Nutrient Removal by *Palmaria palmata* and *Chondrus crispus* in Bioremediation of Aquaculture Effluent

by

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Dedication

This work is dedicated to my wife, Esther and my children, Landon, Julia, and Jerica. May learning and purpose be ever-present elements of our home as we seek to live out God's design for our lives. I love you guys so very much.

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Abstract

Palmaria palmata and *Chondrus crispus* were cultured in the lab at three levels of temperature and two of nitrate, representative of commercial Atlantic halibut farming conditions. Productivity and nitrogen removal by *P. palmata* were greatest at temperatures <10°C. Productivity of *C. crispus* was greatest at >10°C, while nitrogen removal was unaffected by temperature, 6-17°C. When cultured in various nitrate and ammonium combinations, nitrogen uptake was highest when available as ammonium. Both species took up 89-100% of ammonium in 24 hours, but only 23-37% and 55-87% of nitrate was taken up by *P. palmata* and *C. crispus*, respectively. When *P. palmata* was integrated with halibut recirculating aquaculture, productivity and nutrient removal were compromised during summer. During winter, <11°C, nitrogen removal by *P. palmata* was relatively stable at 2.3 gN m^{-2} d^{-1}. For 50% nitrogen removal from halibut aquaculture during winter, a finfish: seaweed biomass of 1: 1 would be required.

List of Abbreviations and Symbols Used

ANOVA	analysis of variance
BH-D	Basin Head strain of *Chondrus crispus*
C	carbon
cm	centimetre
d	day
DW	dry weight
FAO	Food and Agriculture Organization (United Nations)
FW	fresh weight
g	grams
hp	horsepower
ID	inside diameter
IMTA	integrated multi-trophic aquaculture
K	Kelvin
kg	kilograms
kWh	kilowatt hour
L	litre
L:D	hours of light: hours of darkness
m	metre
mm	millimetre
mM	millimoles per litre
mg	milligrams
N	nitrogen
NH_4^+	ammonium
NO_3^-	nitrate
nm	nanometre
P	phosphorus
PO_4^{3+}	phosphate
s	second
SGR	specific growth rate
t	metric tonne
T4	clone of *Chondrus crispus*
vol	volume
W	watt
µM	micromoles per litre
µmol	micromoles
µm	micrometre
°C	degrees Celsius

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Chapter 1: Introduction

In 2009, world aquaculture accounted for an estimated 55.1 million tonnes of food fish, representing 46.8% of global food fish consumption (FAO 2011). At a growth rate of 8.3% per year from 1970-2008, fish production from aquaculture grew at a rate three times that of world meat production (FAO 2010). Aquaculture is now supplying many food fish commodities at levels around 50% of world production (e.g. tilapia, catfish, molluscs), while others are dominating the world market (e.g. carps 89.9%, salmonids 72.8%) (Hall et al. 2011). Such a rate of expansion demands thorough assessment and strategic implementation of responsible farming practices. Ecological impacts must be minimized and resource use efficiencies maximized to ensure longevity of this vital industry and the food security that it represents (Chopin et al. 2001).

Waste production is an unfortunate reality of commercial aquaculture. Effluents of finfish and other heterotrophs in aquaculture are high in organic solids as waste feed and feces, and in dissolved inorganic nutrients in the form of nitrogen and phosphorous (Xu et al. 2008). It is these nutrients which are primarily responsible for eutrophication of waters in the vicinity of intensive fish farms (Boyd et al. 2007). Organic and inorganic wastes can become concentrated to unnatural levels in the vicinity of fish farms, creating ecological imbalances by suppressing certain normally occurring species and favouring others (Chopin et al. 2001). In recirculating (closed) aquaculture systems, nitrogen can accumulate to toxic levels in the form of ammonia, nitrite, and/or nitrate unless flushed from the system by replacement water or removed in the form of biomass.

At best, only one third of the nutrients added to aquaculture systems through feed are assimilated into fish biomass (Troell et al. 1999). Most of the phosphorous

discharged from finfish tanks (50-85%) is tied up in the solid waste and may be filtered mechanically (Timmons et al. 2002). However, seventy to eighty percent of the nitrogen supplied to fish through feed is released to the environment in dissolved form, 80% of which is available to plants and a potential agent of eutrophication (Troell et al. 1999) or compromised fish health in the case of recirculating aquaculture systems.

Integrated Multi-Trophic Aquaculture (IMTA)

Seaweeds were introduced in the 1970s as a potential solution to the nitrogen and phosphorus discharge challenges of commercial aquaculture (Langton et al. 1977; Harlin 1978; Ryther 1977; Ryther et al. 1979). Dubbed integrated multi-trophic aquaculture (IMTA), the evolution of this strategy through research and technology has reached great diversity in its application (Table 1.1). IMTA is described as the cultivation of aquatic species from different trophic levels within a shared water system (Bostock et al. 2010). The objective of this approach is to optimize the use of resources otherwise considered to be waste using species complementary to one another in consumptive and discharge characteristics. Seaweed-integrated aquaculture can lead to reduction of environmental impact by fish farming, increased production and farm income, creation of jobs, and improvement of public image concerning intensive aquaculture (Schuenhoff et al. 2006).

In addition to the possible environmental impacts of nitrogen discharge, recirculating systems must continually treat the nitrogen metabolites given off by fish, converting harmful ammonia to less-harmful nitrate (Timmons et al. 2002). Nitrification by aerobic bacterial bioreactors is the most widely used method of nitrogen treatment in closed systems (Timmons et al. 2002). Though bacterial bioreactors occupy a small area for their relative capacity, they generate virtually no usable biomass, are net consumers of

Table 1.1 Comparison of land-based seaweed-fish integration.

Macroalgal species	Nutrient source	Tank vol (L)	Flow (vol/d)	Temp (°C)	St. density (g FW L^{-1})	[d] Max yield	N-yield (g m^{-2} d^{-1})	Reference
Palmaria palmata	*H. hippoglossus* [a]	[b] 1250	-	0.5-12	4.7	19.0	2.3	This study
Palmaria palmata	*Scophthalmus maximus*	300	11.3	17-21	2.8	40.2	2.7	Matos et al. 2006
Chondrus crispus	*Scophthalmus maximus*	1500	2.2	17-21	3.3	8.4	0.8	Matos et al. 2006
Chondrus crispus	*Scophthalmus maximus*	1500	2.2	18-21	4.7	36.6	0.9	Matos et al. 2006
Gracilaria bursa pastoris	*Scophthalmus maximus*	1500	2.2	18-21	4.7	31.2	1.6	Matos et al. 2006
Gracilaria bursa pastoris	*Scophthalmus maximus*	1500	2.2	21-23	4.7	27.0	4.3	Matos et al. 2006
Gracilaria bursa pastoris	*Scophthalmus maximus*	1500	2.2	21-23	6.5	5.2	3.5	Matos et al. 2006
Chondrus crispus	NH_4Cl	20	114.0	12-14	6.3	-	0.7	Magnusson et al. 1994
Ulva Lactuca	NH_4Cl	20	114.0	12-14	2.5	-	1.4	Magnusson et al. 1994
Palmaria mollis	$NaNO_3$	3.8	1.0	16	2.6	34.9	1.6	[f] Demet. and Lang. 2004
Gracilaria vermiculophylla	*Scophthalmus rhombus, Dicentrarchus labrax*	1200	2.0	11-20	3.8	23.0	1.4	Abreu et al. 2011
Gracilariopsis longissima	*Sparus aurata*	600	3.5	-	0.8	[e] 270.0	3.2	Hernandez et al. 2006
Gracilaria tenuistipitata	*Onchorynchus mykiss*	[c] 30 000	3.4	22	1.1	[e] 44.0	1.1	[g] Hagl. and Ped. 1993
Ulva lactuca	*Sparus aurata*	2500	2.9	17-19	1.2	[e] 115.0	7.0	Neori et al. 1996
Ulva clathrata	*Litopenaeus vannamei*	250	1.0	22-31	4.1	[e] 70.0	0.4	Copertino et al. 2009
Asparagopsis armata	*Sparus aurata*	110	98.0	14.2	5.0	71.0	4.2	Mata et al. 2010
Ulva rigida	*Sparus aurata*	110	98.0	14.2	4.0	44.0	2.7	Mata et al. 2010
Asparagopsis armata	*Sparus aurata*	110	91.0	21.5	5.0	125.0	7.4	Mata et al. 2010
Ulva rigida	*Sparus aurata*	110	91.0	21.5	4.0	73.0	3.6	Mata et al. 2010
Mixed algal biomass	*Dicentrarchus labrax*	5900	0.2	12-25	9.0	50.0	0.9	Pagand et al. 2000

[a] *Hippoglossus hippoglossus* ; [b] Cages; [c] Pond; [d] g DW m^{-2} d^{-1}; [e] g FW m^{-2} d^{-1}; [f] Demetropoulos and Langdon 2004 b; [g] Haglund and Pedersen 1993

oxygen (Timmons et al. 2002), and contribute to acidification of culture water (Cahill et al. 2010). Conversely, seaweed-based bioreactors are net oxygen producers (Neori et al. 2004), can aid in stabilization of pH by uptake of carbon dioxide (Schuenhoff et al. 2006), and remove nitrogen as biomass (Hayashi et al. 2008) rather than converting it to a low-toxic form to be flushed by water replacement. Cahill et al. (2010) have clearly concluded that seaweed biofiltration is superior to bacterial biofiltration, although the study involved only 150 g of abalone integrated with 70 L seaweed cultivation tubs.

Several examples of recent studies in seaweed-fish integration are presented in Table 1.1. Further to these, Buschmann et al. (1996) found that the red alga *Gracilaria chilensis*[1] removed 50% of ammonium in winter and 90-95% in spring from effluent of a salmon culture system. In a study integrating *Ulva lactuca* (Chlorophyta) with gilthead seabream (*Sparus aurata*[2]) in a recirculating aquaculture system, the seaweed caused a marked increase in dissolved oxygen and pH, and took up most of the ammonium, as well as phosphate (Neori at al. 1996). A settling tank for removal of solids prior to the seaweed tanks was the only other filtering component installed in this recirculation system. Another study of recirculating aquaculture systems reported significant reduction of nutrient loading in fish rearing water when a seaweed cultivation loop was integrated with culture of sea bass (Metaxa et al. 2006). This was accomplished without detrimental impacts on fish health or elevated fish mortality. Phosphorus is not the focus of land-based integration studies since it can be removed from solution more readily than nitrogen, it is less likely than nitrogen to limit algal growth, and it is of no significant threat to the well-being of cultured fish species.

Seaweed Species for IMTA

Abreu et al. (2011) outlined criteria useful in identifying suitable seaweed species for IMTA—wide tolerance of environmental conditions and similar growth characteristics between life-stages, high rates of nutrient uptake with preference for ammonium-nitrogen, and market value for extractive or consumption purposes. The latter is perhaps the most challenging, since species selected for integration must have sufficient value to warrant investment (Bostock et al. 2010). It is also important to select local species for which technology is available, in order to avoid introduction of non-indigenous seaweed varieties (Barrington et al. 2009).

