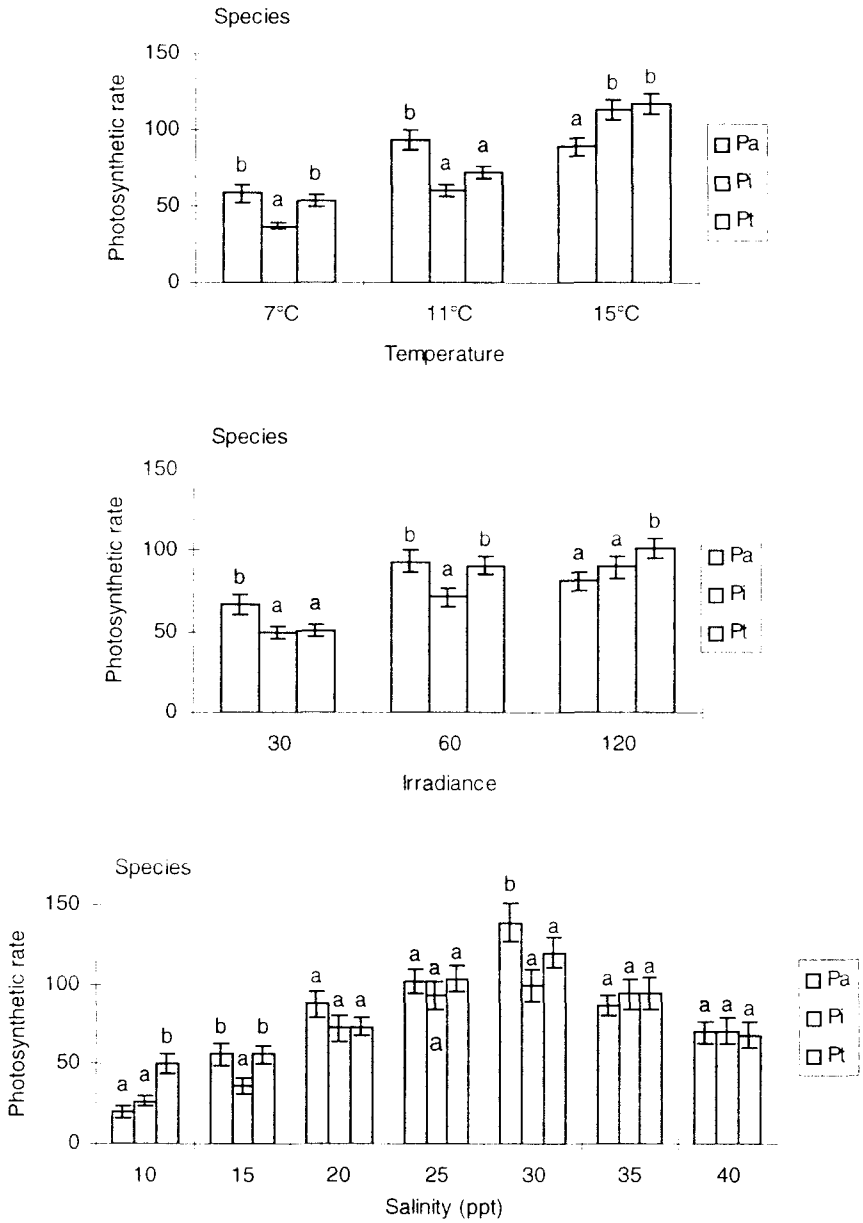

Figure 4.9. Comparison of pooled photosynthetic rates of conchocelis of three species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only between species. Units of parameters tested are: temperature (°C), irradiance (μmol photons m^{-2} s^{-1}) and salinity (ppt).

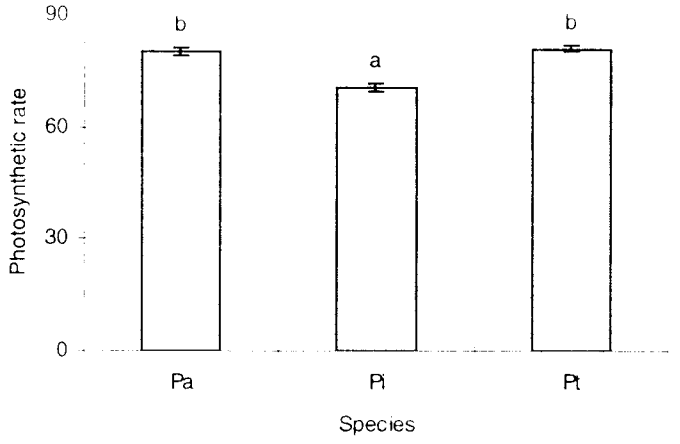

Figure 4.10. Comparison of pooled photosynthetic rates (grand average value) of conchocelis of three species of *Porphyra*. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test.

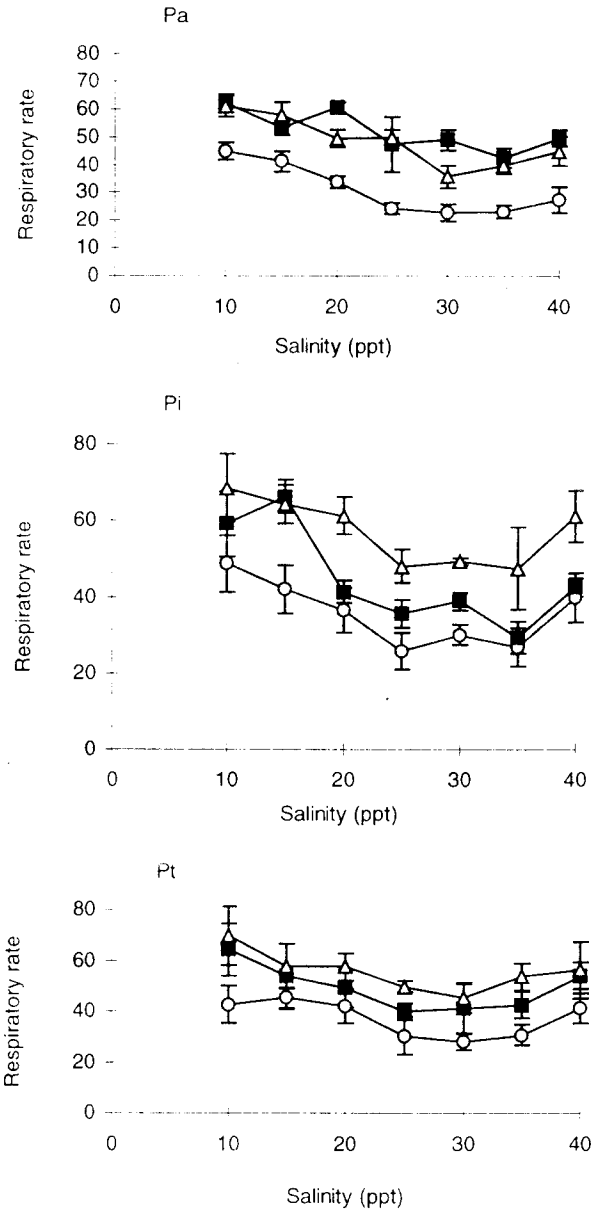

Figure 4.11. Conchocelis respiration of three different *Porphyra* species as a function of salinity (ppt) and temperature (○, 7; ■, 11; Δ, 15°C). Error bars are ± S.E. Respiration is expressed as μmol O_2 g dw^{-1} h^{-1}.

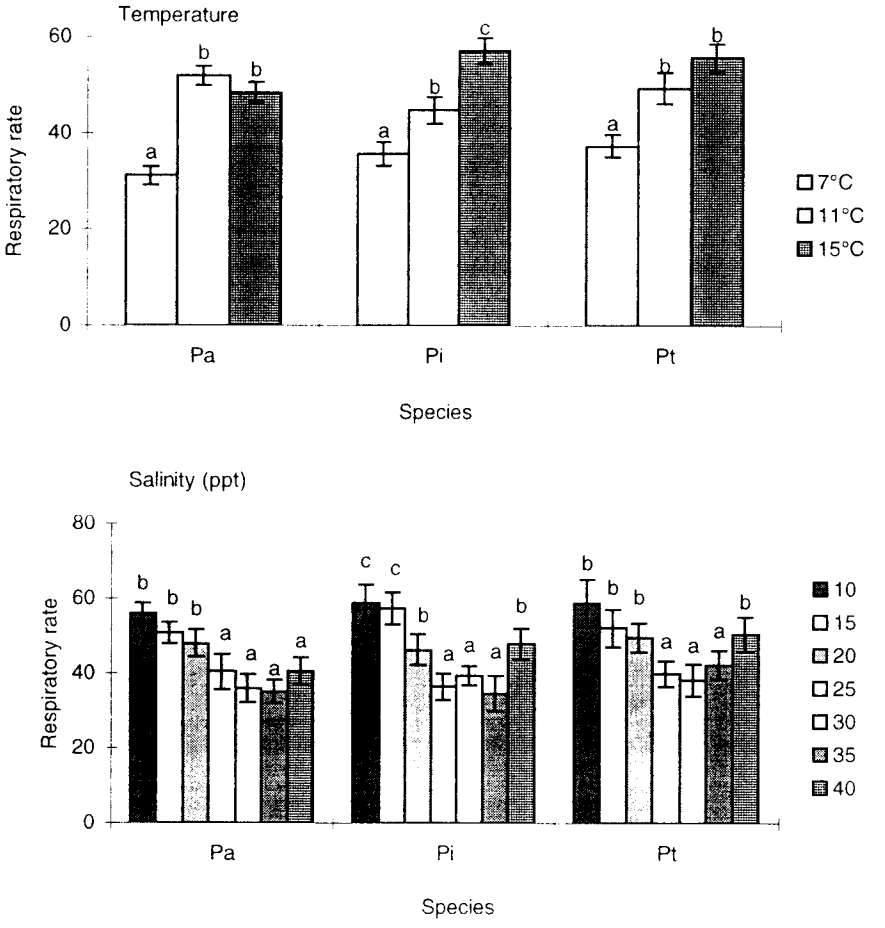

Figure 4.12. Comparison of pooled respiratory rates of conchocelis of three species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only within a species. Units of parameters tested are: temperature (°C) and salinity (ppt).

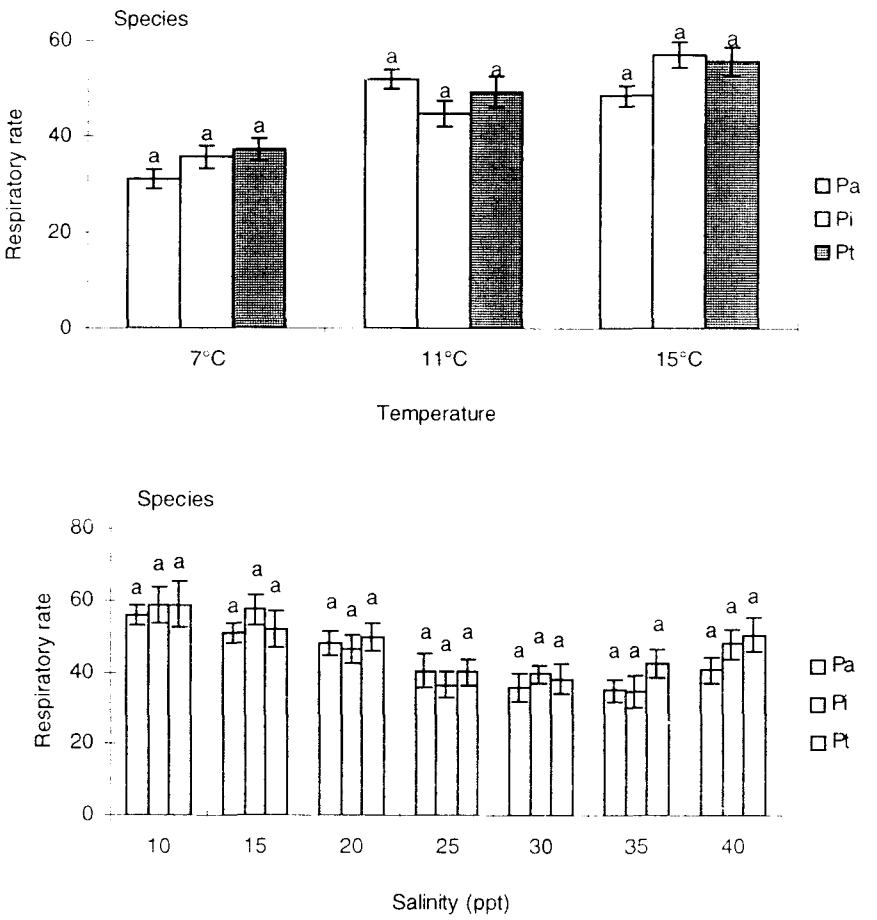

Figure 4.13. Comparison of pooled respiratory rates of conchocelis of three species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.05$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant only between species. Units of parameters tested are: temperature (°C) and salinity (ppt).

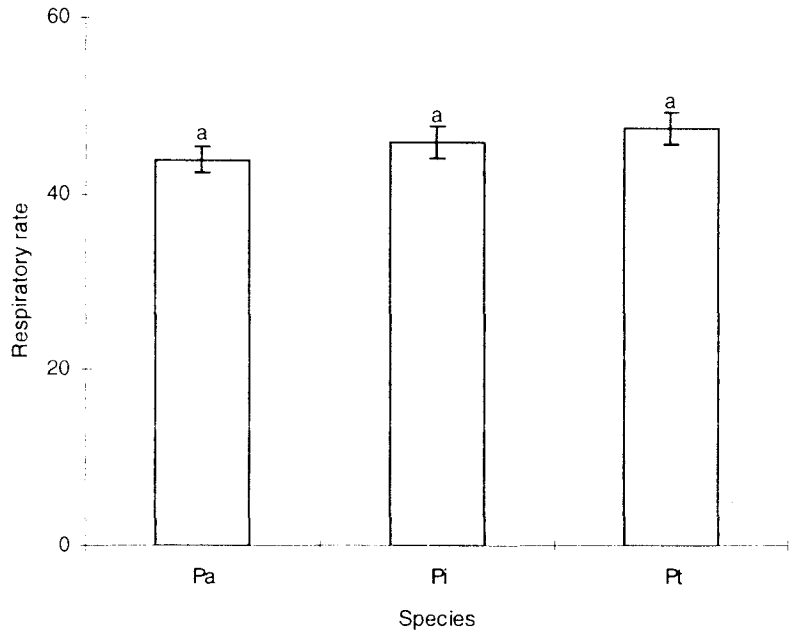

Figure 4.14. Comparison of pooled respiratory rates (grand average value) of conchocelis of three species of *Porphyra*. Error bars are ± S.E. Same letters above the bars indicate no significant differences between these three species.

Chapter 5

Photosynthetic Pigment Content of Four Indigenous Alaskan *Porphyra* Species: Response to Environmental Variables

Abstract

Variations of four photosynthetic pigments in conchosporangia of indigenous Alaskan *Porphyra* species, *P. abbottae* (Pa), *P. pseudolanceolata* (Pe), *P. pseudolinearis* (Pi) and *P. torta* (Pt), were investigated in response to environmental variables. Conchosporangia were cultured under different irradiances of 0, 10, 40 and 160 μmol photons $m^{-2}s^{-1}$ and nutrient concentrations of 0, f/4, f/2 and f for up to 60 days (with temperature and salinity fixed at 11°C and 30ppt).

Phycoerythrin (PE), phycocyanin (PC) , carotenoids (Ca) and chlorophyll *a* (Chl. *a*) contents were extracted and measured by spectrophotometry. PE and PC were the dominant photosynthetic pigments. Phycobiliprotein (PE + PC) comprised 72-95% of total pigments depending on culture conditions, whereas Ca and Chl. *a* accounted for a small percentage of total pigments.

Photosynthetic pigments were significantly affected by irradiance, nutrient concentration and culture duration. For Pa, Pi and Pt, maximal PE (63.2-95.1 mg/g.dw) and PC content (28.8-64.8 mg/g.dw) generally occurred at 10 μmol photons m^{-2} s^{-1}, f/4-f/2 nutrient concentration and 10-20 day culture duration, while Pe had highest PE (73.3 mg/g.dw) and PC content (70.2 mg/g.dw) at 10 μmol photons m^{-2} s^{-1}, f nutrient concentration and 60 day culture duration. For all four species, the highest Ca (3.4 - 6.3 mg/g.dw) and Chl. *a* content (3.6-8.1 mg/g.dw) occurred at 0-10 μmol photons m^{-2} s^{-1}, f/2-f nutrient concentration and 20-30 day culture. There were significant differences in photosynthetic pigment content among the four species. *P.*

abbottae had higher PE content than the other three species and Pe had the highest PC content. Pt had the lowest content for all four kinds of pigment. *Porphyra* conchosporangia generally had higher photosynthetic pigment contents at 0-10μmol photons m^{-2} s^{-1}, f/4-f nutrient concentration. Higher irradiances (40 µmol photons $m^{-2}s^{-1}$), low nutrients and longer culture duration generally caused a decline of photosynthetic pigment content.

Introduction

Because control of the sporophytic stage (*i. e.* conchocelis stage) is very important to successful *Porphyra* aquaculture, the influences of environmental factors on important physiological processes and the biochemical composition of the *Porphyra* conchocelis stage need to be investigated prior to the establishment of a nori mariculture industry. Furthermore, because photosynthetic pigments involve the conversion of light energy to chemical energy in all photosynthetic organisms (Glazer 1977, Grabowski 1978), the study of photosynthetic pigment is an important aspect of physiology of plants. Unfortunately, to date, the physiology and biochemistry of the microscopic life stage has received little attention. Little is understood about effects of environmental factors on the pigment content of *Porphyra* conchocelis stage.