Palmaria palmata (Linnaeus) Weber and Mohr, also known as dulse or sea parsley, has been identified as a suitable species for aquaculture integration based on viable biomass, rapid growth, and promising nutrient uptake rates (Pang and Shan 2008). Dulse is valued as a functional food and nutrient supplement for its high concentration of usable protein (25% DW) and richness of trace elements (Bird and McLachlan 1992). *P. mollis*, a northern Pacific relative of *P. palmata*, has been studied for its application in co-culture with abalone as a fodder and nutrient scrubber (Demetropoulos and Langdon 2004b).

Palmaria palmata is common on all Atlantic coasts of Canada (Bird and McLachlan 1992), occupying the mid-littoral zone to 20 m subtidal depth. Male and tetrasporangial plants of this species are conspicuous, with one to several broad fronds arising from a discoid holdfast (Bird and McLachlan 1992). First identified by van der Meer and Todd (1980), the microscopic female gametophyte is fertilized by non-motile spermatia from large male gametophytes originating in the previous year. Unlike most red algae, there is no carposporophyte generation, and the zygote formed on the female

gametophyte develops directly into the tetrasporophyte (Pang and Lüning 2006). Thalli are generally found attached to rocks or other solid substrate, or may be epiphytic on other macroalgae, such as *Saccharina* sp. (pers obs).

Chondrus crispus Stackhouse is also indigenous to both the eastern and western Atlantic coasts (Bird and McLachlan 1992, Garbary et al. 2011). Morphologically similar male and female gametophytes arise from tetraspores, subsequently yielding carposporophytes on the fronds of female gametophytes (Chen and McLachlan 1971). Tetrasporophytes thus formed will produce the next generation of gametophytes. Commonly known as Irish moss, culture of this species was first investigated for its value as a carrageenophyte (Craigie 1990). High operating costs meant that culture could not compete with harvest of natural *C. crispus* populations and this approach has not been pursued further. However, the commercial culture of Irish moss still exists for the production of a high-value product intended for direct human consumption (Acadian Seaplants Ltd., www.acadianseaplants.com).

Land-based cultivation may also preserve unique wild strains of *Chondrus* and other species for the purpose of stock enhancement. The morphotype of *C. crispus* found at Basin Head, Prince Edward Island, for example, is very sensitive to habitat degradation as it is found only in Canada's Atlantic region (Chopin et al. 1999b). In fact, the strain has experienced a decline of such magnitude to be considered endangered (Sharp et al. 2010). This strain is characterized not only by its distinct morphology, but also its habit of reproduction primarily by fragmentation, with the population dominated by gametophytic thalli (Chopin et al. 1999b; Garbary et al. 2011). Furthermore, it was established in growth trials using hundreds of *C. crispus* that the sporophyte clone of

Basin Head Chondrus (BH-D) has productivity superior to that of clone T4, one of the most productive clones of open-water *C. crispus* (Craigie 1990).

The study described in the following pages investigates some aspects of *P. palmata* and *C. crispus* in IMTA with Atlantic halibut (*Hippoglossus hippoglossus*). The primary objective of this work is identification of the species most suitable for land-based IMTA in this application based on the criteria previously outlined. Chapter 2 reports the effects of nitrogen availability and temperature on productivity and nutrient removal by these two rhodophytes. Conditions were selected which represent the range of conditions encountered at a cold-water, land-based finfish aquaculture facility. This data may contribute to the formulation of a model for seaweed bioremediation of aquaculture effluent. Chapter 3 looks at the impacts of nitrogen source on nutrient removal by *P. palmata* and *C. crispus*. Since both ammonium and nitrate are present in both recirculating aquaculture water and, to some extent, in land-based aquaculture effluent in general, it is important to establish where a seaweed bioreactor should be placed within a water treatment system to have the greatest benefit. Reported in Chapter 4 is the outcome of commercially applied, seaweed-halibut integration using *P. palmata*. These results are directly applicable to other land-based finfish operations for effluent mitigation, while the final chapter provides further discussion of the implications, challenges, and benefits of land-based, integrated aquaculture.

[1] Authorities for algal names may be found at Algaebase (www.algaebase.org).
[2] Authorities for names of fish may be found at Fishbase (www.fishbase.org).

Chapter 2: Bio-remediation Potential of *Chondrus crispus* (Basin Head) and *Palmaria palmata*: Effect of Temperature and High Nitrate on Nutrient Removal

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Abstract

To evaluate the nutrient removal capabilities of two red macroalgae, apical blades were cultured in the lab for four weeks at either 6, 10, or 17°C and nitrate at either 30 or 300 μM. Stocking density was 2.0 g L^{-1}, irradiance 125 μmol photons m^{-2} s^{-1}, photoperiod 16:8 (L:D), and nitrogen:phosphorus ratio 10:1. For both species, highest growth rate was at 300 μM NO_3^- with *Palmaria palmata* growing fastest at 6°C, 5.8% d^{-1}, and *Chondrus crispus* growing best at 17°C, 5.5% d^{-1}. Nitrogen and carbon removal by *P. palmata* was inversely related to temperature, the highest rate at 6°C and 300 μM NO_3^- of 0.47 mg N and 6.3 mg C per gram dry weight per day. By contrast, *C. crispus* removal of N was independent of temperature, with mean removal of 0.49 mgN gDW^{-1} d^{-1} at 300 μM NO_3^-. Highest carbon removal by *C. crispus* was 4.4 mgC gDW^{-1} d^{-1} at 10°C and 300 μM nitrate. Phycoerythrin content of *P. palmata* was independent of temperature, with mean of 23.6 mg gFW^{-1} at 300 μM nitrate. In *C. crispus*, phycoerytherin was different only between 6 and 17°C at 300 μM nitrate, with highest phycoerytherin content of 12.57 mg gFW^{-1} at 17°C.

Introduction

To help address the social demand for environmental sustainability, some salmon farming companies in Atlantic Canada are growing seaweed and mussels around marine net-pens to absorb dissolved and solid waste (Barrington et al. 2010, Chopin et al. 1999a). By comparison, closed containment systems for finfish aquaculture allow better management of solid waste, but are expensive to operate and most currently fail to deal with the dissolved nitrogen and phosphorus (Nobre et al. 2010). At a land-based Atlantic halibut (*Hippoglossus hippoglossus*) farm in Nova Scotia, for example, the dissolved nitrate and phosphorus concentrations can reach 300 and 30 μM respectively, up to 100-fold higher than coastal waters (Scotian Halibut Ltd., unpubl data; Martinez and Rico 2002; Chopin et al. 1999b). The effluent from this facility is sufficient to enhance the growth of intertidal *Ascophyllum nodosum* without inducing local blooms of *Ulva* (White et al. 2011). Using this land-based halibut system as a model, the goal is to determine if red macro-algae are cost-effective bio-remediators of fish farm effluent. *Palmaria palmata* and *Chondrus crispus* were selected because they are common to Atlantic Canada and their culture methods are well known (Morgan et al. 1980a; Craigie 1990). Basin Head *C. crispus* was also chosen because of its high growth rate compared to other highly productive clones (Craigie 1990). The strain is unique to this region, and is distinct morphologically due to its unattached nature (Chopin et al. 1999b). Despite the considerable literature on the growth dynamics of both *C. crispus* and *P. palmata*, their capacity to clean-up fish farm effluent cannot be estimated accurately. This is because previous studies either used low nitrate concentrations (<100 μM), or measured growth but not nutrient uptake (Martinez and Rico 2004; Pang and Lüning 2004; Magnusson et al. 1994), or only tested a narrow range of temperatures (Martinez and Rico 2004; Matos

et al. 2006; Amat and Braud 1990). These species grow best at 6-15°C and 6-18°C for *P. palmata* and *C. crispus*, respectively (Morgan et al. 1980a; Bidwell et al. 1985), though reported optimal temperatures differ between studies.

As a first step to define the bio-remediation capacity of *Chondrus crispus* and *Palmaria palmata*, the present *in vitro* experiment quantified growth rate and removal of nitrogen and carbon over the range of temperature and nitrate concentrations present in the farm effluent. An additional response variable was phycoerythrin, a proteinaceous pigment unique to Rhodophyta and cyanobacteria (Harrison and Hurd 2001). Representing as much as 85-87% of total phycobiliprotein, this pigment accumulates quickly at high nitrogen concentrations, resulting in increased photosynthetic activity and growth (Navarro-Angulo and Robledo 1999; Carmona et al. 2006). In the context of bioremediation, it was important to include phycoerythrin measurement here, since previous studies only quantified it at low nitrate levels (< 100 μM; Chopin et al. 1995; Martinez and Rico 2002).

Materials and Methods

Palmaria palmata was collected in June 2009 from natural populations in the low-intertidal zone of the Bay of Fundy, at Point Prim, Digby County, Nova Scotia (44°69'15.7"N, 65°78'5.4"W). The Basin Head strain of *Chondrus crispus* was obtained from the Sandy Cove Aquaculture Research Station of the National Research Council, Halifax, Nova Scotia, June 2009. Prior to the experiment, both species were stocked to culturing units at the land-based Atlantic halibut nursery site of Scotian Halibut Ltd., Wood's Harbour, Nova Scotia (43°55'80.6"N, 65°73'61.1"W) and grown under high-nutrient recirculating aquaculture seawater conditions.

The experiment was conducted during November 2009 at the Aquaculture Centre of the Nova Scotia Agricultural College, Truro, Nova Scotia. Both species were cultured for four weeks in 1L Florence flasks under saturating light intensities (125 μmol photons m^{-2} s^{-1}; Fortes and Lüning1980; Martinez and Rico 2004). Light was supplied by 32 W, 5000 K white T8 fluorescent lamps (Philips, Somerset, N.J.) on a 16:8 L:D photoperiod (Sagert and Schubert 2000). Irradiance was measured using a light meter (Li-Cor Li-1400 with Li-193SA spherical sensor, Lincoln, NE). Temperature was maintained at 6, 10, or 17°C by sitting the base of the culture flasks in continuous-flow water baths. The culture medium was von Stosch's medium (Guiry and Cunningham 1984) and filtered (0.3 μm) natural seawater from Ketch Harbour, Nova Scotia, containing no nitrogen (N) or phosphorus (P). Culture medium N and P levels were adjusted by addition of nitrate ($NaNO_3$) and phosphate ($NaH_2PO_4 \cdot 2H_2O$) at a molar N:P ratio of 10:1 to ensure that phosphate was not limiting (Shacklock and Craigie 1986). The culture medium was exchanged twice weekly. All glassware was rinsed with 10% HCl followed by distilled water prior to use. Algal biomass was kept in tumbling suspension by continuous aeration via a plastic pipe (3 mm ID).

For three days prior to the start of the experiment *P. palmata* and *C. crispus* were acclimated at 10°C in filtered (0.3 μm) and UV-sterilized, natural seawater without additional nutrients. Then tissue samples were collected in triplicate from both species for subsequent analysis. Following acclimation, 4-7 cm long blades were cut from the apical ends of both *P. palmata* and *C. crispus* fronds (Pang and Lüning 2004). Six to ten blades were stocked in each flask to a total biomass of 2.0 (± 0.1) g L^{-1}.

Experimental design The experiment incorporated a split-plot design with temperature (three levels, 6, 10, and 17°C) as main plots, and nitrate (two levels, 30 and

300 μM) as subplots. A total of six treatment combinations were tested with three replicates for each species. Nitrogen concentrations of 30 and 300 μM in the culture media reflect the highest ambient seawater nitrogen levels during winter and aquaculture total nitrogen levels (Scotian Halibut Ltd. unpubl data), respectively.

Biomass in each flask was weighed (fresh weight, FW) at weekly intervals. FW was obtained after blotting the thalli dry with paper towel and weighing within 2 min. Seaweed biomass in each flask was reduced to initial stocking density (2.0 g L^{-1}) at each weighing, and samples were taken for dry weight and pigment analysis. Dry weight (DW) and moisture content were determined by drying tissue samples to constant weight at 60°C. Specific growth rate (SGR, expressed as % day^{-1}) was calculated using the following formula:

Equation 2.1

$$SGR = \frac{\ln S_2 - \ln S_1}{T_2 - T_1} \times 100$$

where S_1 and S_2 are the fresh weight (g) at days T_1 and T_2, respectively. Specific growth rate was determined weekly.

Dried tissues were ground to powder with an MM301 ball mill (Retsch, Newtown, PA) and then analyzed for total tissue N and carbon (C) using a CNS-1000 Carbon, Sulphur and Nitrogen Analyzer (Leco, St. Joseph, MI). Total tissue N was determined at initial and final time-points of the experiment. Nitrogen removal was calculated according to the equation of Kim et al. (2007):

Equation 2.2

$$\text{N removal, mgN gDW}^{-1}\ \text{d}^{-1} = \frac{((B_t - B_0) \times \text{Tissue N})}{\frac{(B_t + B_0)}{2} \times t} \times \frac{DW}{FW} \times 10^3$$

where B_t and B_0 are biomass (g) at day t (final) and day 0 (initial), respectively. Nitrogen removal, also presented by Schuenhoff et al. (2006) as "nitrogen yield", is an absolute measurement of nitrogen mitigation potential as a function of tissue nitrogen concentration and excess biomass. The same equation was used for C removal, except *Tissue C* was substituted for *Tissue N*. Nitrogen and carbon removal were calculated for the final week of the experiment only when total biomass was removed from the flasks and could be used for tissue analysis. Dry matter of tissue collected during previous weekly biomass surveys was insufficient for this analysis.

Phycoerythrin was extracted using a modification of the Beer and Eshel (1985) protocol. Approximately 100 mg FW of tissue was ground with a mortar and pestle in 0.1 M phosphate buffer (pH 6.5), kept at 4°C, then centrifuged at 19 000 g for 15 min (MSE Harrier 15/80). The supernatant was analyzed using a DR2700 spectrophotometer (Hach, Loveland, Colorado) at 455, 564, and 592 nm. Phycoerythrin content was calculated based on the equations used in Beer and Eshel (1985).

Statistical analysis Data were analyzed using a two-way ANOVA model. Where significant treatment effects were indicated by ANOVA, Tukey's HSD analysis ($p = 0.05$) was used as a post hoc test to determine pairwise relationships between treatment means. Statistical analyses were carried out using SAS statistical software (SAS Institute Inc., Cary, NC, USA) and Minitab 15 (Minitab Inc., State College, PA, USA).

Results

Nitrate availability had a significant effect ($p < 0.001$) on growth rate. Both *P. palmata* and *C. crispus* exhibited higher growth rates at 300 μM nitrate than at 30 μM nitrate at all temperatures. Both species had similar growth rates at 10°C and 300 μM nitrate, 4.7% d^{-1} and 4.5% d^{-1} for *P. palmata* and *C. crispus*, respectively. Highest growth rates of the two species were also similar at 5.8% d^{-1} at 6°C for *P. palmata* and 5.5% d^{-1} at 17°C for *C. crispus*, both at 300 μM nitrate (Fig. 2.1). Temperature also had a significant effect on the growth rate of both species. The growth rate of *P. palmata* was higher at 6 than at 17°C (Fig. 1; $p < 0.05$), with growth rate at 10°C intermediate (not significantly different) to 6 or 17°C. *C. crispus* had the highest growth rate at 17°C, although this was not significantly different from 10°C, with a lower growth rate at 6°C (Fig. 2.1; $p < 0.05$).

Tissue C, tissue N, and C:N ratio in *P. palmata* at the end of the four-week trial were independent of temperature ($p > 0.05$). Nitrate concentration, however, did have a significant effect on tissue N and C:N ratio, resulting in higher N content and lower C:N ratio at 300 μM nitrate. Tissue N ranged from 1.5% (10°C, 30 μM nitrate) to 3.4% (10°C, 300 μM nitrate). Tissue N was about two-fold higher at 300 μM nitrate than at 30 μM nitrate. Tissue C in *P. palmata* was largely independent of temperature and nitrate treatments, except at 17°C and 300 μM nitrate which had a slightly lower C content, 39.1% (Table 2.1). *Chondrus crispus* tissue C, tissue N, and C:N ratio were more affected by both temperature and nitrate concentration than *P. palmata*. Tissue C was inversely related to temperature in all treatments, but only significantly so at 300 μM

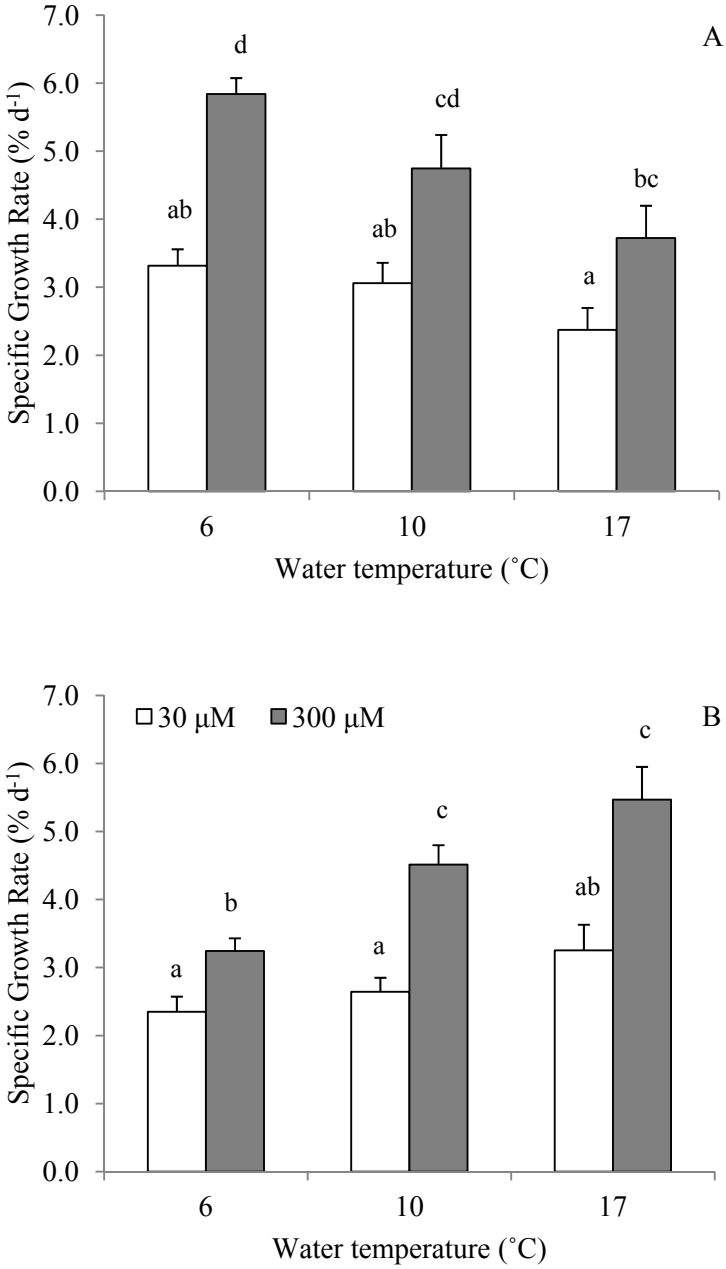

Figure 2.1 Specific growth rate on a wet-weight basis of *Palmaria palmata* (A) and *Chondrus crispus* (B) cultured at 6, 10, or 17˚C and either 30 or 300 µM nitrate. Mean (±SE) of the final three weeks of a four-week trial. Within each species, means sharing the same letter are not significantly different ($p>0.05$).

Table 2.1 Tissue carbon, nitrogen, and C:N ratio of *Palmaria palmata* and *Chondrus crispus* on dry weight basis (means ± SE, start and end of four-week experiment); within each species, values with same letter are not significantly different ($p>0.05$).

	C %		N %		C:N	
μM NO_3^-	30	300	30	300	30	300
P. palmata						
init *	38.7 ±0.47		3.8 ±0.08		10.1 ±0.10	
6˚C	40.9 ±0.28 [a]	40.5 ±0.49 [ab]	1.7 ±0.10 [a]	3.1 ±0.27 [b]	24.2 ±1.57 [a]	13.3 ±0.99 [b]
10˚C	40.8 ±0.37 [a]	40.2 ±0.23 [a]	1.5 ±0.17 [a]	3.4 ±0.14 [b]	28.2 ±2.86 [a]	12.0 ±0.54 [b]
17˚C	40.9 ±0.55 [a]	39.1 ±0.27 [b]	1.5 ±0.07 [a]	3.1 ±0.07 [b]	27.4 ±1.12 [a]	12.5 ±0.19 [b]
C. crispus						
init *	30.5 ±0.34		3.8 ±0.06		8.1 ±0.20	
6˚C	35.4 ±1.13 [ab]	33.4 ±0.13 [a]	1.7 ±0.05 [a]	4.8 ±0.11 [c]	20.8 ±0.42 [a]	7.0 ±0.13 [c]
10˚C	33.6 ±0.38 [ab]	32.6 ±0.12 [b]	1.4 ±0.10 [ab]	3.8 ±0.19 [d]	23.7 ±1.41 [ab]	8.5 ±0.41 [d]
17˚C	32.5 ±0.67 [abc]	31.7 ±0.06 [c]	1.2 ±0.05 [b]	3.8 ±0.05 [d]	26.7 ±0.61 [b]	8.3 ±0.09 [d]

* Initial sample collected from tissues acclimated at 10˚C seawater without additional nutrients for four days.

nitrate, ranging from about 35% at 6°C to 31% at 17°C (Table 2.1). Tissue N in *C. crispus*, like tissue C, was inversely related to temperature with a range from 1.2% (17°C, 30 μM nitrate) to 4.8% (6°C, 300 μM nitrate). Tissue N in *C. crispus* cultivated in 300 μM nitrate was about two-fold higher than in 30 μM nitrate. Lower C:N ratios were observed in both species at 300 μM nitrate than at 30 μM nitrate. The highest ratio (28.21) was in *P. palmata* at 10°C and 30 μM nitrate, while the lowest ratio (6.97) was in *C. crispus* at 6°C and 300 μM nitrate (Table 2.1).

Nitrogen removal rate by both species was two- to five-fold higher in 300 μM nitrate compared to 30 μM depending on temperature (Fig. 2.2). Nitrogen removal by *C. crispus* was independent of temperature, whereas in *P. palmata* it was inversely dependent on temperature (Fig. 2.2). Maximum N removal was similar in both species, around 0.5 mgN gDW^{-1} d^{-1} (Fig. 2.2).

Carbon removal by *P. palmata*, like N removal, was inversely related to water temperature with highest removal of 6.3 mg gDW^{-1} d^{-1} at 6°C and 300 μM nitrate. By contrast, carbon removal by *C. crispus* was positively related to temperature. Highest C removal by *C. crispus,* 4.4 mg gDW^{-1} d^{-1}, was at 10°C and 300 μM nitrate (Fig. 2.3).