Marine red algae like *Porphyra* contain a series of special pigment proteins known as phycobiliproteins (Glazer 1977, 1981, Yu *et al.* 1981). These phycobiliproteins have unique applications in immunology and diagnostic medicine (Loken *et al.* 1977, Mishell *et al.*, 1980, Mota *et al.*, 1978). They can be coupled to specific antibodies, usually as monoclonals. When the antibody attaches to its specific receptor site on a cell or tissue, the latter can readily be visualized by their fluorescence. Other potential applications include the fluorescence-labeling of DNA probes and fluorescence immunoassays of molecules and cells. Phycobiliproteins may also be coupled to enzymes and other proteins, polypeptide, hormones, nucleic acids, drugs, vitamins, etc. (Deisenhofer 1981, Koppel *et al* 1976, Ledbetta *et al.*, 1981, Reihorst *et al.*, 1979, Terhorst *et al.*, 1980, Taylor & Wang 1980). High-purity phycobiliproteins have maintained a market price as high as $25-60 per milligram (Sigma, 1999).

There are several advantages for phycobiliproteins to be extracted from the sporophytic stage of *Porphyra*: (1) there is no limit to the yield of phycobiliprotein products because *Porphyra* conchocelis can be grown abundantly in the lab environment. Therefore,

phycobiliprotein products can be acquired at any time, year round, without going to the field for sample-collecting or depending on the availability of wild agal material (usually marine algae are only available in limited seasonal periods). (2). It is relatively easy to extract the pigments from the uniseriate filaments. (3). high-quality and high-purity phycobiliprotein can be obtained from conchocelis cultures grown under artificially-controlled conditions.

Chlorophyll *a*, phycoerythrin, phycocyanin and carotenoids are the principal light-harvesting pigments in the chloroplasts of marine red algae. In the photosynthetic processes of red algae, accessory pigments (phycoerythrin, phycocyanin and carotenoids) absorb different wavelengths of light and transfer the light energy to chlorophyll a, which converts all absorbed light energy into chemical energy (ATP) and reducing power (NADPH) that are used in the synthesis of organic compounds from carbon dioxide. Therefore, these four harvesting pigments are important in determining physiological responses of *Porphyra* conchocelis stage to environment.

This research was conducted with the following objectives:

(1). to investigate whether or not light has an effect on the pigment content of conchosporangia of Alaskan *Porphyra* species;

(2). to determine the effect of nutrient concentration on the pigment content of conchosporangia;

(3). to determine whether culture age has an effect on the pigment content of the conchosporangia stage;

(4). to investigate interactions among these different factors regarding their effects on the pigment contents;

(5). to compare absorption spectra for photosynthetic pigments from different species of *Porphyra*.

Materials and Methods

Unialgal cultures of each *Porphyra* species (*Porphyra abbottae* Krishnamurthy - strain PaSGS01, *P. pseudolanceolata* Krishnamurthy - strain PeJB03, *P. pseudolinearis* Ueda - strain PiSC14 and *P. torta* Krishnamurthy - strain PtCH13a) were obtained from carpospore release. Mature blades of the gametophyte stage of each species were collected from the field. Blades were washed and scrubbed with sterile seawater to remove surface contamination. The cleaned blades were placed in sterile seawater in petri dishes for carpospore release. After 24-36 hours the blades were removed and the dishes incubated in Provasoli's enriched seawater (PES; McLachlan, 1973) under 16L:8D photoperiod at 11°C. Conchocelis segments (around 110-250 μm) of each species were placed in cell well plates (one piece per well) and incubated at 30ppt salinity and 11°C (100-120 μmol photons m^{-2} s^{-1} irradiance) for the culture of pure genotype conchocelis, which were used for culture of bulk conchocelis materials for experiments. PES enriched seawater culture medium was used for the conchocelis stage. When conchocelis began to develop into the conchosporangia stage, they were incubated at 11°C and 25 μmol photons m^{-2} s^{-1} irradiance with f/2 culture media.

Conchosporangia used for pigment experiments were incubated at 11°C and illuminated with cool-white fluorescent lamps. Irradiance gradients were obtained by wrapping the culture containers with varying layers of white paper. Irradiance was measured by a Li-Cor Radiation Sensor (Li-190SB Quantum Sensor). The pH of the culture medium was adjusted to 7.8 - 8.0 (the ambient pH of the seawater in the inside waters of SE Alaska) using 6 M HCl or 6 M NaOH. The salinity of experimental seawater was set at 30ppt. Culture media were changed every 7 days. Long day (16L: 8D) photoperiods were used. Nutrients were added as an f culture medium concentration (Guillard and Ryther, 1962), which has a nitrogen concentration of 1.747 mM; therefore, nutrient levels of 0, f/4, f/2 and f concentrations represented 0.02, 0.437, 0.874 and

1.747 mM of nitrogen concentration respectively (conchosporangia at 0 nutrient concentration represented those incubated in natural seawater with a nitrogen concentration of 0.02 mM, *i.e.*, no f culture medium was added). In order to ensure sufficient inorganic carbon source available to the conchocelis, culture media were supplemented with 5mM $NaHCO_3$. For pigment experiments, different levels of three environmental factors were employed: nutrient levels of 0, f/4, f/2, f concentration; irradiances of 0, 10, 40, 160 μmol photons m^{-2} s^{-1} ; culture duration of 10, 20, 30, 60 days.

Procedures for measurement and analysis of pigment content:

Porphyra conchosporangia were grown in 200 ml flasks under the varying culture conditions. After being incubated for 10, 20, 30, 60 days, conchosporangia samples were rinsed with sterile seawater and ground in a mortar and pestle at low temperature and low light. Four replicates of conchosporangia samples from each combination of culture conditions were used for pigment measurements and one corresponding sample was used for measurement of the ratio of dry weight to fresh weight. Pigments were extracted at 4°C temperature and in the dark for 18 hr to ensure a complete extraction. Water-soluble accessory pigments-phycoerythrin (PE) and phycocyanin (PC) were measured and analyzed on the basis of their absorption peak values at the wavelengths of maximum light absorption (568nm and 620 nm wavelength were used for PE and PC measurements) after samples were ground and extracted with 0.05M sodium phosphate buffer solution (pH 6.7) and centrifuged at about 14,000g for 30 minutes. About 5-7 mg (fresh weight) of conchosporangia was extracted for PE and PC measurements. Chlorophyll *a* and carotenoid contents were measured and analyzed on the basis of their absorption peak values at the wavelengths of maximum light absorption (445 nm and 670 nm were used for chlorophyll *a* and carotenoid measurements) after the samples were ground and extracted by organic solvent (acetone, 90%) with one drop of saturated $MgCO_3$ added and centrifuged at about 14,000g for 30

minutes. About 4-6 mg (fresh weight) of conchosporangia was used for chlorophyll a and carotenoid measurements. Volume of extracted pigment solution was set to 2 ml for pigment measurements. Specific extinction coefficients used to calculate pigment amount in red seaweeds pigment extracts were obtained from O'hEocha (1971). Pigment absorbances were determined using a Gilford spectrophotometer 250.

The following formulae were used for the estimation of pigment contents in conchosporangia samples on the basis of the absorbances of the pigment extracts at specified wavelengths and their corresponding specific extinction coefficients:

Phycoerythrin (mg $g.dw^{-1}$) = (246.9 A_{568} - 91.0 A_{620})/sample amount (mg.dw)

Phycocyanin (mg $g.dw^{-1}$) = (303.0 A_{620} - 3.28 A_{568})/sample amount (mg.dw)

Carotenoids (mg $g.dw^{-1}$) = (7.14 A_{445} - 3.85 A_{670})/sample amount (mg.dw)

Chlorophyll *a* (mg $g.dw^{-1}$) = (19.8 A_{670})/sample amount (mg.dw)

Statistical analyses of the experimental data

In pigment content experiments, four species were used (*P. abbottae, P. pseudolanceolata, P. pseudolinearis and P. torta*). For each species, the experiment included irradiance (0, 10, 40, 160 µmol photons m^{-2} s^{-1}), nutrition concentration (0, f/4, f/2, f) and incubation period (10, 20, 30, 60 days). There were sixty-four complete combinations of different levels of these factors with four replicates per treatment for a total of = 4 x 4 x 4 x 4 = 256 data for data analysis of each species and each kind of pigment. A three-way model I ANOVA was performed to analyze the influences of these factors including potential interactions on each kind of pigment content of the conchosporangia for each species of *Porphyra* by using S-Plus 3.1 for windows (Statistical Sciences, Inc. 1993).

The Newman-Keuls multiple comparison test (Zar, 1996) was performed to identify which factors were important in controlling pigment contents of the conchosporangia of *Porphyra*. Statistical power analysis for main effect factors was conducted according to Cohen's methods (Cohen, 1988).

Results

Comparison of absorption spectra

PE absorption has two peaks, one lower peak at about 495 nm and another main peak at 568 nm. PC has an absorption peak at 620nm. The peak absorption of chlorophyll a occurs at 670 nm and carotenoid has maximal absorption at 445 nm with a shoulder absorption at 475 nm. Pigments extracted from the conchosporangia of all four species of *Porphyra* tested have uniform peak absorptions at corresponding wavelengths, there are no differences in wavelengths of peak absorption between different species (Figure 5.1).

Variations of photosynthetic pigments

1. *Porphyra abbottae*

Both phycoerythrin (PE) and phycocyanin (PC) content of the conchosporangia of *Porphyra abbottae* were significantly influenced by all three factors including some interactions between these factors (Figure 5.2, Figure 5.6, Table 5.1, Table 5.2). Conchosporangial cultures with no nutrients added had the lowest content of phycoerythrin and phycocyanin. Both PE and PC content declined with an increase in irradiance. An irradiance of 40 μmol photons m^{-2} s^{-1} caused a significant decline of phycoerythrin and phycocyanin contents. At a relatively high irradiance, cultures with no nutrients added produced little PE and PC (Figure 5.2, Figure 5.6, Figure 5.18, Figure 5.19). The most suitable irradiance was 10 μmol photons m^{-2} s^{-1}, at which maximal pigment content was generally achieved, particularly with the nutrient concentrations

between f/4 and f/2. Cultures in darkness usually had the second highest phycobilin content. Both phycoerythrin and phycocyanin content decreased with culture duration (Figure 5.2, Figure 5.6, Figure 5.18, Figure 5.19). The highest phycoerythrin and phycocyanin content (95.07 and 61.02 mg g.dw^{-1}, respectively) occurred at 10 μmol photons m^{-2} s^{-1}, f/2 nutrient concentration after 10 days of culture.

Both carotenoid and chlorophyll *a* content of the conchosporangia of *Porphyra abbottae* were significantly influenced by all three factors, with interactions affecting chlorophyll *a* content but not carotenoid content (Figure 5.10, Figure 5.14, Table 5.3, Table 5.4). Conchosporangial cultures with no nutrients added generally had significantly lower content of carotenoid (Ca) and chlorophyll *a* (Chl. *a*) after longer culture duration (20-60 days), but not for 10-day cultures. At high irradiances, cultures with no nutrients added had the lowest carotenoid and chlorophyll *a* content (Figure 5.10, Figure 5.14, Figure 5.20, Figure 5.21).

Carotenoid and chlorophyll *a* content of *Porphyra abbottae* also varied with different light environments. Cultures in darkness or at a low irradiance (10 μmol photons m^{-2} s^{-1}) had the highest content of carotenoid and chlorophyll *a*. Irradiances $\geq$ 40 μmol photons m^{-2} s^{-1} resulted in a remarkable decline (Figure 5.10, Figure 5.14).

Carotenoid and chlorophyll *a* content also decreased with culture duration, with the lowest for 60-day culture duration but no significant variation for 30-60 days of culture (Figure 5.10, Figure 5.14, Figure 5.20, Figure 5.21).

The maximal carotenoid and chlorophyll *a* contents (6.3 and 8.2 mg g.dw^{-1}, respectively) were achieved at 0 μmol photons m^{-2} s^{-1}, f/4- f/2 nutrient concentration and 10-20day culture duration.

***Porphyra pseudolanceolata* (Pe).**

Phycoerythrin content of the conchosporangia of *Porphyra pseudolanceolata* was only affected by nutrients and light, with interactions occurring between nutrient and light or nutrient and culture duration, whereas phycocyanin (PC) content was influenced by all three factors, including their interactions (Figure 5.3, Figure 5.7, Table 5.1, Table 5.2).

Conchosporangial cultures with no nutrients added generally had the lowest content of phycoerythrin and phycocyanin. For example, at 40 μmol photons m^{-2} s^{-1} of irradiance, phycoerythrin and phycocyanin content in cultures with no nutrients added decreased to below 9-35 and 10-31 mg g.dw^{-1} respectively (Figure 5.3, Figure 5.7, Figure 5.18, Figure 5.19).

In comparison with *P. abbottae*, when nutrients were provided *P. pseudolanceolata* had an obvious optimal irradiance (10 μmol photons m^{-2} s^{-1}) at which the highest contents of phycoerythrin and phycocyanin were obtained, particularly for the longer duration of cultures (20-60 days) and higher concentrations of nutrient (f/2-f).

Unlike *P. abbottae*, cultures in the dark usually did not have the second highest PE or PC content and had significantly lower PE or PC content than at 10 μmol photons m^{-2} s^{-1} irradiance. There was a relatively high PE and PC content at higher irradiances (40-160 μmol photons m^{-2} s^{-1}) when nutrients were added at higher concentrations (f/2-f). In contrast to *P. abbottae*, PE and PC content did not decrease with culture duration, conversely, there was a slight increase for 20-60day culture (Figure 5.3, Figure 5.7, Figure 5.18, Figure 5.19). The highest phycoerythrin and phycocyanin contents (73 and 71 mg g.dw^{-1}, respectively) both occurred at 10 μmol photons m^{-2} s^{-1} , f nutrient concentration and 60-day culture duration.

Carotenoid content of the conchosporangia of *P. pseudolanceolata* was affected by nutrient level and culture duration but not light. However, there was an interaction between light and culture duration. chlorophyll *a* content were influenced by all three factors with the same interaction as in carotenoid content (Figure 5.11, Figure 5.15, Table 5.3, Table 5.4).

Conchosporangial cultures with no nutrients added generally had low contents of carotenoid and chlorophyll *a* for 20-60day culture duration, but not for 10day culture. There was a significant difference in chlorophyll *a* content between different light environments, with significantly higher content occurring in dark environment or at 10 μmol photons m^{-2} s^{-1}.

Nutrients between f/4 and f concentrations did not significantly affect the carotenoid and chlorophyll *a* contents of *P. pseudolanceolata,* which had a pooled mean of carotenoid and chlorophyll *a* of 2.3-2.5 and 3.0-3.3 mg $g.dw^{-1}$, respectively. However, cultures with no nutrients added had significantly lower content of carotenoid and chlorophyll *a* than those with nutrients added.

Basically, carotenoid and chlorophyll *a* content of *P. pseudolanceolata* declined with culture duration, except significantly higher content occurred for 20 day cultures (Figure 5.20, Figure 5.21). The highest carotenoid and chlorophyll *a* content were achieved at 0 μmol photons m^{-2} s^{-1} , f nutrient concentration and 20day culture duration (Figure 5.11, Figure 5.15).

***Porphyra pseudolinearis* (Pi)**

All three factors, including two-factor interactions, influenced phycoerythrin and phycocyanin contents of the conchosporangia of *Porphyra pseudolinearis* (Figure 5.4, Figure 5.8, Table 5.1, Table 5.2).

Conchosporangial cultures with no nutrients added had the lowest content of phycoerythrin and phycocyanin, especially with an increase in irradiance (Figure 5.4, Figure 5.8, Figure 5.18, Figure 5.19). The conchosporangia of *P. pseudolinearis* appeared to be sensitive to higher irradiances. For instance, 40 μmol photons m^{-2} s^{-1} resulted in a significant decline of phycoerythrin and phycocyanin content (Figure 5.4, Figure 5.8, Figure 5.18, Figure 5.19).

Like *P. pseudolanceolata*, when nutrients were provided, *P. pseudolinearis* had an optimal irradiance (10 μmol photons m^{-2} s^{-1}) at which more phycoerythrin and phycocyanin

were produced, particularly for cultures with f/4-f/2 nutrient concentration. Similar to *P. pseudolanceolata*, cultures in darkness usually had the second highest PE content, but they were not significantly lower than at 10 μmol photons m^{-2} s^{-1} (Figure 5.4, Figure 5.18). Also similar to *P. abbottae,* cultures in the dark generally had the highest PC content, but they were not significantly higher than at 10 μmol photons m^{-2} s^{-1} (Figure 5.8, Figure 5.19). There was a relatively high PE and PC content at higher irradiances when nutrients were added at concentrations between f/4 and f/2. In contrast to *P. abbottae*, PE and PC contents increased for 20-60day cultures (Figure 5.4, Figure 5.8, Figure 5.18, Figure 5.19).

Both the highest phycoerythrin and phycocyanin content occurred at 10 μmol photons m^{-2} s^{-1} , f/2 nutrient concentration and 20day culture duration.

Both carotenoid and chlorophyll *a* contents of the conchosporangia of *Porphyra pseudolinearis* were influenced by all three factors tested, including interactions between these factors (Figure 5.12, Figure 5.16, Table 5.3, Table 5.4).

Conchosporangial cultures with no nutrients added generally had lower content of carotenoid and chlorophyll *a*, particularly for longer culture durations (Figure 5.12, Figure 5.16, Figure 5.20, Figure 5.21). At higher irradiances, cultures with no nutrients added had especially low carotenoid and chlorophyll *a* contents (Figure 5.12, Figure 5.16).

Carotenoid and chlorophyll *a* content of *P. pseudolinearis* varied with different light environments. Cultures in darkness or at the low irradiance with nutrients generally had higher carotenoid and chlorophyll *a*. High irradiances resulted in a remarkable decline of carotenoid and chlorophyll *a* (Figure 5.12, Figure 5.16, Figure 5.20, Figure 5.21).