Phosphate was not a limiting factor for either *P. palmata* or *C. crispus* in the current study. Available phosphate was not exhausted by either species at any of the treatments over three or four days between water exchanges. Phosphate uptake, as a measurement of depletion in the media, was many-fold greater at 30 μM phosphate compared to 3 μM phosphate at all temperatures for both species (data not shown).

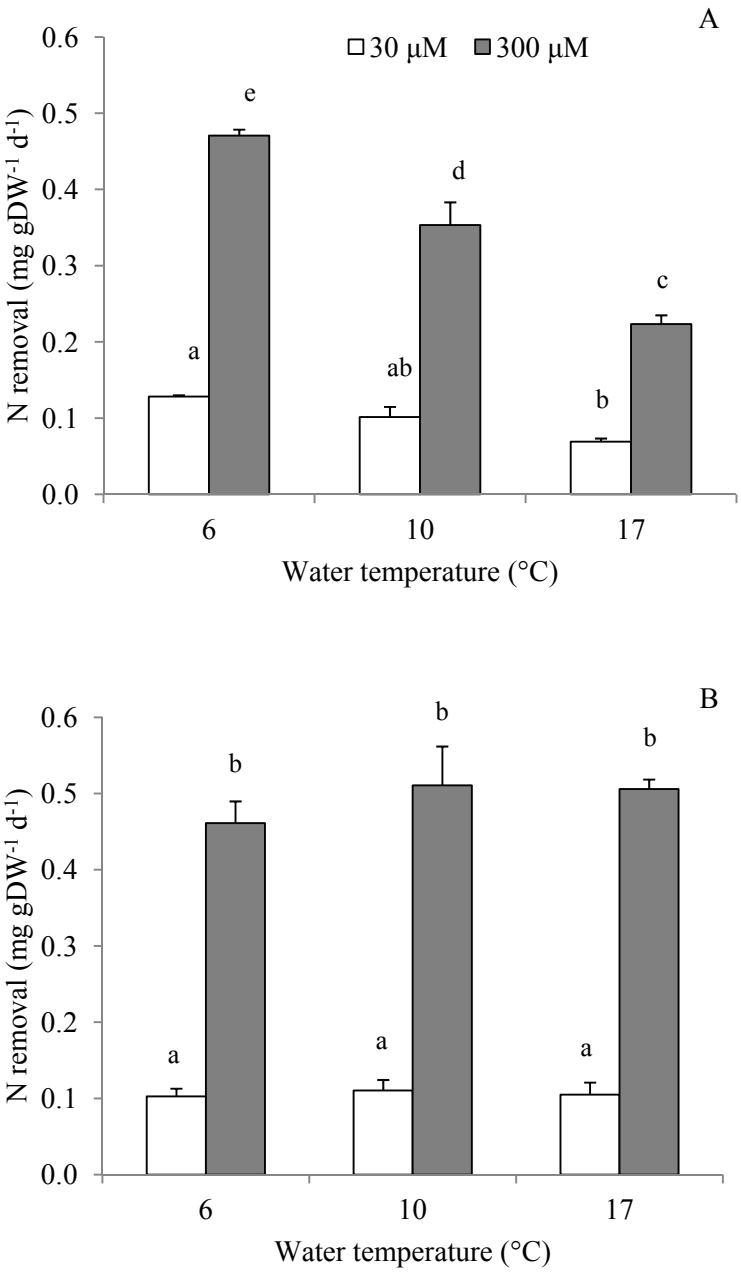

Figure 2.2 Nitrogen removal by *Palmaria palmata* (A) and *Chondrus crispus* (B) cultured at 6, 10, or 17˚C and either 30 or 300 µM nitrate (mean ± SE, n=3). Within each species, means sharing the same letter are not significantly different ($p>0.05$).

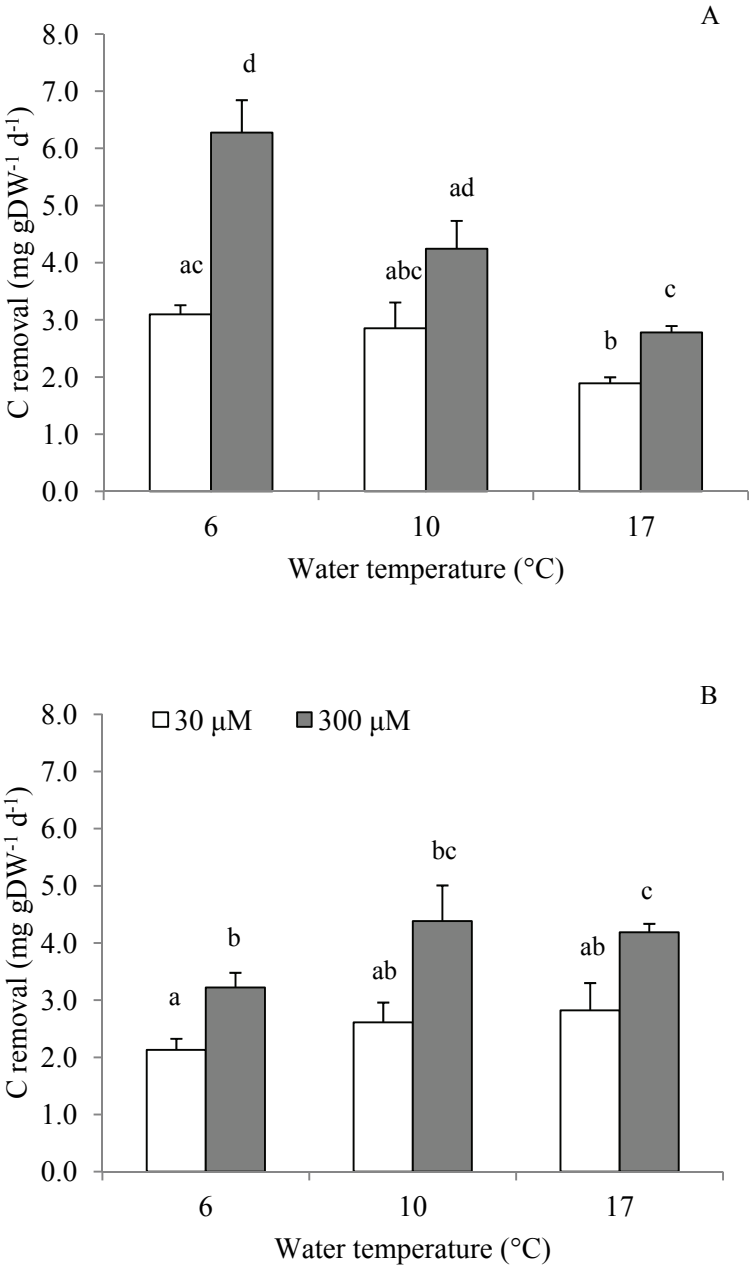

Figure 2.3 Carbon removal by *Palmaria palmata* (A) and *Chondrus crispus* (B) cultured at 6, 10, or 17˚C and 30 or 300 µM nitrate (mean ± SE, n=3). Bars with same letter are not significantly different ($p>0.05$) within that species.

Phycoerythrin at the end of the trial was higher in both species at 300 μM than at 30 μM nitrate (Fig. 4, $p<0.001$). Phycoerythrin in *P. palmata* was independent of temperature ($p > 0.05$), while interaction of temperature and nitrate concentration was significant ($p < 0.001$) for *C. crispus*. Higher phycoerythrin content occurred at 10 and 17°C and 300 μM nitrate than at 6°C and 300 μM nitrate ($p < 0.001$). Highest phycoerythrin of 28.3 mg gFW^{-1} was observed at 17°C and 300 μM nitrate for *P. palmata*. *Chondrus crispus* exhibited consistently lower phycoerythrin content than *P. palmata*, with highest phycoerythrin of 12.6 mg gFW^{-1} at 17°C and 300 μM nitrate. Phycoerythrin content in both species was highest when the tissue N contents were high ($r^2 = 0.859$ for *P. palmata*; $r^2 = 0.683$ for *C. crispus*, Fig. 2.5). At 30 μM nitrate, pigment loss occurred in both *P. palmata* and *C. crispus* at the tips of fronds where the frond is thinner while the basal part of these fronds still contained a significant amount of pigment (Fig. 2.6).

Morphological changes occurred in both species at high temperatures. In *P. palmata*, 300 μM nitrate resulted in curling of fronds at 10 and 17°C, with the same morphological change less prominent at 6°C, and 30 μM nitrate at all temperatures (Fig. 2.6). In *C. crispus* at 17°C, new blades formed around the entire perimeter of the fronds at week three of the experiment (Fig. 2.6). Development of these bladelets was more pronounced as temperature increased, and was independent of nutrient concentration.

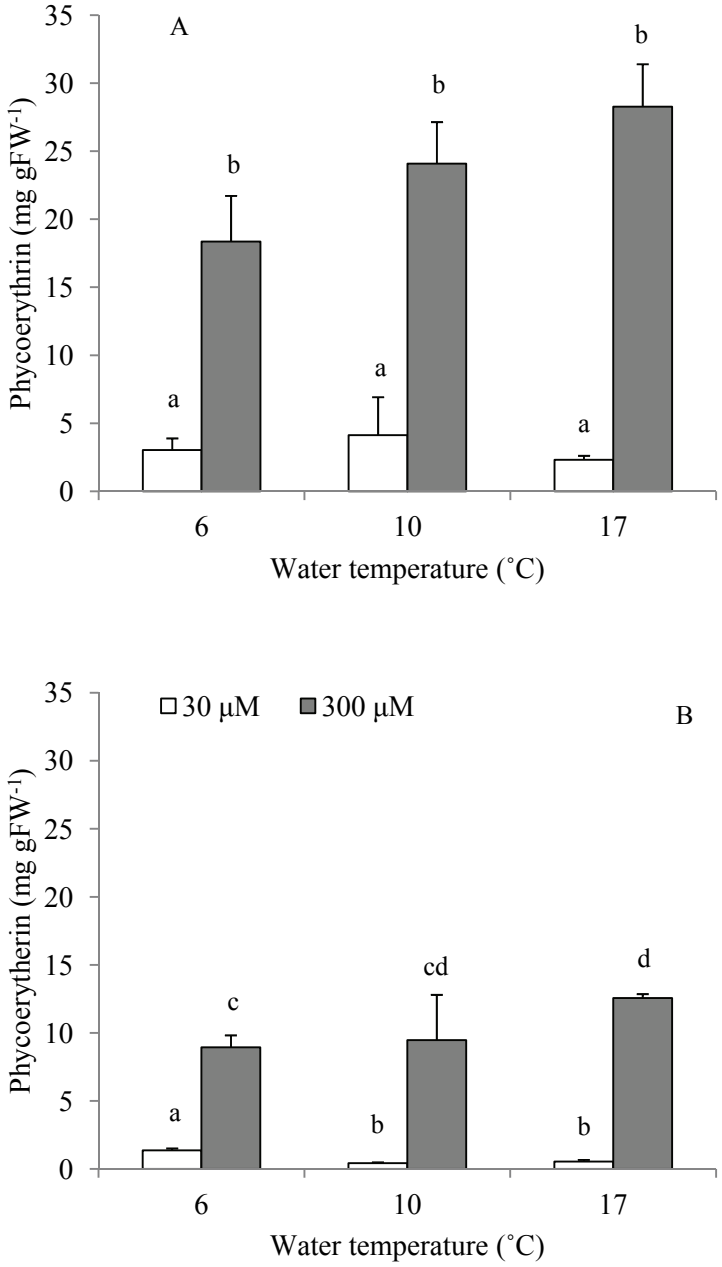

Figure 2.4 Phycoerythrin content of *Palmaria palmata* (A) and *Chondrus crispus* (B) cultured for four weeks at 6, 10, or 17˚C and 30 or 300 µM nitrate (mean ± SE). Bars with same letter are not significantly different ($p>0.05$) within that species.

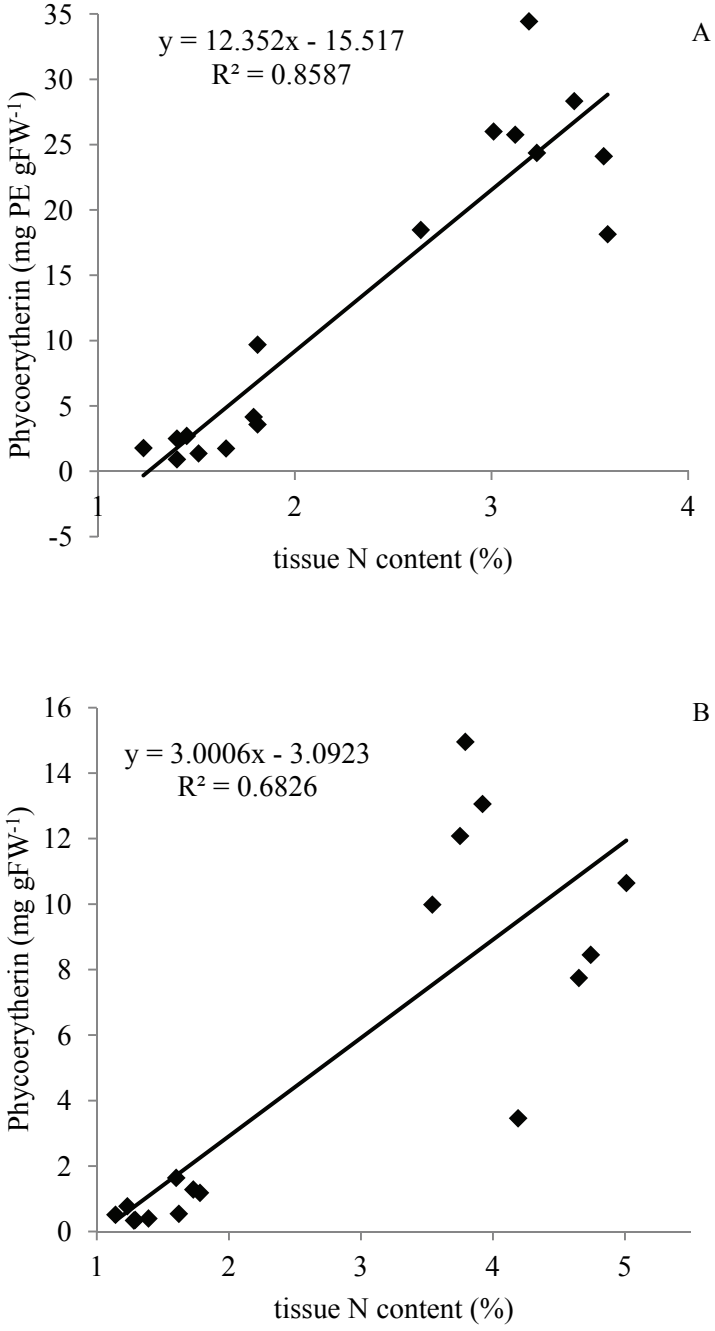

Figure 2.5 Phycoerythrin versus tissue nitrogen contents of *Palmaria palmata* (A) and *Chondrus crispus* (B).

Discussion

Nitrogen and carbon removal by *C. crispus* was largely unaffected by temperature even though specific growth rate was optimal at temperatures 10°C and higher. *Palmaria palmata* clearly favours temperatures at or below 10°C, while productivity and nutrient removal by *C. crispus* was apparently not saturated at 17°C. These species may be co-cultivated to broaden the range of environmental conditions in which an integrated system may be effective (Kim et al. 2007).

The inverse relationship between nitrogen removal and temperature for *P. palmata* is partially attributed to decrease in growth rate, despite its unchanging tissue nitrogen content. Tissue N concentrations for *P. palmata* in the present study are similar to 1.50-3.05%·DW when cultured at 2.0 mM nitrate wk^{-1} and 10°C (Morgan and Simpson 1981b). *Palmaria palmata* may store N when growth is limited by temperature (Martinez and Rico 2002). This was the case in the present study as tissue N did not decrease proportional to growth rate. However, it was not enough to mitigate the effect that slower growth rate had on N removal at 10 and 17°C.

In a study by Craigie (1990) using *C. crispus*, N uptake rate more than doubled between 5.5 and 15.5°C. Amat and Braud (1990) observed a similar effect of temperature on *C. crispus*, N uptake increasing with temperature though only substantially up to 11°C with little increase in N uptake at higher temperature. Assuming that the N taken up in these studies was either stored or used for growth, and since the current study accounted for both stored N and growth in calculation of N removal, the N removal in this study should also have increased. Basin Head *C. crispus* seems to respond differently to temperature than other strains since temperature did not affect N removal in this strain.

In *C. crispus*, the independence between nitrogen removal and temperature may be attributed to tissue nitrogen increase of 25% (relative difference) between 17 and 6°C, mitigating the effects that slower growth at 6 and 10°C would have had on nitrogen removal. Higher tissue N is associated with slower growth in many species of macroalgae (Asare et al. 1983). Tissue nitrogen in *C. crispus*, clone T4, was 5.2, 3.9, and 3.3% DW at 6, 10, and 15°C, respectively when nitrate was supplied in bi-weekly pulses of 500 μM (Simpson and Shacklock 1979). The method of estimating N removal used in the current study is a more direct measurement of the absolute N mitigation capacity of an alga compared to nutrient uptake as in Craigie (1990) and Amat and Braud (1990). This method, therefore, may be more useful for bioremediation applications where N elimination as opposed to nutrition is of greater interest.

Maximum nitrogen removal rates in both species, about 0.5 mgN gDW^{-1} d^{-1}, were within the same order of magnitude range of four species of *Porphyra* at 250 μM nitrogen (Kim et al. 2007). Nitrogen removal varied between zero in *P. leucostica* at 20°C and 25 μM ammonium to 1.3 mgN gDW^{-1} d^{-1} in *P. umbilicalis* at 10°C and 250 μM ammonium (Kim et al. 2007). This two-fold higher rate of N removal at optimum conditions for certain *Porphyra* species compared to *P. palmata* and *C. crispus* tested here is partially explained by faster growth rate (10-15% d^{-1}) associated with a different thallus anatomy (Kim et al. 2007). The *Porphyra* species have membranous thalli, one or two cell layers thick and a high surface area to volume ratio. Thallus anatomy of *P. palmata* and *C. crispus* consist of a cortex with multiple cell layers, and a medulla of spherical-shaped or thick-walled filamentous cells in *P. palmata* and *C. crispus*, respectively (Bird and McLachlan 1992). *Gracilaria vermiculophylla* removed nitrogen from an integrated turbot-seaweed system at a rate of 0.44 mgN gFW^{-1} d^{-1} (Abreu et al. 2011). On dry

weight basis, nitrogen removal of either *C. crispus* or *P. palmata* in the present study surpassed that of *G. vermiculophylla* by approximately four-fold.

Phycoerythrin content at 30 μM nitrate in the current study was similar to wild samples of *P. palmata* and *C. crispus* in 15-25 μM nitrate (Martinez and Rico 2002; Chopin et al. 1995). By contrast, in 300 μM nitrate the ten-fold higher phycoerythrin content has not been reported previously. The similar phycoerythrin content of *P. palmata* at all temperatures indicates this species can store nitrogen in the form of pigments independent of plant growth. In comparison, phycoerythrin content of *C. crispus* was lower at temperature and nitrogen sub-optimal for growth (Fig. 2.4), but tissue N increased under the same conditions (Table 2.1). Tissue N was strongly correlated to phycoerythrin content in *P. palmata*, but the same relationship was not as strong in *C. crispus*. C. crispus, then, may have a mechanism more important than phycoerytherin for the storage of N, while the primary N storage location in *P. palmata* may be phycoerythrin. This is not unlike the red alga *Gracilaria cornea* in which phycoerythrin accounted for as much as 60% of total soluble protein (Navarro-Angulo and Robledo 1999), allowing the plants to grow for seven days under low N concentrations. *Chaetomorpha linum* also stores surplus N as pigment and this N reserve is the first to be depleted when insufficient N is available in the medium (Menéndez et al. 2002). Similarly, for both *P. palmata* and *C. crispus*, pigment loss was noticeable within the first week of cultivation at 30 μM nitrate. C:N ratios >20 for both species at all temperatures and 30 μM nitrate suggest that the seaweed was nitrogen limited under these treatments (Dean and Hurd 2007).

Morphological changes similar to those observed in this study at 300 μM nitrate have been observed in *P. palmata* when grown in effluent of Atlantic halibut aquaculture (P. Corey personal observation). This may be a consequence of unnaturally high nutrient availability to which the plants are not adapted. If the change of morphology was simply a curling effect, self-shading may increase, thus reducing the surface area available for photosynthesis without increasing nutrient uptake, since thallus surface area remains unaffected. Fronds of *Iridaea cordata*, a Rhodophyte with similar morphology to that of *P. palmata*, exhibited this same curling morphology when exposed to UVB radiation (Navarro et al. 2010). If the morphology change is a ruffling effect, then the surface area available for nutrient uptake may be increased. *Laminaria longicruris* was more productive in a sheltered locality where it had frilled morphology compared to plants in an exposed area (Gerard and Mann 1979). This provided them with a larger surface area for nutrient uptake and photosynthesis than the plants at the exposed locality.

Morphological effects of temperature are typical in *C. crispus*. Plants cultured at higher temperature (15-20°C) are more compact, with thinner fronds and apices than plants cultured at sub-optimal temperatures (Simpson and Shacklock 1979). The T4 strain of the afore-mentioned study is morphologically distinct from the Basin Head strain of *C. crispus* used in our investigation (Chopin et al. 1999b). The greater frond complexity observed in *C. crispu*s at 20°C has been suggested to improve nutrient uptake capacity by increasing the surface area (Kübler and Dudgeon 1996). Similarly, Basin Head *C. crispus* has increased surface area that could increase productivity (McCurdy 1980). A similar effect may be accomplished by formation of marginal bladelets as in the current study.