Cultures in darkness usually had the highest levels of carotenoid and chlorophyll *a* , but these were not significantly higher than that at 10 μmol photons m^{-2} s^{-1} (Figure 5.20, Figure 5.21). Statistical tests showed no effect of culture duration (Figure 5.20, Figure 5.21).

The peak contents were achieved for carotenoid (5.1 mg g.dw^{-1}) at 10 μmol photons m^{-2} s^{-1}, f/2 nutrient concentration and 30day culture duration and for chlorophyll *a* (7.2 mg g.dw^{-1}) at 0 μmol photons m^{-2} s^{-1}, 0 nutrient concentration and 30day culture duration.

***Porphyra torta* (Pt)**

All three factors, including their interactions influenced phycoerythrin and phycocyanin contents of the conchosporangia of *Porphyra torta* (Figure 5.5, Figure 5.9, Table 5.1, Table 5.2).

Conchosporangial cultures with no nutrients added had significantly lower content of phycoerythrin and phycocyanin (Figure 5.18, Figure 5.19). But unlike the other three species, phycocyanin content in cultures with no nutrients added did not have an obvious declining trend with an increase in irradiance (Figure 5.5, Figure 5.9). Cultures with nutrients added were sensitive to light environments, with significantly higher PE and PC contents occurring at 10 μmol photons m^{-2} s^{-1}.

Unlike the other three species, cultures of *P. torta* under dark environment had significantly lower PE and PC contents (Figure 5.5, Figure 5.9, Figure 5.18, Figure 5.19). There was a relatively reasonably high PE and PC content at higher irradiances (40-160 μmol photons m^{-2} s^{-1}) when nutrients were provided.

As for *P. abbottae*, PE content of *P. torta* significantly decreased with culture duration. However, in contrast to *P. abbottae*, there was an obvious increase in PC content for a longer culture (Figure 5.5, Figure 5.9, Figure 5.18, Figure 5.19).

The highest phycobilin content occurred at 10 μmol photons m^{-2} s^{-1}, f/2 nutrient concentration and 10-day culture duration (for PE content) and 10 μmol photons m^{-2} s^{-1}, f nutrient concentration and 60-day culture duration (for PC content).

All three factors tested had effects on carotenoid content but only nutrient and light affected chlorophyll *a* content of the conchosporangia of *Porphyra*. There were no interactions occurring among factors (Figure 5.13, Figure 5.17, Table 5.3, Table 5.4).

Conchosporangial cultures with no nutrients added generally had low contents of carotenoid and chlorophyll *a* (Figure 5.13, Figure 5.17, Figure 5.20, Figure 5.21).

Unlike the other three species, the conchosporangia of *P. torta* contained more carotenoid and chlorophyll *a* under dark environment than the light environment. Cultures under the light environment had little variation in carotenoid and chlorophyll *a* contents (Figure 5.13, Figure 5.17, Figure 5.20, Figure 5.21).

Like *P. pseudolinearis,* there was no effect of culture duration for chlorophyll *a*.

The maximal pigment contents were achieved at 0 μmol photons m^{-2} s^{-1}, f nutrient concentration and 10day culture duration (for carotenoid content) and 0 μmol photons m^{-2} s^{-1}, f nutrient concentration and 30-day culture duration (for chlorophyll *a* content), respectively.

The effect difference between species

Comparison of pooled pigment content of four species of *Porphyra* for each parameter tested (for comparison of effect difference between species) is shown in the right column of Figure 5.18. to Figure 5.21. These pooled data analyses showed that for PE content, Pa generally had the highest PE content at each level of all three factors (nutrient concentration, irradiance and culture duration), with Pe and Pi having the second highest and Pt having the lowest PE content. However, these four species exhibited no differences in PE content between 40 and 160 µmol photons m^{-2} s^{-1} (Figure 5.18). Pe generally had the highest PC production , with Pi and Pa having the second highest and Pt having the lowest PC production for almost all levels of nutrient and irradiance (with exception of PC content under dark environment).

There were also significant differences in PC content between species for culture duration. Pt had the lowest PC content for all culture periods. During a 10 day culture Pa had the highest PC content. For 20-30day culture, Pa, Pe and Pi had significantly higher PC content than Pt, but no differences in PC content existed between these three species. For the longest culture duration (60 day), Pe had the highest PC content, with Pi having the second highest (Figure 5.19). Overall, Pa and Pi had significantly higher Ca and Chl. *a* contents than the other two species for comparison at all levels of three factors (with exception of 160 µmol photons m^{-2} s^{-1} irradiance). Pt had the lowest Ca and Chl. *a* content at all levels of three factors (Figure 5.20, Figure 5.21).

Conchosporangia cultures with nutrient added had significantly higher PE content than cultures with no nutrient added. Significant higher PE content (grand mean 56.5 mg $g.dw^{-1}$) was obtained at 10 µmol photons m^{-2} s^{-1}, with the second highest PE content being under dark environment. PE content decreased with culture duration (Figure 5.22). Pe had the highest PC content (grand mean 36.5 mg $g.dw^{-1}$), with Pa and Pi the second highest and Pt having the lowest PC content (grand mean 15 mg $g.dw^{-1}$). There was also a significant difference in PC content between cultures with nutrient added and cultures with no nutrient added (grand mean 19 vs. 32 mg $g.dw^{-1}$).

Similarly, significantly higher PC content (grand mean 38.5 mg $g.dw^{-1}$) was obtained at 10 µmol photons m^{-2} s^{-1}, with the second highest PC content occurring under dark environment. PC content also decreased with culture duration (Figure 5.23). Comparison of grand mean value for both Ca and Chl. *a* followed similar results. Pa and Pi had significantly higher Ca and Chl. *a* contents than the other two species.

Cultures with no nutrient added had significantly lower Ca and Chl. *a* contents than those with nutrient added (grand mean 2.2 vs. 3.0 mg $g.dw^{-1}$ for Ca content, and 2.7 vs. 3..6 mg $g.dw^{-1}$

for Chl. *a* content). Cultures under dark environment and at 10 μmol photons m^{-2} s^{-1} had significantly higher Ca and Chl. *a* contents than those at higher irradiances (Figure 5.24, Figure 5.25).

Statistical power (1- ß) analysis for the experiments of pigment content

The results of statistical power (1- ß) analysis indicated that main effect factors (nutrient concentration, light, duration) have high power values (>0.80) when the minimum detectable difference in means for pigment content of conchosporangia is set at 15% (Table 5.5, Table 5.6, Table 5.7, Table 5.8).

Discussion

Hannach (1989) reported that the spectral absorbances of *P. abbottae* gametophytes from Washington State increased in low light and high nutrient levels. Based on previously determined growth rates, she postulated that nutrient saturated *P. abbottae* blades can synthesize photosynthetic pigments in excess of immediate needs, with main allocation being given to the phycobiliproteins, especially phycocyanin . She reported that *P. abbottae* blades grown under different conditions contained 4.7-7.2 mg Chl. *a* g dw^{-1}, 23.2-40.6 mg PE g dw^{-1} and 13.3-22.6 mg PC g dw^{-1} if a conventional conversion coefficient of 10 was used for the ratio of fresh weight to dry weight of red algae. It appears that both sporophytic and gametophytic stages of *P. abbottae* have similar chlorophyll *a* content. However, compared with her results, the conchosporangia have a much higher content of phycobilin (phycoerythrin and phycocyanin) based on my experimental results.

In a field investigation of the green seaweed *Codium fragile*, total chlorophyll levels were found to vary inversely with the depth, namely, the amount of available light (Wassman 1973). This conclusion is similar to my experimental results, which showed that chlorophyll *a* content of

Porphyra conchsporangia was highest at low light (10 μmol photons m^{-2} s^{-1}), with significant decline at higher irradiances. Furthermore, my experimental results also indicated a similar variation in phycobiliprotein contents. This is a phenomenon worth-discussing., what are its physiological, ecological and biological implications? What is its mechanism? Here are some possible interpretations. In red algal cells, the photosynthetic pigments are associated closely with proteins in the thylakoid membranes of chloroplasts to form light-harvesting complexes (*e.g.*, phycobiliproteins are on the surfaces of thylakoids and further organized into granular phycobilisomes, which are the principal light-harvesting structures and transfer light energy to chlorophyll *a* embedded in the thylakoid membrane). Occurrence of photosynthetic activity must rely upon pigment-protein complexes, which structurally are biological macro-molecules and needs some time for their synthesis in plant cells. Unlike sun plants or other plants which can, obtain regular light, cryptic *Porphyra* sporophytes have relatively few chances to access light because of living in the shell substrates. Therefore, as an adaptation mechanism, one possible reason that they maintain high contents of photosynthetic pigments under low light or darkness is in order to catch and utilize light, *i.e.*, their photosynthetic pigments are ready for light harvesting at any time when light becomes available. This could be interpreted as increasing pigments to maximize numbers of photons collected, an advantage for benthic algae.

Another likely reason is that high content of phycobiliprotein is possibly related to some potential pathways of nitrogen uptake, utilization and storage. These photosynthetic pigment complexes contain a significant amount of nitrogen in their chromophores and their proteins that are bonded with pigments (O'Carra 1965). In some studies, color and content of photosynthetic pigments in *Porphyra* blades have been used as indicators of nitrogen supply and availability in the field cultivation waters (Amano & Noda 1978, Fujita & Migita 1984). These stored

components may be used for growth and other necessary physiological processes when nitrogen supply becomes insufficient for marine algae (Lobban & Harrison 1994, Hwang *et al.*, 1987).

Color and content of photosynthetic pigments can be used to determine the physiological responses of *Porphyra* sporophytes. From my experimental observations, there was a distinct pigmentation of the conchosporangia phase under optimal culture conditions, where the color of the conchocelis appeared particularly dark brown-red (an indication of best and healthiest conchosporangia cultures) due to the abundant amount of phycobilin in plant cells. This is in contrast to only very a slight red color or bleached pale color occurring under unfavorable or extremely-stressed culture conditions such as those absent nutrients added and those exposed to high irradiance.

Because photosynthetic pigments are essential for plants to perform photosynthetic process, variations of pigment content likely can determine growth, development , physiological responses and survival of plants (Fortes & Luning 1980, Zavodnik 1987). My experimental findings showed that photosynthetic pigments of the conchosporangia for four species of indigenous *Porphyra* are significantly influenced by environmental factors such as irradiance, nutrient concentration and culture duration, including some interactions among these factors, with salinity and temperature fixed at 30ppt and 11°C respectively. Pigment content of the conchosporangia appear to be sensitive to environmental change and indicate physiological responses of sporophytic stage of *Porphyra* to environmental fluctuation. Since relatively low content and little variation of carotenoid and chlorophyll *a* occurred under all culture conditions, the change of accessory pigments (phycoerythrin and phycocyanin) is principally responsible for variations of the photosynthetic pigments. Accessory pigments could possibly determine the magnitude of any physiological responses of *Porphyra* conchosporangia.

Overall, the conchosporangia of all four species of *Porphyra* contained higher levels of photosynthetic pigments, especially phycoerythrin and phycocyanin at a low irradiance (10 μmol photons m^{-2} s^{-1}) or even in a dark environment. So unique a physiological trait is evidence that *Porphyra* conchosporangia have the adaptability to a low irradiance or the dark environment.

Many studies have indicated that nutrients, especially nitrogen could affect both growth and quality of *Porphyra* blades. My experimental results also indicated that nutrients are very important for sporophytic stage of *Porphyra*. Under the light environment, conchosporangia grown in media with nutrients added had much higher contents of photosynthetic pigments and evidently exhibited a healthy brown-red color, in contrast to cultures with no nutrients added that had very low amounts of photosynthetic pigments and were bleached. Nitrogen supply in coastal waters are related to the seasonal occurrence, causing variations of growth and abundance of marine algae (Hanisak 1983, Hannach 1989, Grobe, Yarish & Davison 1998, Wheeler & North 1980). Because in natural habitats *Porphyra* sporophytes occur mainly during the period of summer season for the most of species, without doubt, their occurrence during this season would encounter the limiting nitrogen availability. For instance, in Alaska, a drastic decline of nutrient concentration usually occurs during the late spring and summer as the result of frequent phytoplankton blooms. Hence, shortage of nutrient supply during this period would potentially depress growth, development and survival of natural populations of *Porphyra* sporophytes.

Sufficient nutrient supply is necessary to promote higher production of phycobilin for *Porphyra* conchosporangia. However, different species exhibited differences in nutrient requirements. For example, higher nutrient concentration (f concentration) might be needed for *P. pseudolanceolata*. For the other three species, intermediate nutrient concentrations (f/4-f/2) were basically sufficient for high pigment content. Culture duration also should be taken into consideration in order to maximize phycobilin production. *P. abbottae* tended to synthesize

significantly less photosynthetic pigments with prolonged culture duration, in contrast to the other three species having a relative constant pigment production throughout the entire period of culture.

The conchosporangial stage is critical to the successful mariculture of *Porphyra*. The possibility of conchospore maturation and release, to a great extent, rests on whether or not the best cultures of the conchosporangia are grown. The optimal culture conditions at which the highest production of phycoerythrin and phycocyanin occurred could provide high quality and healthy conchosporangia for successful cultivation of these indigenous *Porphyra* species for food production purposes or for phycobilin extraction.

Conclusions

Four kinds of photosynthetic pigments in the conchosporangia of *Porphyra* are significantly influenced by environmental factors such as irradiance, nutrient concentration and culture duration, including some interactions among these factors. Pigment contents of the conchosporangia are sensitive to environmental conditions and can be used to indicate the physiological state of the sporophytic stage of *Porphyra*.

Phycobilins (phycoerythrin and phycocyanin) are the major components of total pigments, there are relatively small amounts of carotenoid and chlorophyll *a* in the cells of *Porphyra* sporophytes. *Porphyra* conchosporangia have the apparent adaptability to low irradiances and perhaps even to complete darkness. The conchosporangia of four species of *Porphyra* produced and maintained high contents of photosynthetic pigments, especially phycoerythrin and phycocyanin at low light (10 μmol photons m^{-2} s^{-1}) or in a dark environment. Such a physiological trait, derived likely from historical adaptation to environments and the process of natural selection, could possess important biological implication for them to survive

and persist in habitats with limited light. Variation and magnitude of pigment contents of the conchosporangia vary considerably from species to species in response to varying environments.

Sufficient nutrients are necessary for high production of photosynthetic pigments. However, different species exhibited differences in nutrient requirements.

P. abbottae tends to synthesize significantly less photosynthetic pigments with prolonged culture duration, in contrast to the other three species, which generally have a relative constancy in pigment production throughout the entire period of culture.

The conchosporangia stage is critical to successful mariculture of *Porphyra*. The possibility of conchospore maturation and release, to a great extent, rests on whether or not healthy cultures of the conchosporangia are grown. The optimal culture conditions at which the highest production of phycoerythrin and phycocyanin occur could provide high quality and healthy conchosporangia for successful mariculture of these indigenous *Porphyra* species or for the purposes of phycobilin extracts.

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Table 5.1. ANOVA table for phycoerythrin content of the conchosporangia of four different *Porphyra* species at combinations of nutrient concentration (Nc), irradiance (Light) and culture duration (day). [a] 0, f/4, f/2, f; [b] 0, 10, 40, 160 μmol photons m^{-2} s^{-1}; [c] 10, 20, 30, 60 days. (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Nutrient[a]	3	29251.47	9750.49	59.67**
Light[b]	3	56923.70	18974.57	116.12**
Day[c]	3	47115.78	15705.26	96.11**
Nc x Light	9	12038.16	1337.57	8.19**
Nc x Day	9	2648.46	294.27	1.80
Light x Day	9	1985.22	220.58	1.35
Nc x Light x Day	27	8092.99	299.74	1.83*
Residuals	192	31374.09	163.41	
P. pseudolanceolata				
Nutrient[a]	3	25264.14	8421.38	95.90**
Light[b]	3	7886.83	2628.94	29.94**
Day[c]	3	150.75	50.25	0.57
Nc x Light	9	8387.13	931.90	10.61**
Nc x Day	9	2979.42	331.05	3.77**
Light x Day	9	1507.65	167.52	1.91
Nc x Light x Day	27	3390.62	125.58	1.43
Residuals	192	16861.10	87.82	
P. pseudolinearis				
Nutrient[a]	3	35291.62	11763.87	119.25**
Light[b]	3	24063.84	8021.28	81.31**
Day[c]	3	1734.97	578.32	5.86**
Nc x Light	9	11989.34	1332.15	13.50**
Nc x Day	9	4058.94	450.99	4.57**
Light x Day	9	1791.95	199.11	2.02*
Nc x Light x Day	27	3050.22	112.97	1.15
Residuals	192	18940.94	98.65	

(Continued Table 5.1.)

Source of variation	df	Sum of squares	Mean square	F
P. torta				
Nutrient[a]	3	8096.91	2698.97	31.00**
Light[b]	3	17579.17	5859.72	67.31**
Day[c]	3	15102.35	5034.12	57.83**
Nc x Light	9	4301.83	477.98	5.49**
Nc x Day	9	1701.54	189.06	2.17*
Light x Day	9	5622.43	624.71	7.18**
Nc x Light x Day	27	3746.94	138.78	1.59*
Residuals	192	16714.84	87.06	

Table 5.2. ANOVA table for phycocyanin content of the conchosporangia of four different *Porphyra* species at combinations of nutrient concentration (Nc), irradiance (Light) and culture duration (day). [a] 0, f/4, f/2, f; [b] 0, 10, 40, 160 μmol photons m^{-2} s^{-1}; [c] 10, 20, 30, 60 days. (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Nutrient[a]	3	6520.24	2173.41	44.35**
Light[b]	3	25892.80	8630.93	176.11**
Day[c]	3	23185.40	7728.47	157.70**
Nc x Light	9	2645.01	293.89	6.00**
Nc x Day	9	531.83	59.09	1.21
Light x Day	9	1743.89	193.77	3.95**
Nc x Light x Day	27	2494.35	92.38	1.89**
Residuals	192	9409.62	49.01	
P. pseudolanceolata				
Nutrient[a]	3	13290.40	4430.13	85.07**
Light[b]	3	20415.47	6805.16	130.68**
Day[c]	3	505.67	168.56	3.24*
Nc x Light	9	5796.90	644.10	12.37**
Nc x Day	9	1364.74	151.64	2.91**
Light x Day	9	3721.60	413.51	7.94**
Nc x Light x Day	27	2267.19	83.97	1.61*
Residuals	192	9998.42	52.08	
P. pseudolinearis				
Nutrient[a]	3	11886.83	3962.28	69.75**
Light[b]	3	44793.45	14931.15	262.83**
Day[c]	3	7952.78	2650.93	46.66**
Nc x Light	9	7088.97	787.66	13.87**
Nc x Day	9	1339.64	148.85	2.62**
Light x Day	9	3665.41	407.27	7.17**
Nc x Light x Day	27	2139.92	79.26	1.40
Residuals	192	10907.32	56.81	

(Continued Table 5.2.)