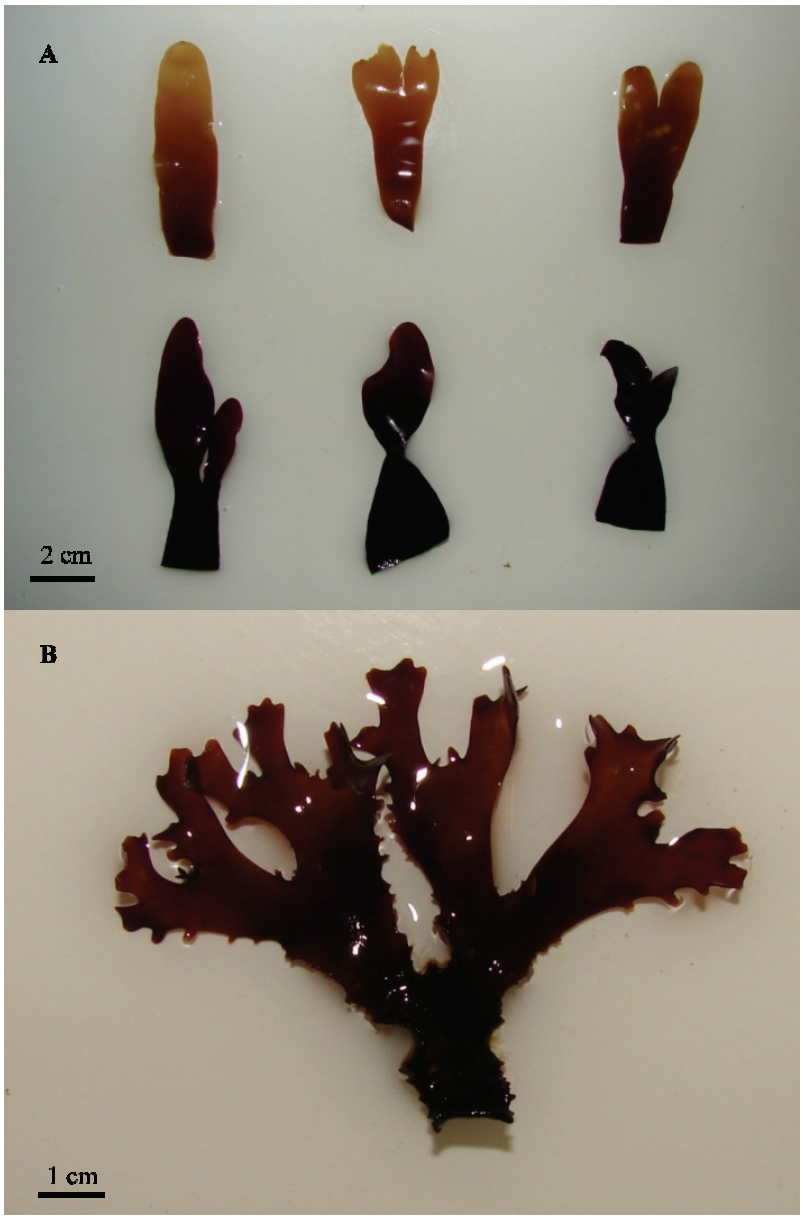

Figure 2.6 (A) *Palmaria palmata* cultured at 6, 10, or 17˚C (left to right, respectively) and 30 or 300 µM nitrate (top and bottom, respectively) on day 21 of a 28-day culture experiment; (B) *Chondrus crispus* cultured at 17˚C and 300 µM nitrate, day 19 of a 28-day growth experiment.

Optimal temperature for growth of *P. palmata* is between 6 and 10°C, but the species will grow well between 6 and 15°C (Morgan et al. 1980a). In the current study, growth was apparently compromised at temperatures exceeding 10°C. Point Prim in the Bay of Fundy has water temperatures that rarely exceed 12°C, and one would expect the species from here to be cold-adapted. For *C. crispus*, estimates of the optimal temperature for growth vary considerably from 10-15°C (Fortes and Lüning 1980; Laycock et al. 1981), to 15-20°C (Simpson and Shacklock 1979), to 6-18°C (Bidwell et al. 1985). These differences may be based on physiological adaptation to the local environmental conditions from which each strain originated (Troell et al. 2009). Basin Head lagoon is regularly exposed to temperatures > 25°C during July-August (Chopin et al. 1999b), whereas plants from outside of this habitat would rarely be exposed to seawater > 20°C. This may explain why the *C. crispus* reared in the present study grew best at 17°C. Hence, one would expect this strain of *Chondrus* to be adapted for growth at high temperatures.

The growth profile and nutrient uptake capacity of *C. crispus*, with respect to temperature, corresponds better than *P. palmata* to the growth profile of Atlantic halibut, which is optimal between 10 and 14°C (Hallaråker et al. 1995; Jonassen et al. 2000). *P. palmata* may be the more suitable species at temperatures ≤10°C. Nevertheless, inclusion of *P. palmata* could represent greater economic return since it commands a higher market price than *C. crispus*. Viability of seaweed-finfish integration may be accomplished by integration of higher value seaweeds at the expense of reduced nutrient removal (Matos et al. 2006).

Bioremediation by *P. palmata* and *C. crispus* in finfish aquaculture may be accomplished best by complementary use of both species. Overlap of productive temperature ranges and morphological effects of high nutrient environments may be exploited to further increase nutrient removal efficiency by these species within an integrated aquaculture system. Results reported here are first steps in evaluation of these two red macroalgae for integration with land-based Atlantic halibut aquaculture. *In situ* experiments at a land based Atlantic halibut farm using halibut effluent and ambient sunlight will be needed to see if *C. crispus* and *P. palmata* can be used in a commercial scale, integrated multi-trophic aquaculture system.

Chapter 3: Nitrogen Uptake and Nutrient Removal by *Palmaria palmata* and *Chondrus crispus* in Ammonium and Nitrate Fertilization

Abstract

The ability of a seaweed to take up nitrogen as ammonium or nitrate will determine the best location for a seaweed biofilter within a water treatment system. *Palmaria palmata* and *Chondrus crispus* were cultivated for four weeks at 10°C and 300/0, 0/300, 150/150, 270/30, or 30/270 μM NO_3^- / NH_4^+ for a total nitrogen (N) concentration of 300 μM. Stocking density was 2.0 g L^{-1}, irradiance 125 μmol photons m^{-2} s^{-1}, photoperiod 16:8 (L:D), and molar nitrogen:phosphorus ratio of 10:1. Growth during the final week of the experiment was not affected by nitrogen source in either of the species, with mean specific growth rate of 4.3 and 3.7% d^{-1} for *P. palmata* and *C. crispus*, respectively. For both species, total nitrogen uptake was lowest at 300 μM NO_3^-, 1.6 mgN gDW^{-1} d^{-1} for *P. palmata* and 3.4 mgN gDW^{-1} d^{-1} for *C. crispus*. Over a 24-hour period, 23-37% of the available nitrate and 91-100% of available ammonium were taken up by *P. palmata*. In the same period, *C. crispus* took up 55-87% of available nitrate and 89-100% of ammonium. Tissue N in *P. palmata* was highest at 270 and 300 μM NH_4^+, 4.1%, while nitrogen source did not have a significant effect on tissue N of *C. crispus* with mean of 4.6%. For greatest bioremediation benefit, a seaweed biofilter using these species should be placed prior to bacterial biofiltration for exposure to greater proportions of ammonium than nitrate.

Introduction

In recirculating aquaculture systems, the return of nutrient-rich water to the fish can have detrimental impacts on their growth and performance. The waste nitrogen of an untreated aquaculture system consists primarily of ammonium/ammonia (Timmons et al. 2002), un-ionized ammonia (NH_3) being the more toxic to finfish at concentrations below 0.05 $mg \cdot L^{-1}$ (Person-Le Ruyet et al. 1997; Foss et al. 2009). Bacterial biofilters are the most common method of NH_3 treatment, converting harmful NH_3 to less harmful nitrate (NO_3^-) (Timmons et al. 2002). The fluidized-sand-bed biofilter, in particular, is energy-intensive, is a net oxygen consumer, and provides no usable secondary by-product. Seaweed cultivation, on the other hand, is a net oxygen producer during daylight hours and may provide marketable biomass to mitigate production costs.

The most important sources of nitrogen for seaweeds are ammonium (NH_4^+), NO_3^-, and, to a lesser extent, nitrite (NO_2^-) (Lobban and Harrison 1996). Ammonium is thought to be superior to the others since it can be directly assimilated into amino acids while nitrate must be reduced prior to amino acid construction (Barsanti and Bualtieri 2006). Known studies in nitrate and ammonium uptake by *P. palmata* and *C. crispus* to date have made use of single-nitrogen-source media—i.e. experiments were conducted under either NO_3^--absent or NH_4^+-absent conditions. Morgan and Simpson (1981b) fertilized *P. palmata* cultures at a rate of 2.0 mM nitrogen per week as either nitrate or ammonium and found that growth of this species was supported more by nitrate than ammonium. In another study using NH_4^+, NO_2^-, and NO_3^- separately, *P. palmata* had similar affinity for both ammonium and nitrate (Martinez and Rico 2004). For *C. crispus*, growth is similar using either ammonium or nitrate as the nitrogen source (Neish and Shacklock 1971). Matos et al. (2006) cultivated the same species in effluent of an

integrated finfish culture system, but did not include concentrations of nitrate in analysis of growth and nitrogen uptake.

Demetropoulos and Langdon (2004a) used a combined-nitrogen medium of ammonium and nitrate in culture of *Palmaria mollis*. Growth was not significantly different when compared to seaweed cultured using nitrate only. However, in the same study, growth of this species was significantly reduced when ammonium was used as the only nitrogen source. *Palmaria palmata* has also been identified as an ammonium-sensitive species for its lesser tolerance of ammonium compared to nitrate (Morgan and Simpson 1981b).

The location of seaweed "biofilters" in relation to bacterial biofilters within a recirculating aquaculture system depends very much on the species of seaweed selected for bioremediation and the nitrogen uptake and growth characteristics of those species within dynamic nitrogen conditions. The objective of this study is to evaluate the nutrient uptake and growth responses of two red macroalgae species within mixed-nitrogen-source media and the application of those species to land-based, integrated multi-trophic aquaculture.

Materials and Methods

Palmaria palmata was collected in June 2009 from natural populations in the low-intertidal zone of the Bay of Fundy, at Point Prim, Digby County, Nova Scotia (44°69'15.7"N, 65°78'5.4"W). The Basin Head strain of *Chondrus crispus* was obtained from the Sandy Cove Aquaculture Research Station of the National Research Council, Halifax, Nova Scotia, June 2009. Both species were stocked to culturing units at the land-based Atlantic halibut nursery site of Scotian Halibut Ltd., Wood's Harbour, Nova

Scotia (43°55'80.6"N, 65°73'61.1"W) and grown under recirculating aquaculture seawater conditions until used for experiments.

The experiment used *P. palmata* in late-February to March 2010, and the experiment was repeated with *C. crispus* in March 2011. Prior to both experiments, algal materials were held in effluent of Atlantic halibut aquaculture. Since the experiments were conducted in similar seasons, both species were exposed to prolonged temperatures of 2° to 4°C prior to experimentation.

Palmaria palmata and *C. crispus* were acclimated at 10°C in filtered (0.3 μm) and UV-sterilized, natural seawater from Ketch Harbour, Nova Scotia, without additional nutrients, exchanged twice daily for three days prior to start of the experiment. Post-acclimation tissue samples were collected in triplicate from each of the two species for biochemical analysis. Following acclimation, 4-6 cm long blades were cut from the apical ends of both *P. palmata* and *C. crispus* fronds (Pang and Lüning 2004). Seven to twelve blades were stocked in each flask to a total biomass of 2.0 g L^{-1}.