Source of variation	df	Sum of squares	Mean square	F
P. torta				
Nutrient[a]	3	1299.67	433.22	89.23**
Light[b]	3	4063.94	1354.65	279.01**
Day[c]	3	1039.86	346.62	71.39**
Nc x Light	9	671.27	74.59	15.36**
Nc x Day	9	215.67	23.96	4.94**
Light x Day	9	349.23	38.80	7.99**
Nc x Light x Day	27	466.76	17.29	3.56**
Residuals	192	932.20	4.86	

Table 5.3. ANOVA table for carotenoid content of the conchosporangia of four different *Porphyra* species at combinations of nutrient concentration (Nc), irradiance (Light) and culture duration (day). [a] 0, f/4, f/2, f; [b] 0, 10, 40, 160 μmol photons m^{-2} s^{-1}; [c] 10, 20, 30, 60 days. (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Nutrient[a]	3	26.8694	8.9565	5.424**
Light[b]	3	389.5719	129.8573	78.639**
Day[c]	3	57.8462	19.2821	11.677**
Nc x Light	9	12.4006	1.3778	0.834
Nc x Day	9	14.6073	1.6230	0.983
Light x Day	9	8.8640	0.9849	0.596
Nc x Light x Day	27	7.3458	0.2721	0.165
Residuals	192	317.0494	1.6513	
P. pseudolanceolata				
Nutrient[a]	3	20.6513	6.8838	14.544**
Light[b]	3	3.4866	1.1622	2.455
Day[c]	3	23.9163	7.9721	16.843**
Nc x Light	9	4.8898	0.5433	1.148
Nc x Day	9	2.2420	0.2491	0.526
Light x Day	9	11.6384	1.2932	2.732**
Nc x Light x Day	27	7.8932	0.2923	0.618
Residuals	192	90.8746	0.4733	
P. pseudolinearis				
Nutrient[a]	3	74.6694	24.8898	46.391**
Light[b]	3	72.4945	24.1648	45.040**
Day[c]	3	5.4269	1.8090	3.372*
Nc x Light	9	23.4517	2.6057	4.857**
Nc x Day	9	16.6328	1.8481	3.445**
Light x Day	9	16.1365	1.7929	3.342**
Nc x Light x Day	27	20.0840	0.7439	1.386
Residuals	192	103.0122	0.5365	

(Continued Table 5.3.)

Source of variation	df	Sum of squares	Mean square	F
P. torta				
Nutrient[a]	3	9.9316	3.3105	6.758**
Light[b]	3	58.2839	19.4280	39.661**
Day[c]	3	10.1840	3.3947	6.930**
Nc x Light	9	5.9471	0.6608	1.349
Nc x Day	9	0.5849	0.0650	0.133
Light x Day	9	4.4003	0.4889	0.998
Nc x Light x Day	27	6.5450	0.2424	0.495
Residuals	192	94.0521	0.4899	

Table 5.4. ANOVA table for chlorophyll *a* content of the conchosporangia of four different *Porphyra* species at combinations of nutrient concentration (Nc), irradiance (Light) and culture duration (day). [a] 0, f/4, f/2, f; [b] 0, 10, 40, 160 μmol photons m^{-2} s^{-1}; [c] 10, 20, 30, 60 days. (*P<0.05; **P<0.01).

Source of variation	df	Sum of squares	Mean square	F
P. abbottae				
Nutrient[a]	3	39.3185	13.1062	18.203**
Light[b]	3	832.2614	277.4205	385.302**
Day[c]	3	109.0687	36.3562	50.494**
Nc x Light	9	36.9991	4.1110	5.710**
Nc x Day	9	22.0478	2.4498	3.402**
Light x Day	9	26.5725	2.9525	4.101**
Nc x Light x Day	27	21.1674	0.7840	1.089
Residuals	192	138.2416	0.7200	
P. pseudolanceolata				
Nutrient[a]	3	34.7435	11.5812	18.994**
Light[b]	3	42.5447	14.1816	23.258**
Day[c]	3	33.3750	11.1250	18.246**
Nc x Light	9	6.0534	0.6726	1.103
Nc x Day	9	4.0953	0.4550	0.746
Light x Day	9	34.1028	3.7892	6.214**
Nc x Light x Day	27	18.3748	0.6805	1.116
Residuals	192	117.0698	0.6097	
P. pseudolinearis				
Nutrient[a]	3	77.7924	25.9308	27.179**
Light[b]	3	318.3588	106.1196	111.227**
Day[c]	3	24.0444	8.0148	8.401**
Nc x Light	9	50.9408	5.6601	5.932**
Nc x Day	9	33.4742	3.7194	3.898**
Light x Day	9	32.3669	3.5963	3.769**
Nc x Light x Day	27	45.1706	1.6730	1.754*
Residuals	192	183.1837	0.9541	

(Continued Table 5.4.)

Source of variation	df	Sum of squares	Mean square	F
P. torta				
Nutrient[a]	3	9.5275	3.1758	5.805**
Light[b]	3	135.0219	45.0073	82.272**
Day[c]	3	4.1820	1.3940	2.548
Nc x Light	9	6.0129	0.6681	1.221
Nc x Day	9	1.3153	0.1461	0.267
Light x Day	9	5.1561	0.5729	1.047
Nc x Light x Day	27	9.6414	0.3571	0.653
Residuals	192	105.0350	0.5471	

Table 5. 5. Statistical power (1- ß) based on the results of variance analysis for experiments of the effects of environmental factors on the phycoerythrin content of *Porphyra* conchosporangia. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) foı each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05. (** P<0.01 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Nutrient	3	59.67**	3	49	0.3208	0.97
Light	3	116.12**	3	49	0.3527	>0.99
Day	3	96.11**	3	49	0.3403	0.98
Porphyra pseudolanceolata						
Nutrient	3	95.90**	3	49	0.5142	>0.99
Light	3	29.94**	3	49	0.4312	>0.99
Day	3	0.57	3	49	0.4052	>0.99
Porphyra pseudolinearis						
Nutrient	3	119.25**	3	49	0.3878	>0.99
Light	3	81.31**	3	49	0.3584	>0.99
Day	3	5.86**	3	49	0.3155	0.97
Porphyra torta						
Nutrient	3	31.00**	3	49	0.3321	0.98
Light	3	67.31**	3	49	0.3595	>0.99
Day	3	57.83**	3	49	0.3517	>0.99

Table 5. 6. Statistical power (1- ß) based on the results of variance analysis for experiments of the effects of environmental factors on the phycocyanin content of *Porphyra* conchosporangia. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05.
(** P<0.01, * P<0.05 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Nutrient	3	44.35**	3	49	0.2912	0.94
Light	3	176.11**	3	49	0.3466	0.98
Day	3	157.70**	3	49	0.3369	0.98
Porphyra pseudolanceolata						
Nutrient	3	85.07**	3	49	0.4109	>0.99
Light	3	130.68**	3	49	0.4488	>0.99
Day	3	3.24*	3	49	0.3618	>0.99
Porphyra pseudolinearis						
Nutrient	3	69.75**	3	49	0.3878	>0.99
Light	3	262.83**	3	49	0.3584	>0.99
Day	3	46.66**	3	49	0.3155	0.97
Porphyra torta						
Nutrient	3	89.23**	3	49	0.2776	0.91
Light	3	279.01**	3	49	0.3653	>0.99
Day	3	71.39**	3	49	0.2708	0.89

Table 5. 7. Statistical power (1- ß) based on the results of variance analysis for experiments of the effects of environmental factors on the carotenoids content of *Porphyra* conchosporangia. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05.
(** P<0.01, * P<0.05 for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Nutrient	3	5.424**	3	49	0.3102	0.96
Light	3	78.639**	3	49	0.4180	>0.99
Day	3	11.677**	3	49	0.3164	0.97
Porphyra pseudolanceolata						
Nutrient	3	14.544**	3	49	0.4362	>0.99
Light	3	2.455	3	49	0.4125	>0.99
Day	3	16.843**	3	49	0.4412	>0.99
Porphyra pseudolinearis						
Nutrient	3	46.391**	3	49	0.4993	>0.99
Light	3	45.040**	3	49	0.4972	>0.99
Day	3	3.372*	3	49	0.4432	>0.99
Porphyra torta						
Nutrient	3	6.758**	3	49	0.3135	0.96
Light	3	39.661**	3	49	0.3666	>0.99
Day	3	6.930**	3	49	0.3137	0.96

Table 5. 8. Statistical power (1- ß) based on the results of variance analysis for experiments of the effects of environmental factors on the chlorophyll a content of *Porphyra* conchosporangia. Power values are determined by specific values of the degree of freedom (u), effect size index (f) and sample size (n) for each main effect. Desired minimum detectable difference in means is set at 15%. Significant criterion α is equal to 0.05.
(** P<0.01for F test).

		F test				Power
Effect	df	F	u	n	f	
Porphyra abbottae						
Nutrient	3	18.203**	3	49	0.3153	0.97
Light	3	385.30**	3	49	0.5475	>0.99
Day	3	50.494**	3	49	0.3250	0.97
Porphyra pseudolanceolata						
Nutrient	3	18.994**	3	49	0.4236	>0.99
Light	3	23.258**	3	49	0.4302	>0.99
Duration	3	18.246**	3	49	0.4225	>0.99
Porphyra pseudolinearis						
Nutrient	3	27.179**	3	49	0.3834	>0.99
Light	3	111.23**	3	49	0.4755	>0.99
Day	3	8.401**	3	49	0.3692	>0.99
Porphyra torta						
Nutrient	3	5.805**	3	49	0.2563	0.86
Light	3	82.27**	3	49	0.3524	>0.99
Day	3	2.548	3	49	0.2537	0.85

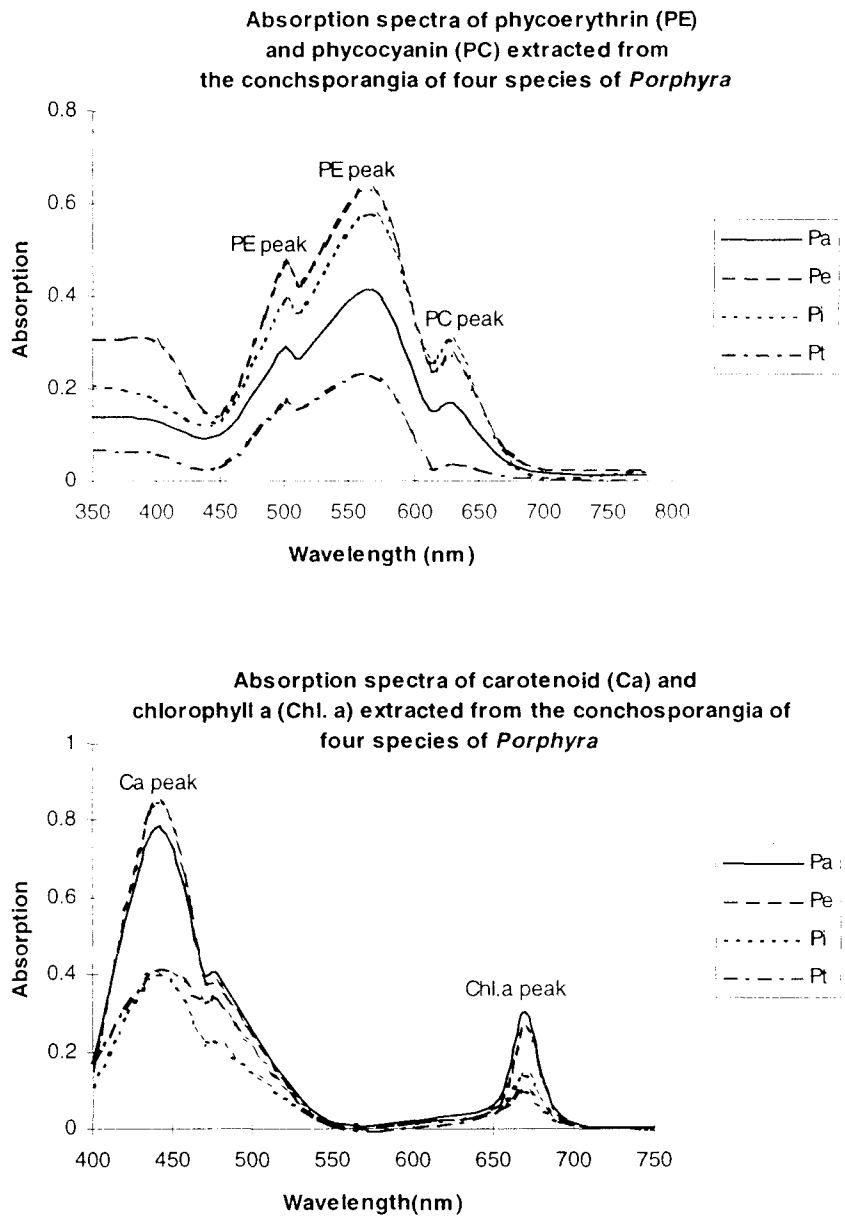

Figure 5.1. Comparison of absorption spectra of photosynthetic pigments extracted from the conchosporangia of four species of Alaskan *Porphyra*.

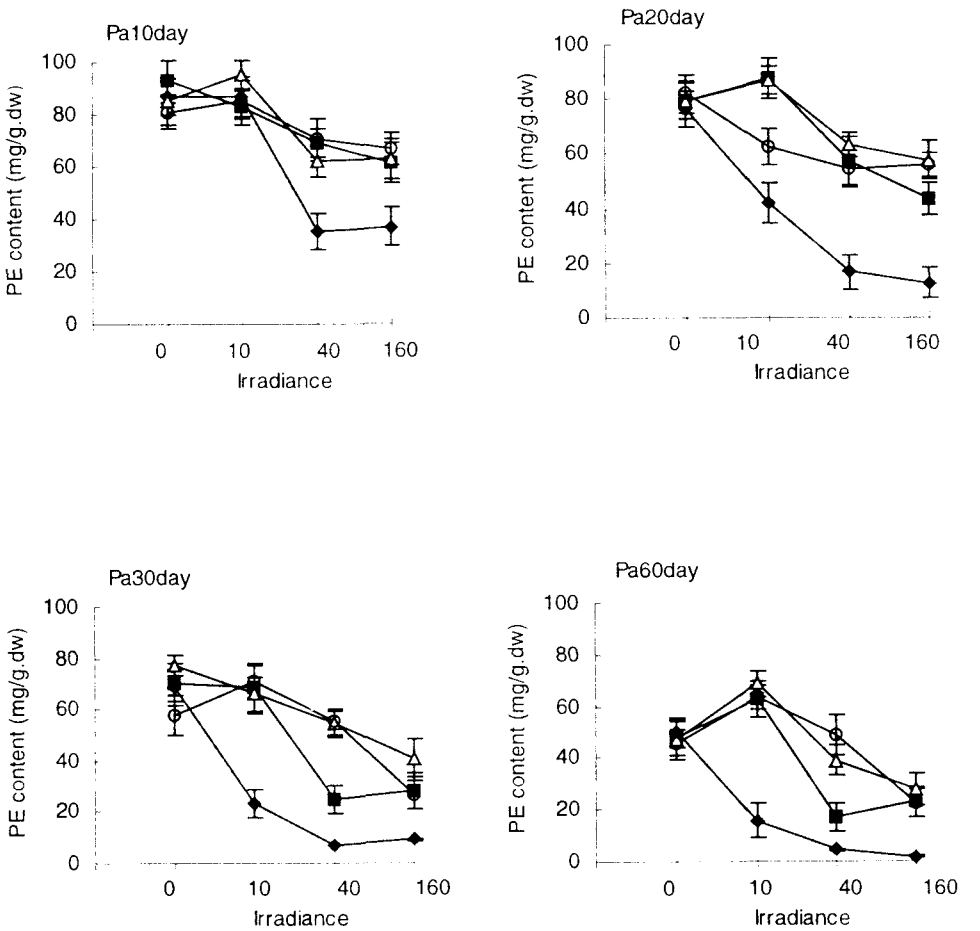

Figure 5.2. *Porphyra abbottae* (Pa). Phycoerythrin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

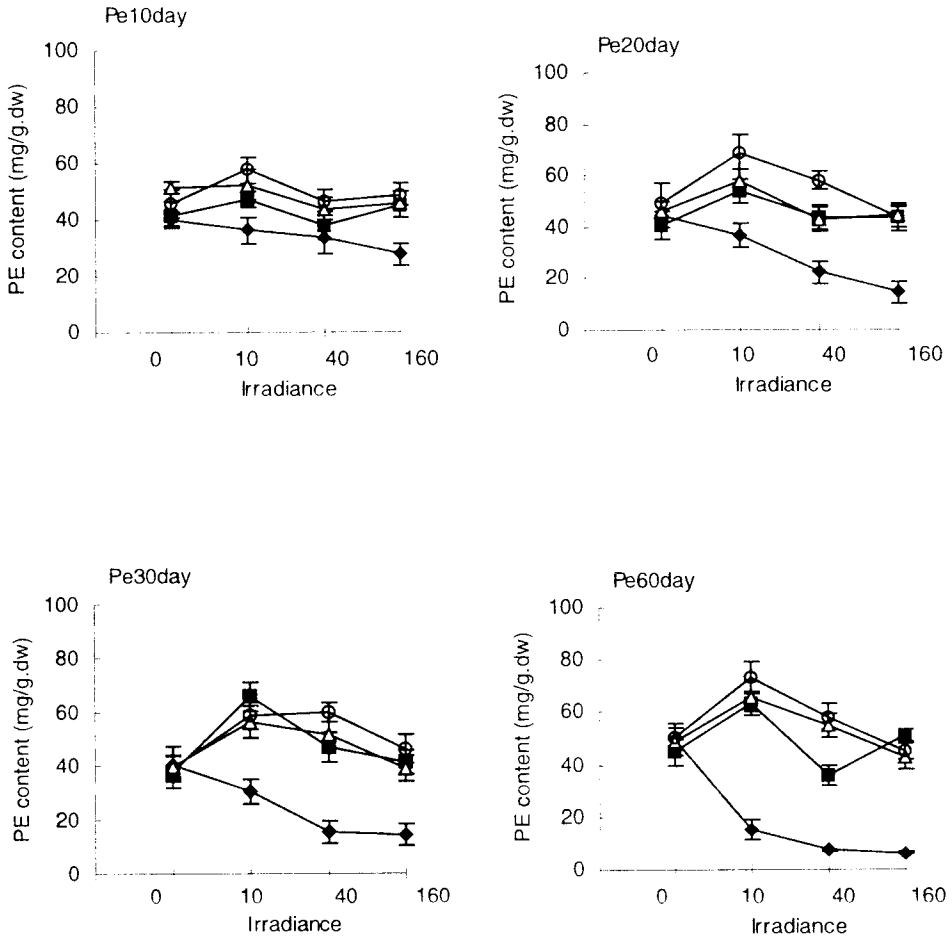

Figure 5.3. *Porphyra pseudolanceolata* (Pe). Phycoerythrin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

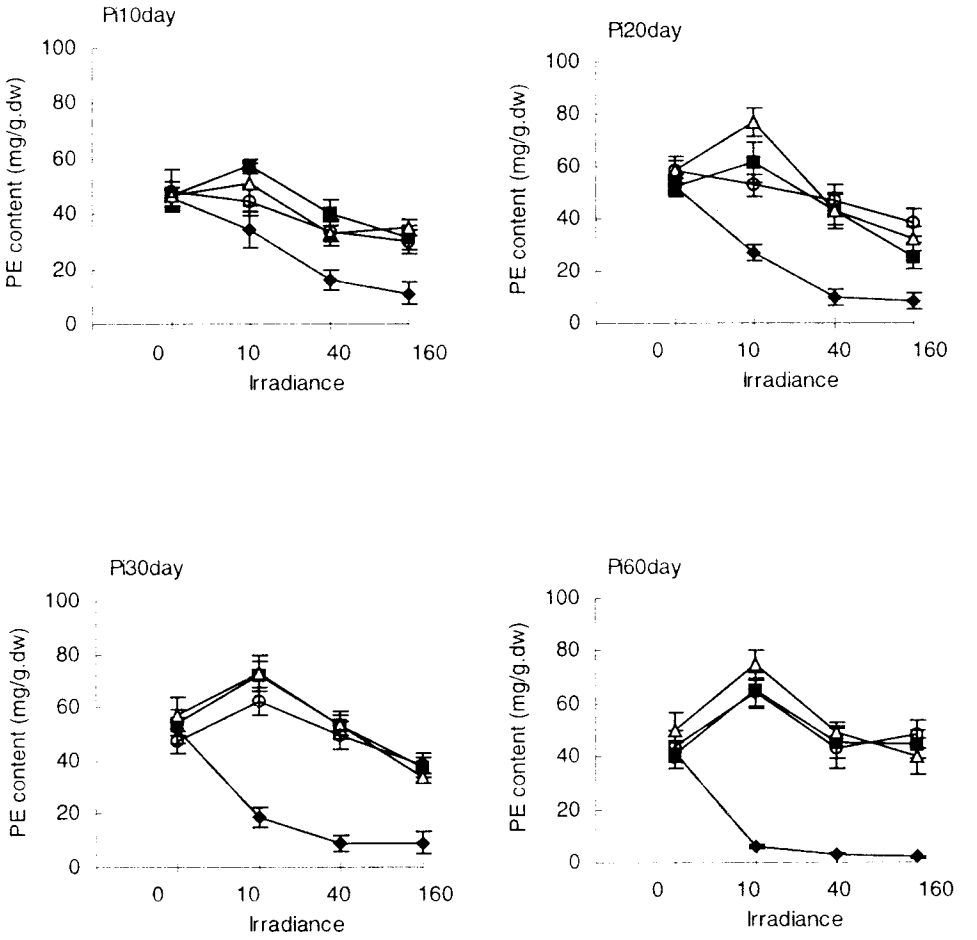

Figure 5.4. *Porphyra pseudolinearis* (Pi). Phycoerythrin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

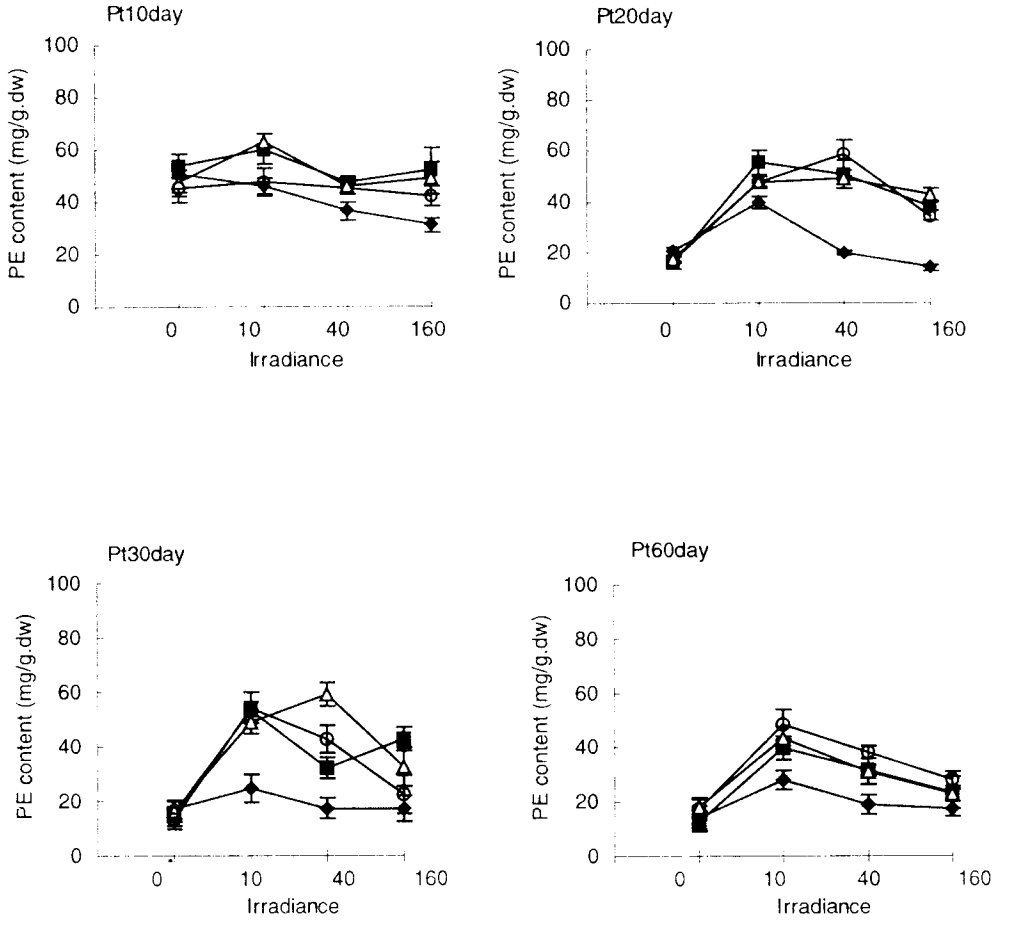

Figure 5.5. *Porphyra torta* (Pt). Phycoerythrin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

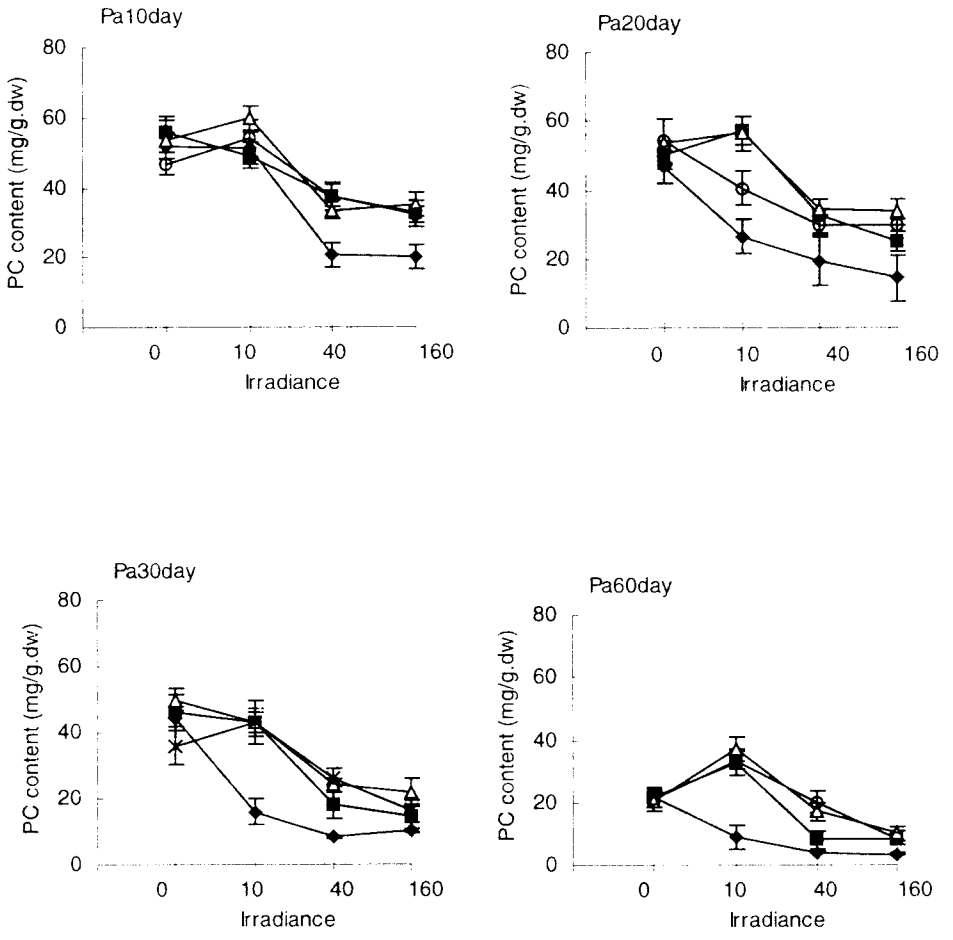

Figure 5.6. *Porphyra abbottae* (Pa). Phycocyanin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

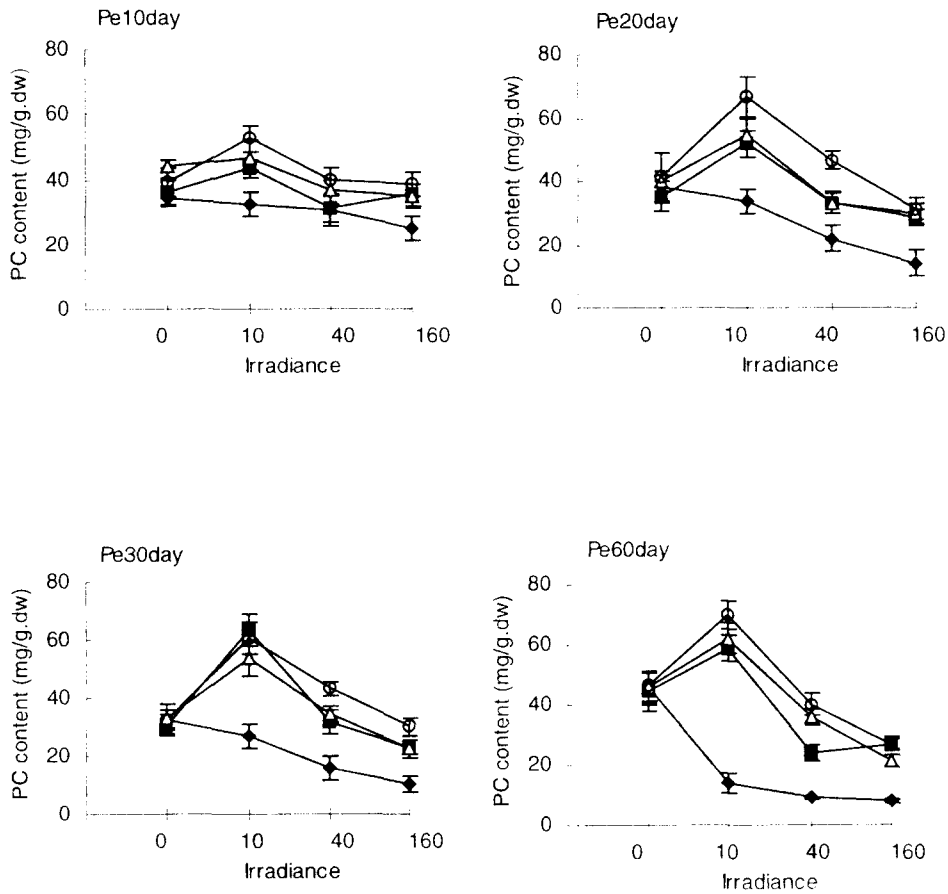

Figure 5.7. *Porphyra pseudolanceolata* (Pe). Phycocyanin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

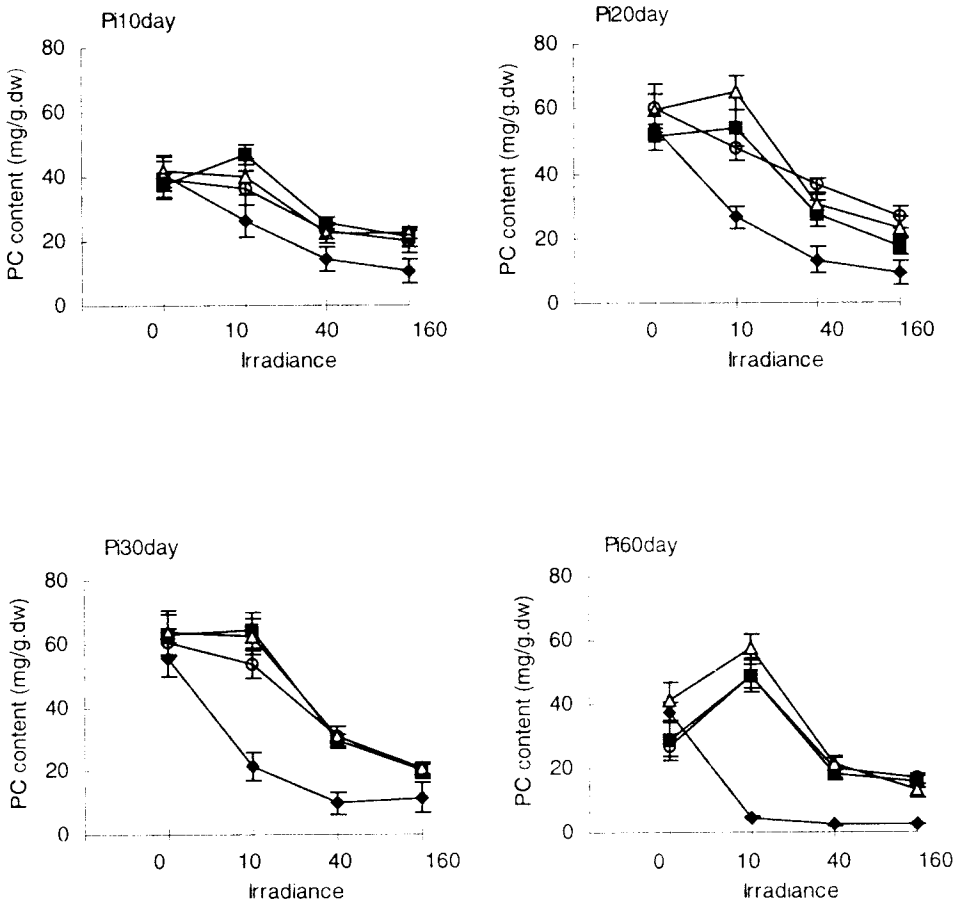

Figure 5.8. *Porphyra pseudolinearis* (Pi). Phycocyanin content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