A four-week experiment was conducted in 1 L Florence flasks under saturating light intensities (125 μmol photons m^{-2} s^{-1}; Fortes and Lüning 1980; Martinez and Rico 2004) at the Aquaculture Centre of Nova Scotia Agricultural College. Light was supplied by 32 W, 5000 K white T8 fluorescent lamps (Philips, Somerset, NJ). Irradiance was measured using a light meter (Li-Cor Li-1400 with Li-193SA spherical sensor, Lincoln, NE). Photoperiod was 16:8 (L:D, Sagert and Schubert 2000). Temperature was maintained at 10°C by continuous-flow water bath. The culture medium was exchanged twice weekly using von Stosch's medium (Guiry and Cunningham 1984) without nitrogen (N) and phosphorous (P). N and P levels were controlled by addition of nitrate

($NaNO_3$) and/or ammonium (NH_4Cl) and phosphate ($NaH_2PO_4 \cdot 2H_2O$) to the culture medium at a molar N:P ratio of 10:1 to ensure that phosphate was not limiting (Shacklock and Craigie 1986). Algal biomass was kept in tumbling suspension by continuous aeration.

Experimental design The experiment incorporated a completely randomized block design with five levels of nitrogen treatment (Table 3.1) and six replicates for each treatment. Control flasks—i.e. without seaweed—for treatments containing ammonium were maintained for the duration of the experiment with *P. palmata* and for the first two water exchanges (one week) of the experiment involving *C. crispus*. The control flasks enabled measurement of possible ammonium loss by volatilization. One flask for each treatment was held in each of six temperature-controlled water baths (tanks). The nitrogen concentrations of the culture media (300 μM in total) reflect halibut recirculating aquaculture total nitrogen levels (Scotian Halibut Ltd. unpubl. data). The experiment was conducted at the Aquaculture Centre of the Nova Scotia Agricultural College, Truro, Nova Scotia.

Table 3.1 Nutrient treatments offered to *Palmaria palmata* and *Chondrus crispus* for four weeks.

	Nutrient concentration (μM)		
Treatment	NO_3^-	NH_4^+	PO_4^{3+}
1	300	0	30
2	0	300	30
3	150	150	30
4	270	30	30
5	30	270	30

Biomass in each flask was weighed (fresh weight, FW) at weekly intervals. FW was obtained after blotting the thalli dry with paper towel and weighing within 2 min. Seaweed biomass in each flask was reduced to initial stocking density (2.0 g L^{-1}) at each weighing, and samples were taken for dry weight (DW) and tissue analysis. DW and moisture content were determined by drying tissue samples to constant weight at 60°C. Specific growth rate (SGR, expressed as % day^{-1}) was calculated weekly using Equation 2.1.

Water samples were collected at each medium exchange, semi-weekly for four weeks. Nitrate, ammonium, and phosphate uptake was calculated as the difference between initial and final concentrations of these nutrients in the media. This is different from nitrogen and carbon removal described in Chapter 2 (p. 13), where mitigation potential is a measure of tissue nutrient concentration and excess biomass. Nitrate concentration was determined by nitrite cadmium reduction modified from Jones (1984). The method of Liddicoat et al. (1975) was followed for analysis of ammonium concentration, and phosphate concentration was determined following Parsons et al. (1984). At each water exchange, culture flasks were rinsed with a 10% HCl solution to eliminate secondary algal growth, presence of nitrifying bacteria, and possible nutrient contamination.

In weeks three and four of each experiment following medium exchange around 6:00 pm, water samples were collected at 12-hour intervals over a 24-hour period (6:00 am and 6:00 pm next day). In effect, three water samples were obtained for each of these periods.

Dried tissues were ground to powder with an MM301 ball mill (Retsch, Newtown, PA) and then analyzed for total tissue N and carbon (C) using a CNS-1000 Carbon,

Sulphur and Nitrogen Analyzer (Leco, St. Joseph, MI). N removal was calculated using equation 2.2 (Kim et al. 2007). The same calculation was used for C removal, substituting *Tissue C* for *Tissue N*. N and C removal were calculated for the final week of the experiment only, because there was inadequate dry matter from the other sampling dates for complete tissue analysis.

Statistical analysis Data were analyzed using a two-way ANOVA model, using nitrogen combination for treatment effect and tank as the blocking factor. Where significant treatment effects were indicated by ANOVA, Tukey's HSD analysis ($p = 0.05$) was used as a post hoc test to determine pairwise relationships between treatment means. For the ammonium control treatment, initial and final ammonium concentrations were compared by two-sample t-test. Statistical analyses were carried out using SAS statistical software (SAS Institute Inc., Cary, NC, USA) and Minitab 15 (Minitab Inc., State College, PA, USA).

Results

Palmaria palmata

Nitrogen source had a significant effect on specific growth rate ($P < 0.0001$, $F = 7.64$) in the first week of the experiment, growth being highest at conditions of 150 - 300 μM nitrate. Growth rate was not significantly different ($P > 0.05$) between treatments during subsequent weeks. For all treatments, growth rate increased until week two, to a maximum of 8.9% d^{-1} (mean of all treatments). Growth rate decreased to an average of 4.3% d^{-1} during week four (Fig. 3.1).

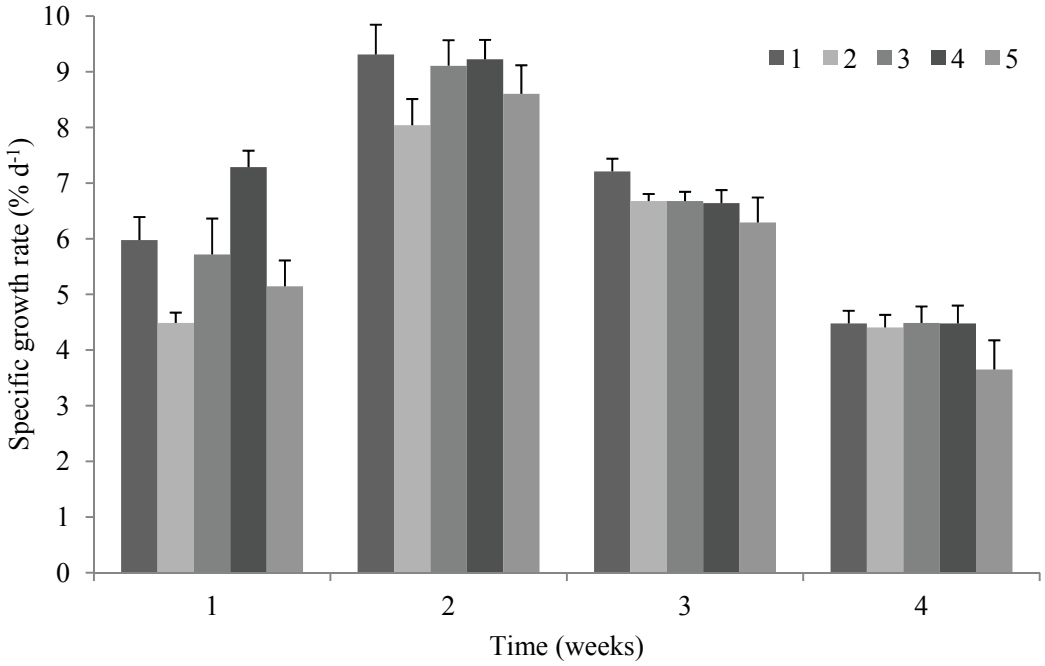

Figure 3.1 Specific growth rate (SGR) of *Palmaria palmata* in 1 L culture flasks at 1. 300 μM NO_3^-/0 μM NH_4^+, 2. 0 μM NO_3^-/300 μM NH_4^+, 3. 150 μM NO_3^-/150 μM NH_4^+, 4. 270 μM NO_3^-/30 μM NH_4^+, or 5. 30 μM NO_3^-/270 μM NH_4^+, over a four-week culture experiment (mean ±SE, n=6).

Nitrogen uptake data is reported for the 24-hour sampling periods following medium change in week four. Twenty-three to thirty-seven percent of nitrate was taken up by the plants, while 91-100% of ammonium was taken up from the media (Fig. 3.2). When uptake of nitrate- and ammonium-nitrogen were compared at similar concentrations of each nutrient, ammonium was taken up 1.5 to 7 times faster than nitrate (Fig. 3.3). Ammonium uptake was highest at 0 μM NO^{3-}/300 μM NH_4^+ with a value of 12.0 μmol NH_4^+ gDW^{-1} hr^{-1}. Highest nitrate uptake during the same period was 4.4 μmol NO_3^- gDW^{-1} hr^{-1} at 270 μM NO^{3-}/30 μM NH_4^+.

Total nitrogen uptake was highest in flasks enriched with ammonium ($P < 0.001$, $F = 20.39$) and was directly related to the concentration of ammonium available to the plants (Fig. 3.4). Highest uptake rate during this sampling period was a mean 4.5 mgN gDW^{-1} d^{-1} for seaweed cultured at 0 μM nitrate/300 μM ammonium and 30 μM nitrate/270 μM ammonium. The lowest nitrogen uptake of 1.6 mgN gDW^{-1} d^{-1} occurred at 300 μM nitrate. The changes in NH_4^+ in all control flasks (without seaweed) were not significant. Therefore, it is assumed that NH_4^+ uptake in all treatments was attributed to *P. palmata*.

Samples from the ammonium control treatments were analyzed for each week of the experiment. Only two of the final ammonium concentrations differed from the initial concentration after four days of continuous aeration ($P < 0.001$), though the difference was shown as an increase from initial to final ammonium concentration on both occasions (Appendix VII). It is believed that these were ambiguous results, since all other samples from ammonium control flasks did not differ between initial and final concentrations ($P > 0.05$).

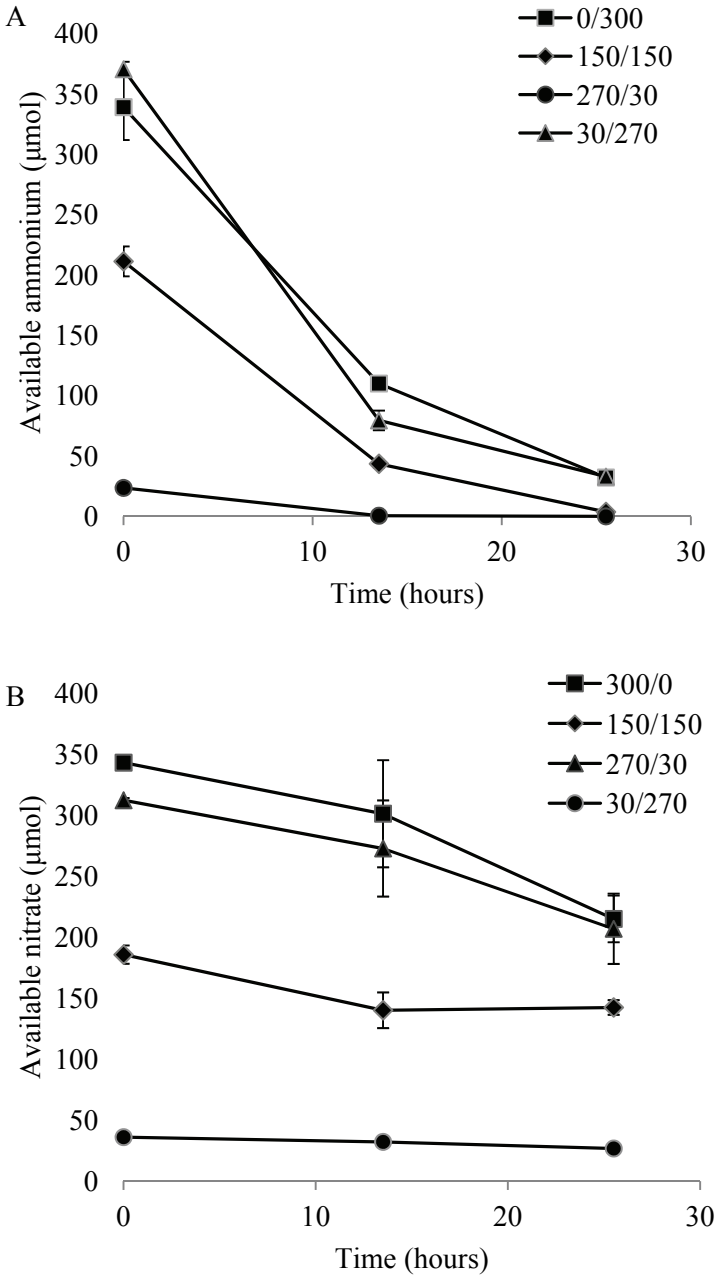

Figure 3.2 Depletion of ammonium (A) and nitrate (B) by 2 g FW *Palmaria palmata* in 1 L culture flasks at various combinations of nitrogen media (NO_3^- /NH_4^+). Water samples were collected over a 24-hour sampling period in week four of a four-week culture experiment (mean ±SE).