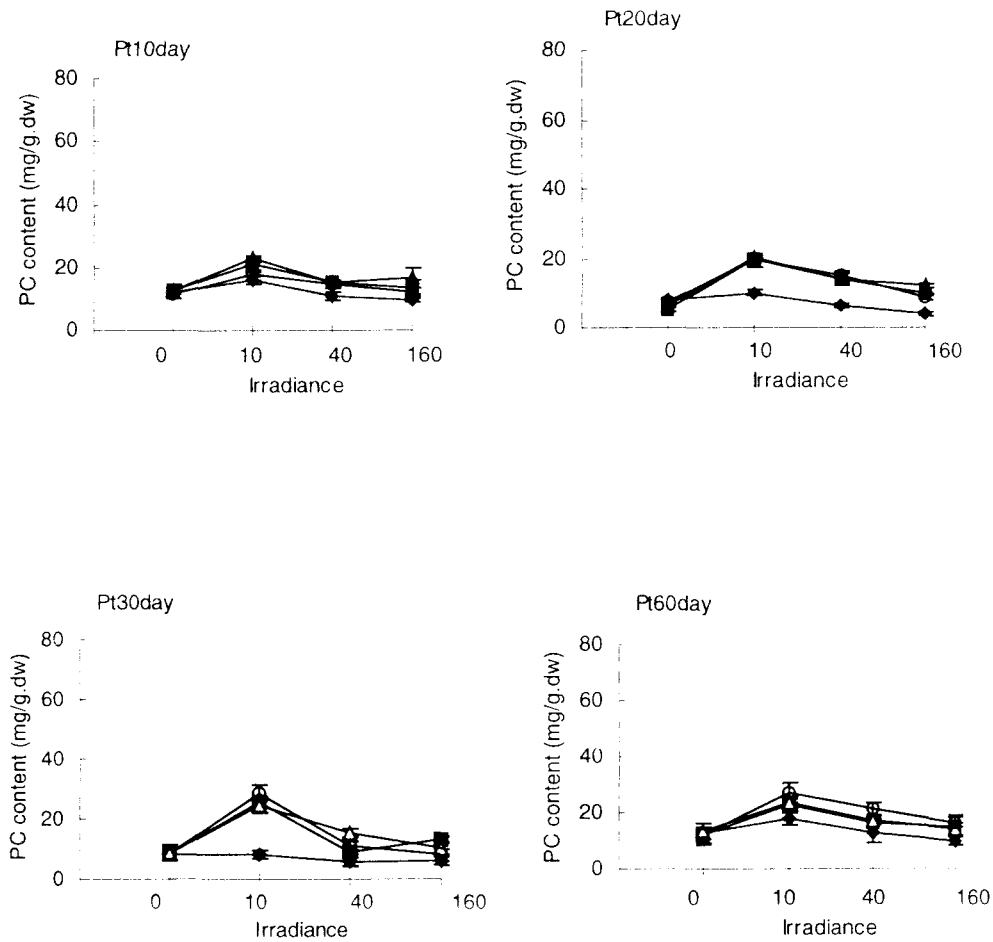

Figure 5.9. *Porphyra torta* (Pt). Phycocyanin content of the conchosporangia as a function ofirradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

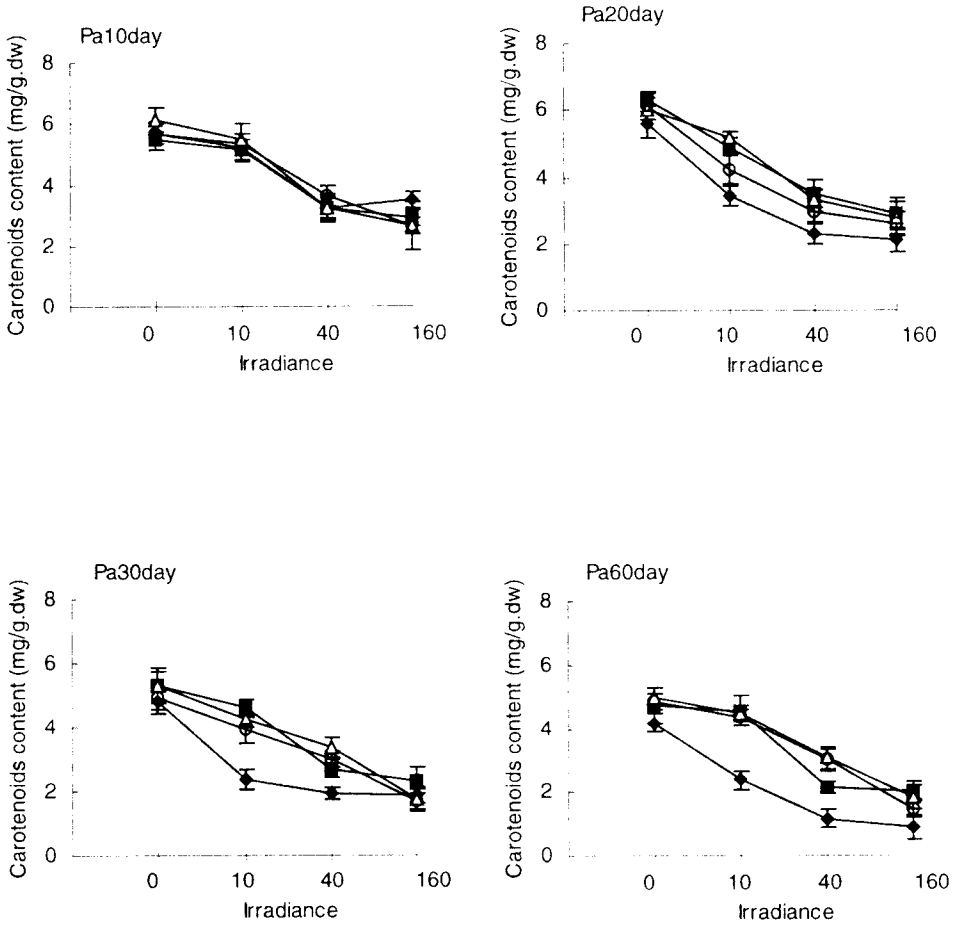

Figure 5.10. *Porphyra abbottae* (Pa). Carotenoid content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

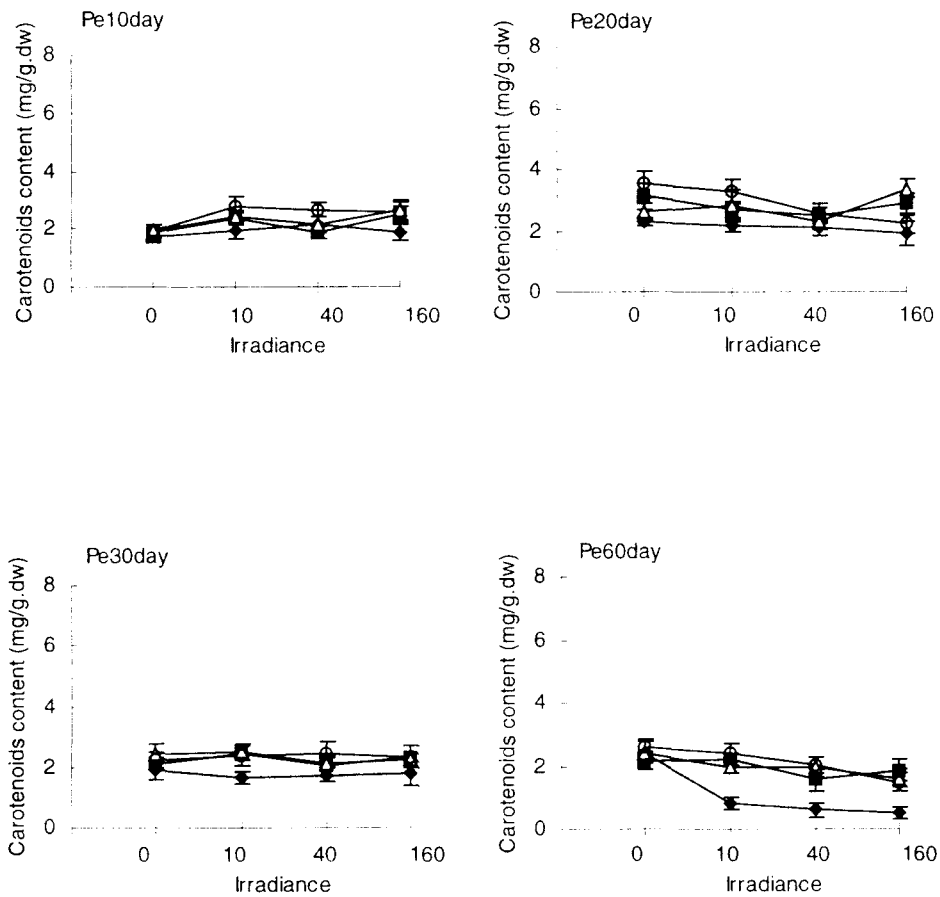

Figure 5.11. *Porphyra pseudolanceolata* (Pe). Carotenoid content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; ○, f) and culture duration. Error bars are ± S.E.

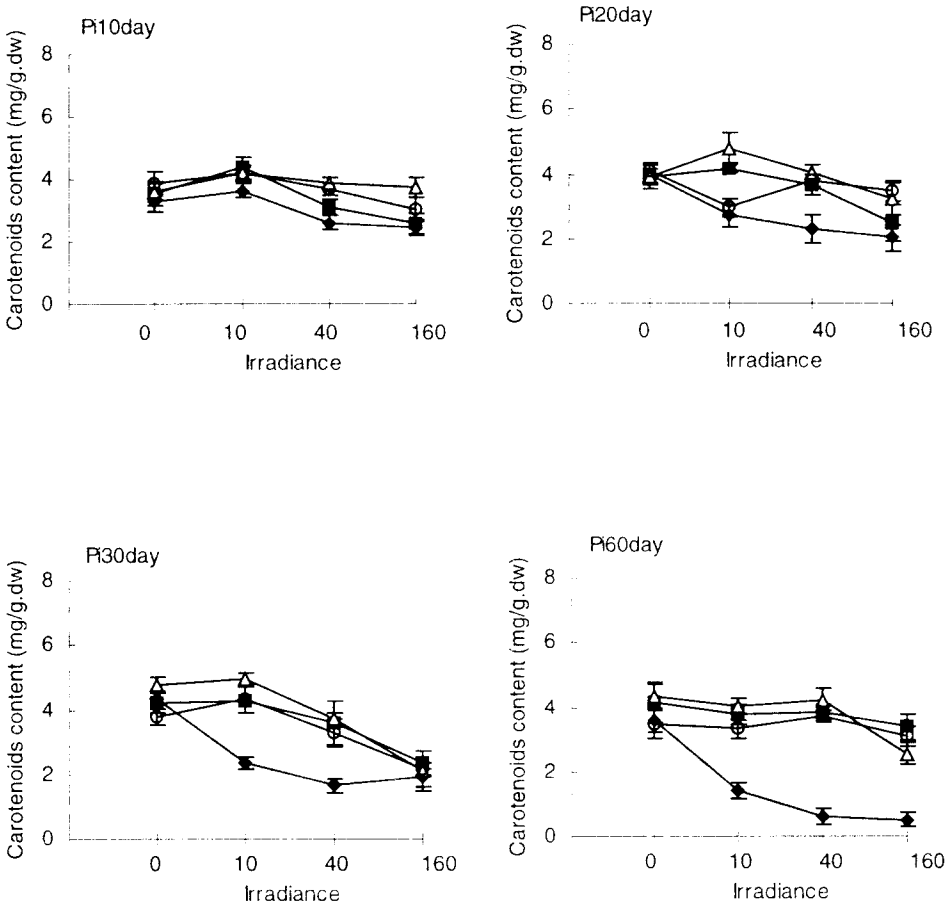

Figure 5.12. *Porphyra pseudolinearis* (Pi). Carotenoid content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

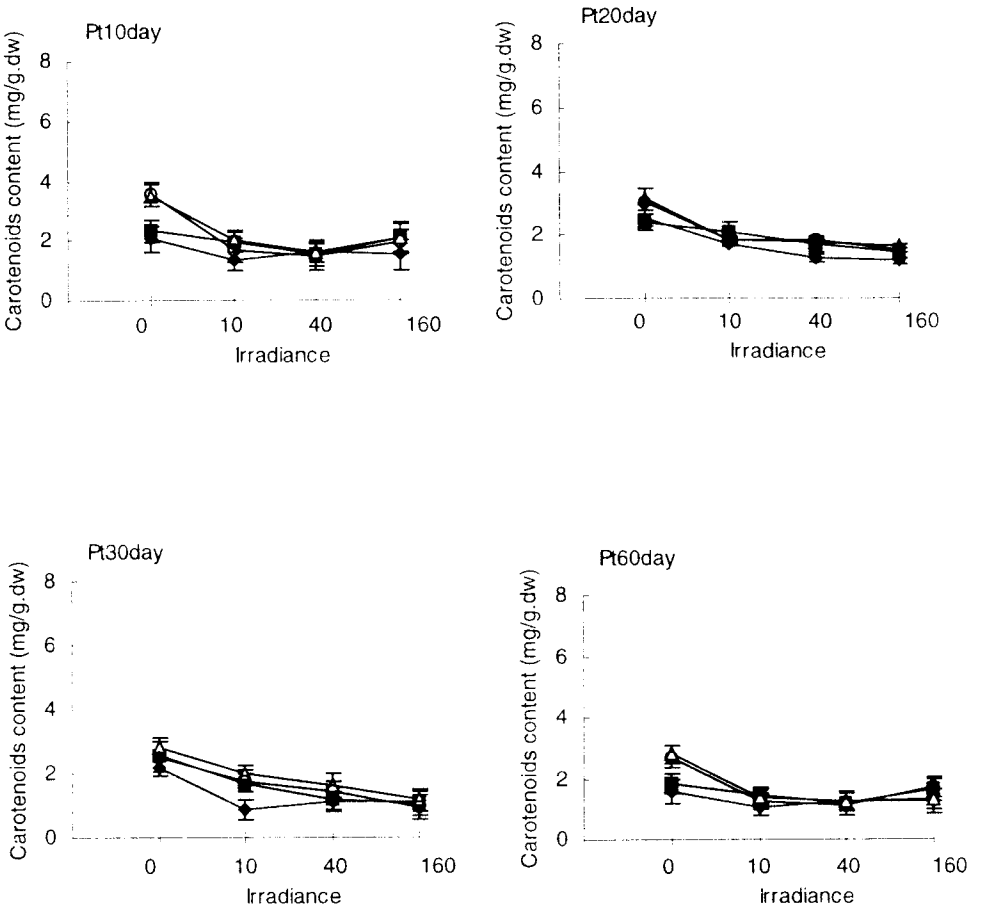

Figure 5.13. *Porphyra torta* (Pt). Carotenoid content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

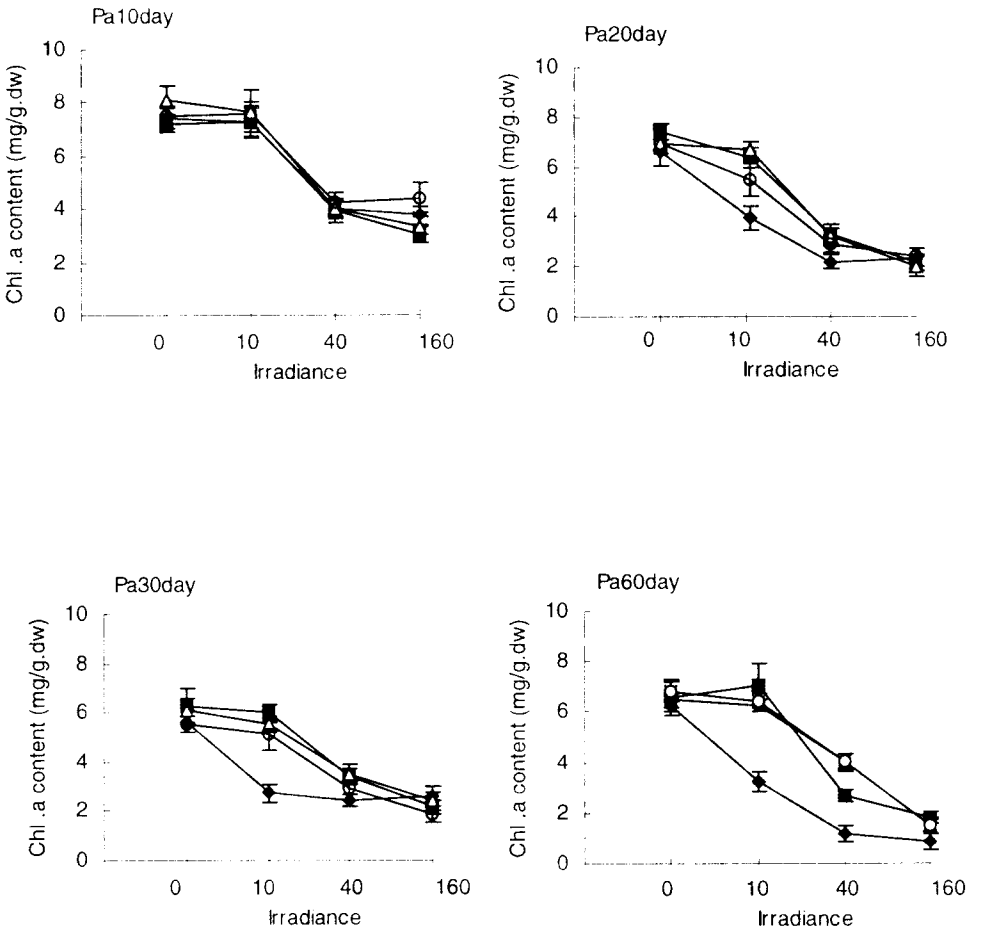

Figure 5.14. *Porphyra abbottae* (Pa). Chlorophyll *a* content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

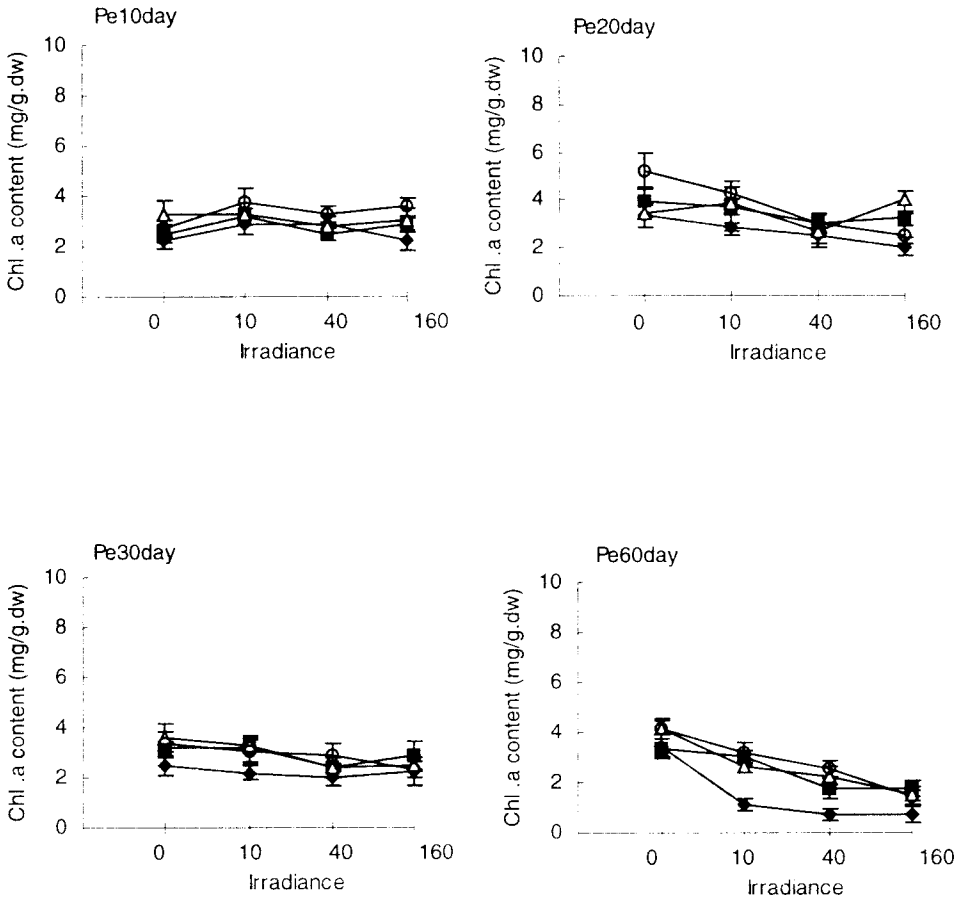

Figure 5.15. *Porphyra pseudolanceolata* (Pe). Chlorophyll *a* content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

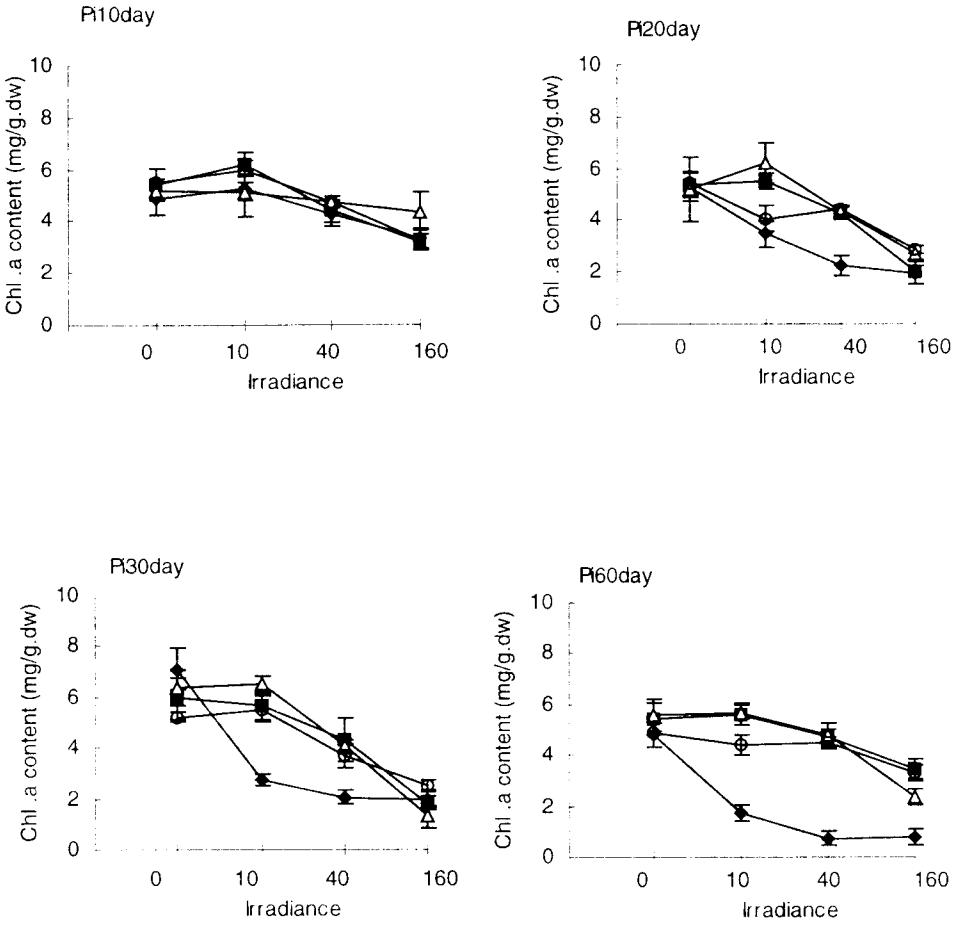

Figure 5.16. *Porphyra pseudolinearis* (Pi). Chlorophyll *a* content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■ , f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

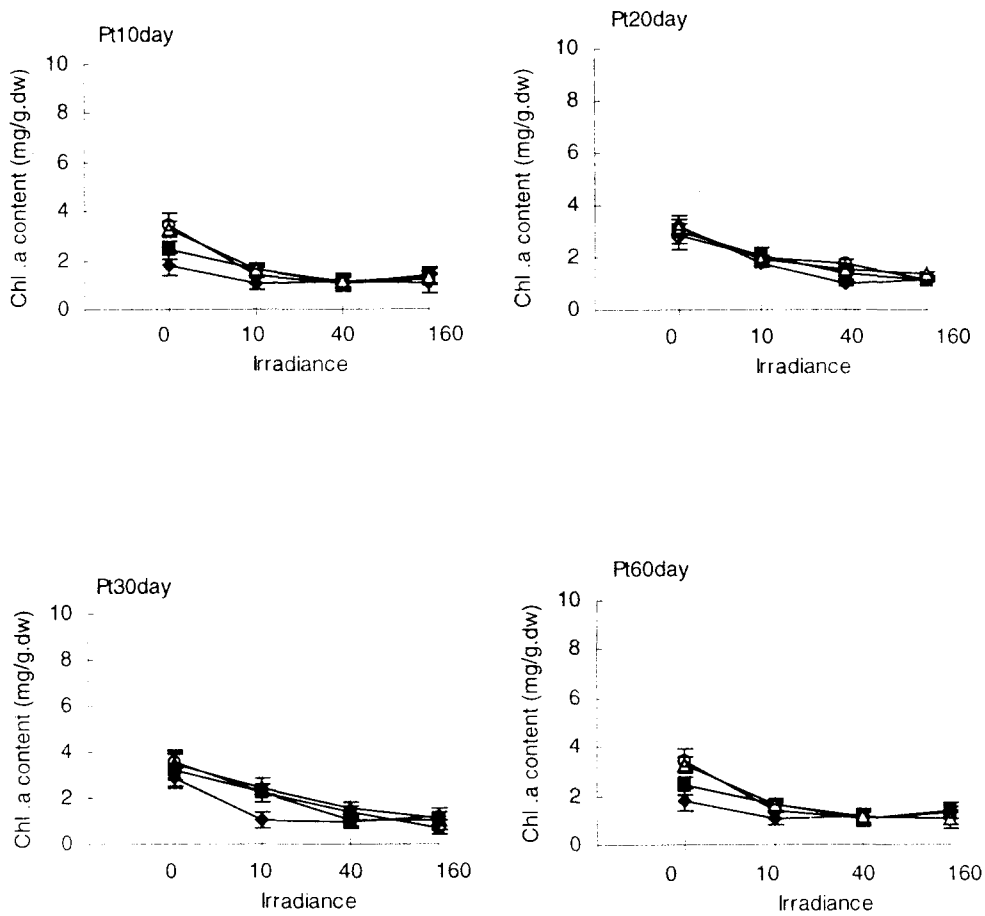

Figure 5.17. *Porphyra torta* (Pt). Chlorophyll *a* content of the conchosporangia as a function of irradiance, nutrient concentration (◆, 0; ■, f/4; Δ, f/2; O, f) and culture duration. Error bars are ± S.E.

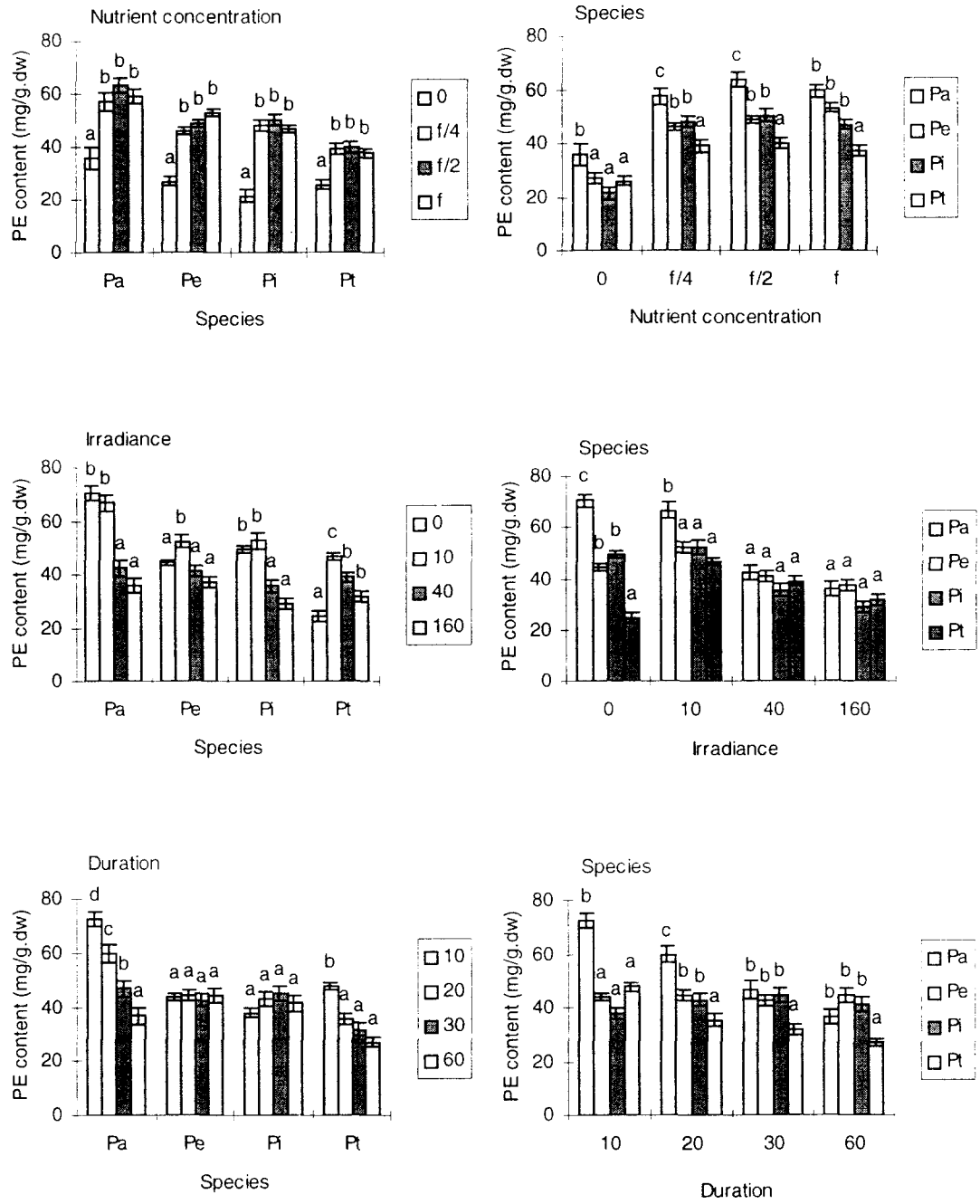

Figure 5.18. Comparison of pooled phycoerythrin content of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant within a species (for the figures on the left) and relevant between species (for the figures on the right). Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

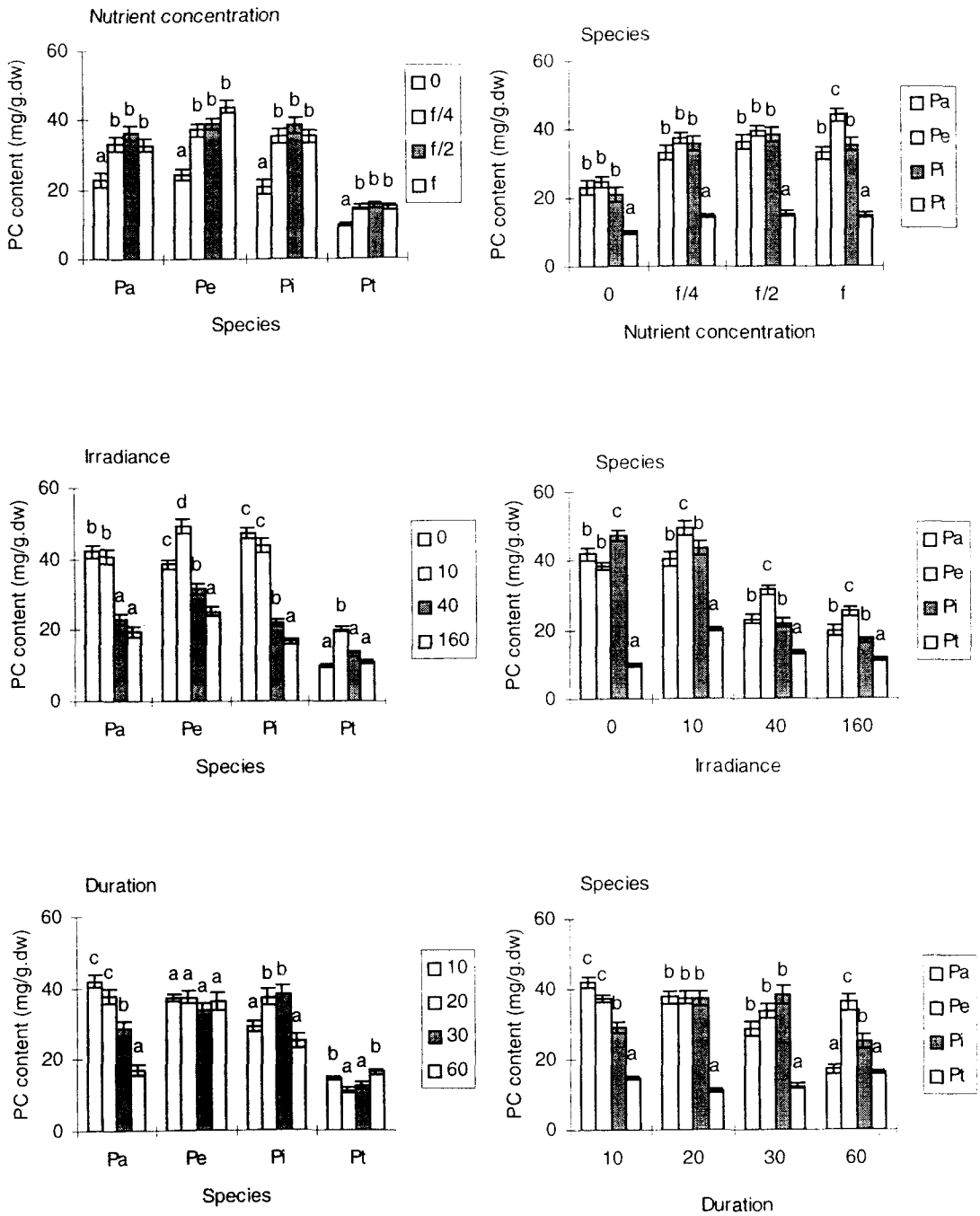

Figure 5.19. Comparison of pooled phycocyanin content of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference (P<0.01) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant within a species (for the figures on the left) and relevant between species (for the figures on the right). Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

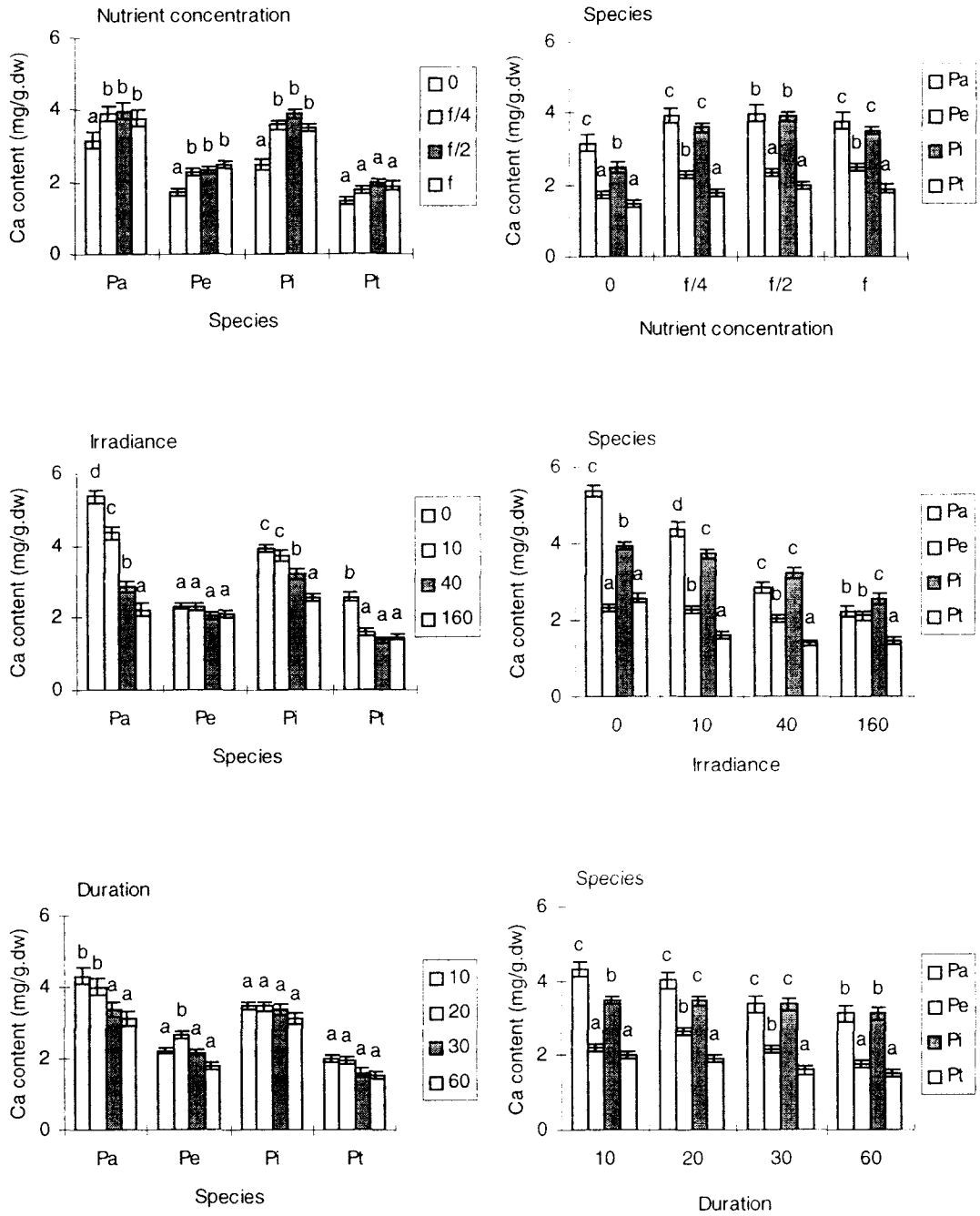

Figure 5.20. Comparison of pooled carotenoid content of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant within a species (for the figures on the left) and relevant between species (for the figures on the right). Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

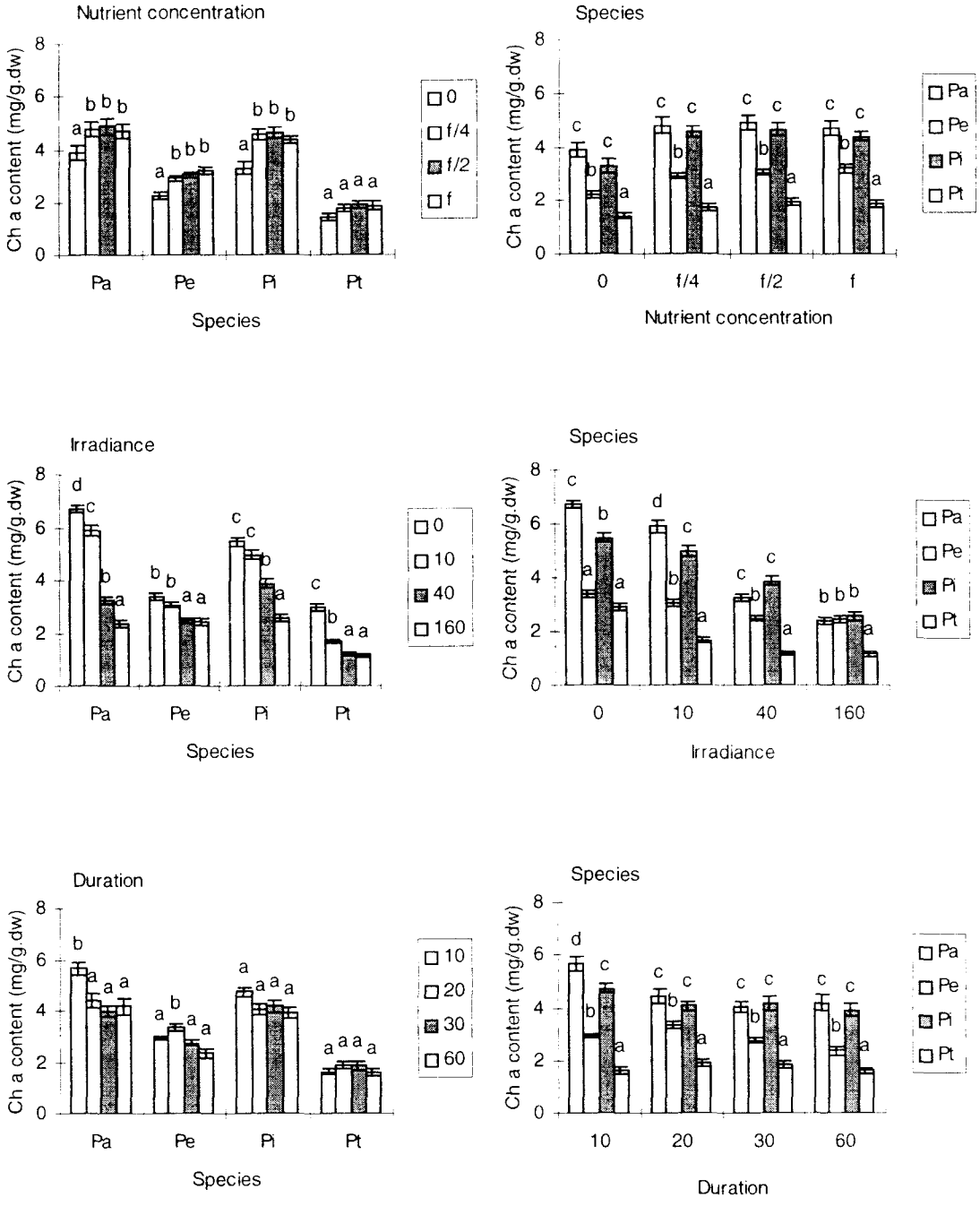

Figure 5.21. Comparison of pooled chlorophyll *a* content of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference (P<0.01) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant within a species (for the figures on the left) and relevant between species (for the figures on the right). Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

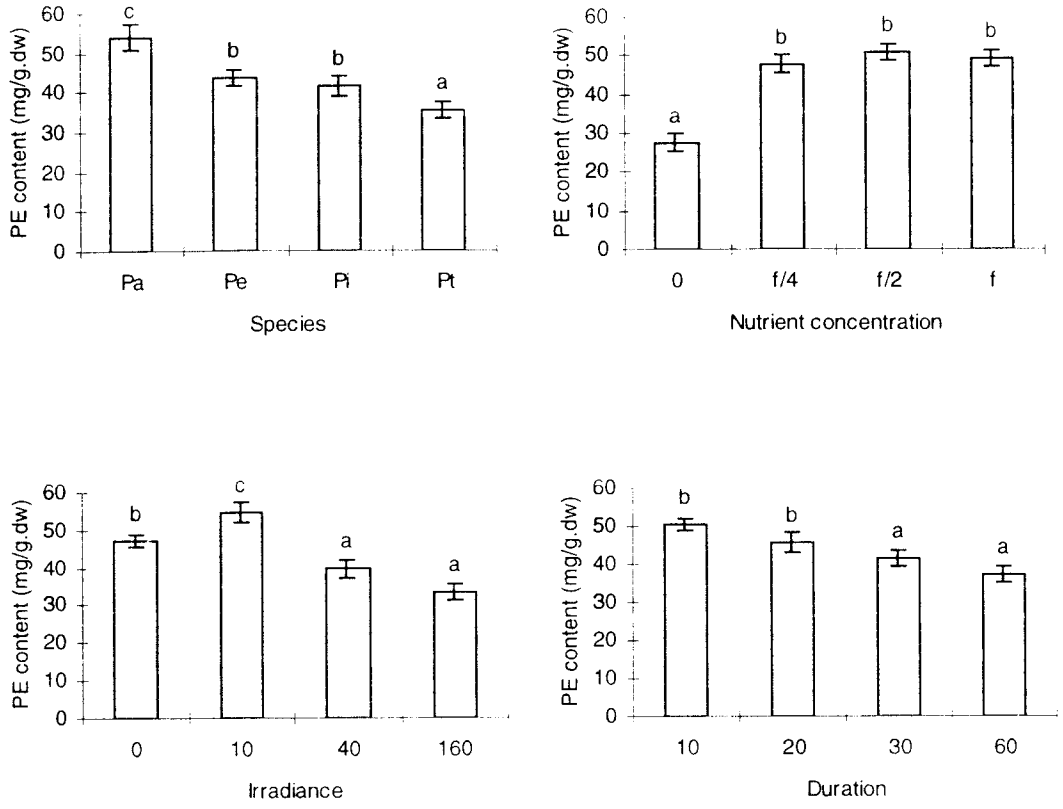

Figure 5.22. Comparison of pooled phycoerythrin content (grand average value) of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

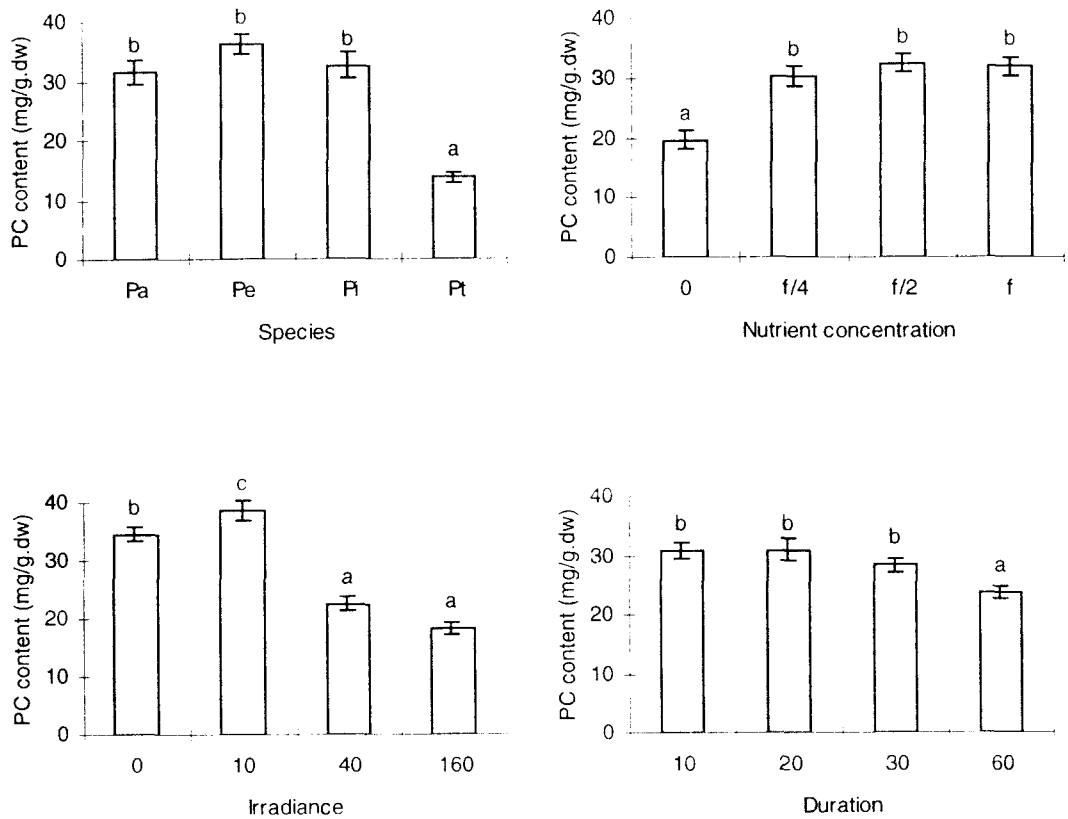

Figure 5.23. Comparison of pooled phycocyanin content (grand average value) of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

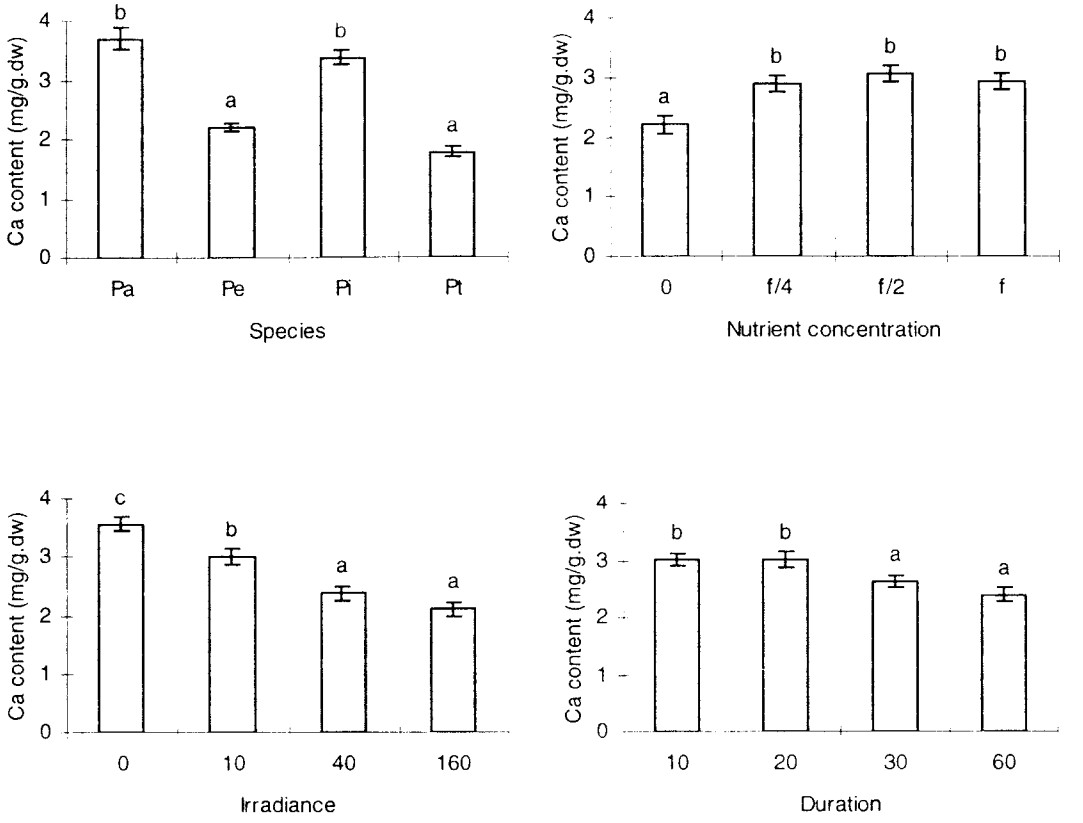

Figure 5.24. Comparison of pooled carotenoid content (grand average value) of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).

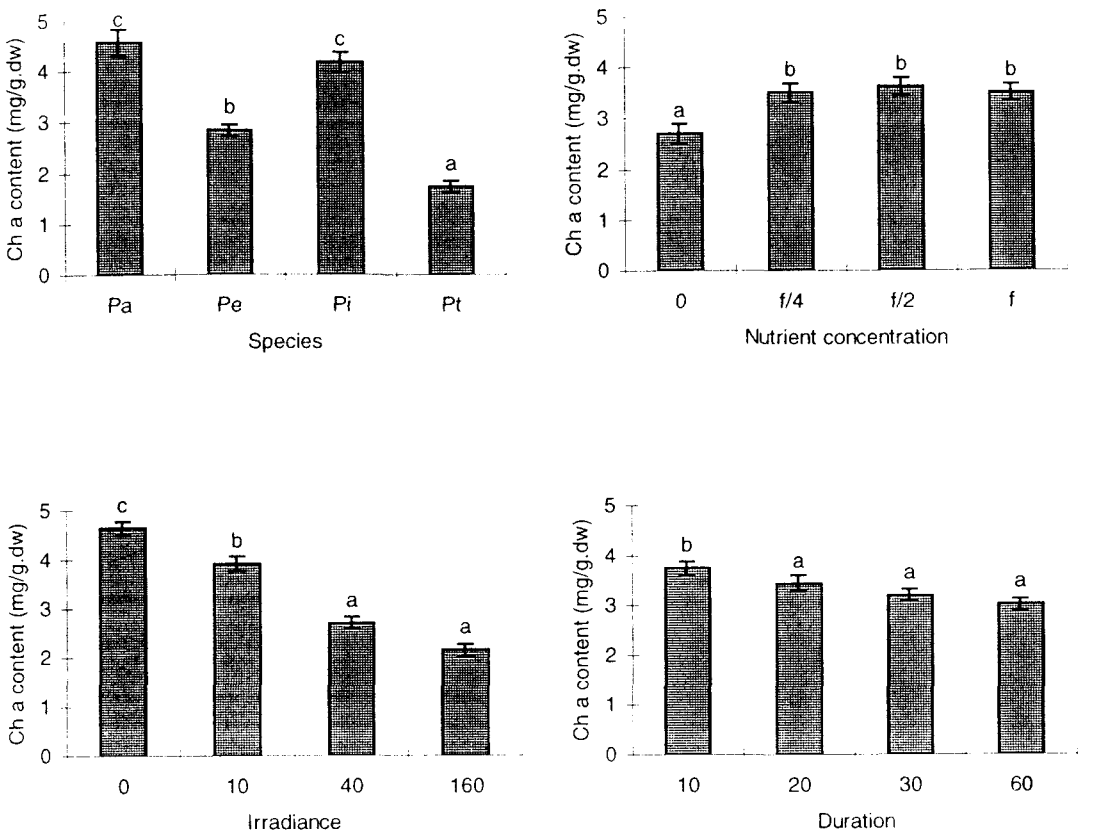

Figure 5.25. Comparison of pooled chlorophyll *a* content (grand average value) of the conchosporangia of four species of *Porphyra* for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Units of parameters tested are: irradiance (μmol photons m^{-2} s^{-1}), nutrient concentration (expressed as the f fraction) and culture duration (day).