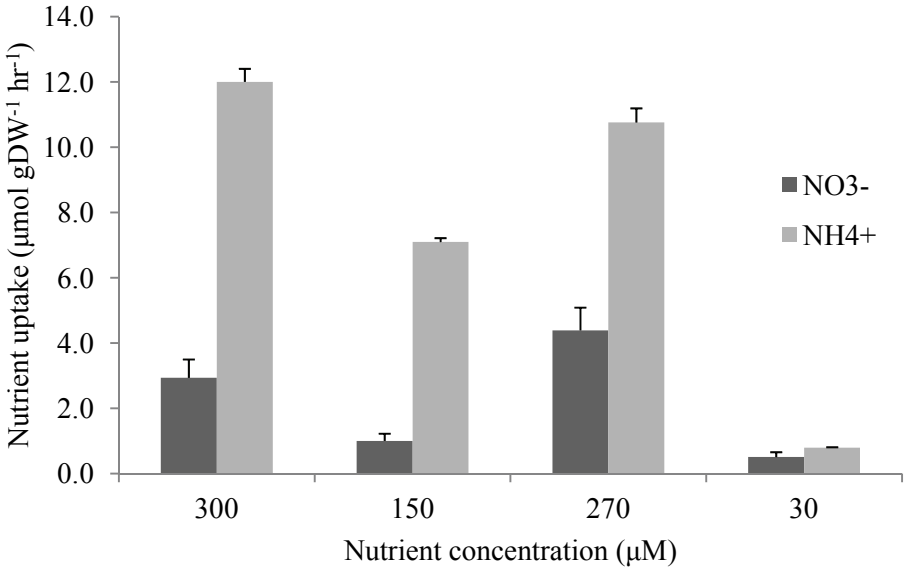

Figure 3.3 Nutrient uptake by *Palmaria palmata* over a 24-hour sampling period. Total nitrogen in media of all treatments was 300 µM as nitrate and/or ammonium. Mean of measurements from weeks three and four of a four-week culture experiment (mean ±SE).

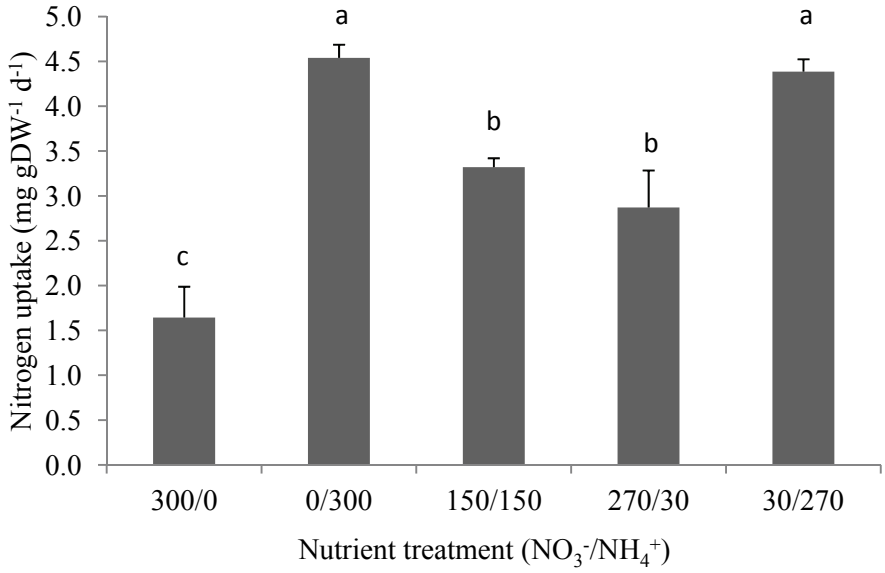

Figure 3.4 Nitrogen uptake by *Palmaria palmata* at various combinations of nitrogen media over a 24-hour sampling period. Mean of measurements from weeks three and four of a four-week culture experiment (mean ±SE; means sharing same letter are not significantly different, $p < 0.05$).

Nitrogen source in the media did not have a significant effect on tissue carbon in *Palmaria palmata* (P = 0.967, F = 0.14), though tissue carbon increased by 14.3% compared to initial samples. Tissue nitrogen was significantly affected by nitrogen source (P = 0.000, F = 18.13). Tissue N was highest in the seaweed cultured in media containing high levels (270 and 300 μM) of ammonium (Table 3.2), with tissue N of 4.1%. Fronds cultured at 30 μM and 150 μM NH_4^+ were not significantly different from one another with mean tissue N of 3.2%. This is a reduction of 28.2% from initial tissue N of 4.4%. Since tissue C was not different between treatments, C:N ratio was significantly different. Mean C:N ratio of fronds cultured at high levels of ammonium was 9.2, while that of the other treatments was 11.61.

Similar to tissue N, nitrogen removal was highest at 300 μM ammonium media concentration (P = 0.006, F = 5.05) and lowest in treatments containing higher levels of nitrate (150, 270, and 300 μM; Fig. 3.5.A). However, nitrogen removal at 270 μM ammonium was not significantly different from any of the other treatments. Nitrogen removal at 0 μM NO_3^-/300 μM NH_4^+ was 0.55 mg gDW^{-1} d^{-1}. Nitrogen removal was not significantly different in treatments containing nitrate, with mean removal of 0.46 mg gDW^{-1} d^{-1}.

Despite the fact that growth rate and tissue carbon were not significantly different in week four of the experiment, source of nitrogen had a significant effect on carbon removal by *P. palmata* (P = 0.014, F = 4.11). Highest carbon removal was 5.6 mg gDW^{-1} d^{-1} at 300 μM NO_3^-/0 μM NH_4^+ and lowest carbon removal occurred at 30 μM NO_3^-/270 μM NH_4^+ with a value of 4.5 mg gDW^{-1} d^{-1} (Fig. 3.5.B).

Table 3.2 Tissue carbon and nitrogen and C:N ratio of *Palmaria palmata* on dry weight basis (means ± SE, week four of four-week experiment; values with same letter are not significantly different ($p>0.05$) within that species).

Culture media		C (%)	N (%)		C:N	
NO_3^- (µm)	NH_4^+ (µm)	Mean	Mean		Mean	
initial *		31.0 ±1.23	4.4 ±0.36		7.3 ±0.69	
300	0	36.6 ±0.41	3.1 ±0.11	a	11.9 ±0.35	a
0	300	36.8 ±0.40	4.1 ±0.09	b	9.0 ±0.23	b
150	150	36.6 ±0.39	3.3 ±0.10	a	11.1 ±0.33	a
270	30	37.0 ±0.38	3.1 ±0.08	a	11.8 ±0.24	a
30	270	36.9 ±0.62	4.0 ±0.16	b	9.3 ±0.37	b

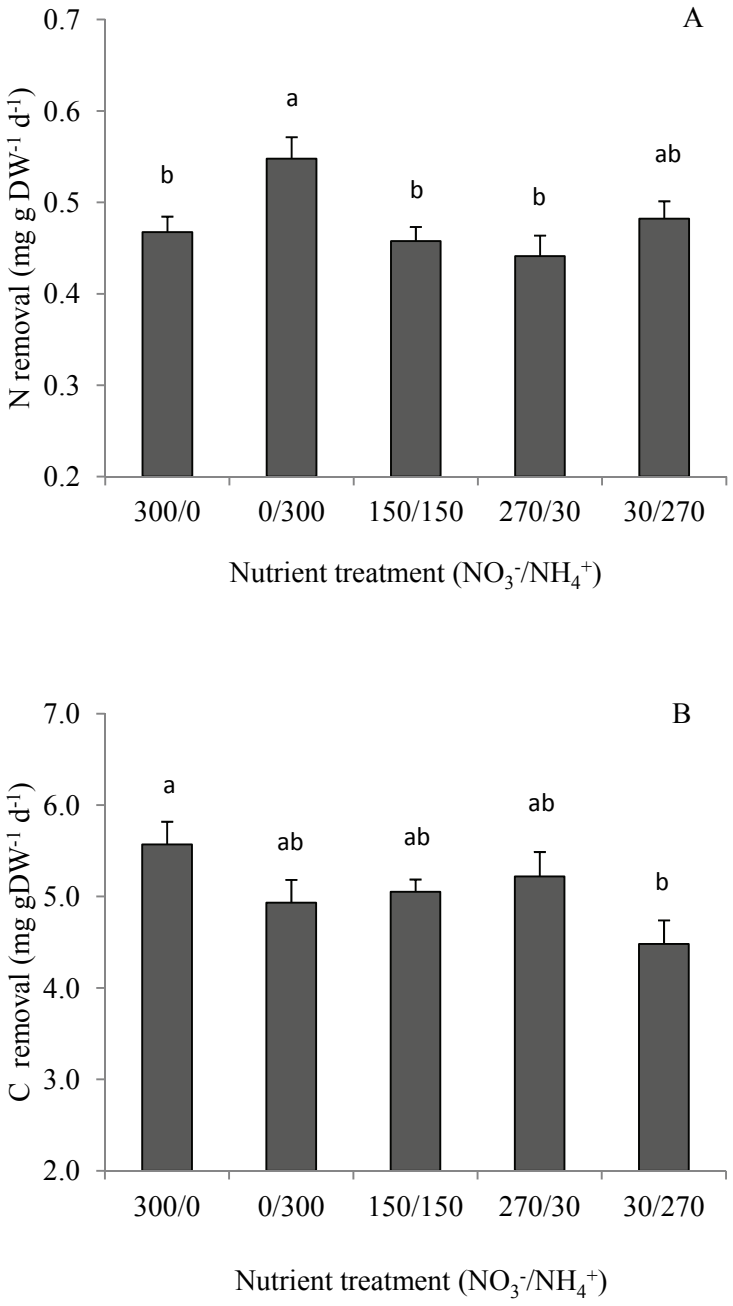

Figure 3.5 Nitrogen removal (A) and carbon removal (B) as a function of tissue nutrient concentration and productivity by *Palmaria palmata* at various combinations of nitrogen media (mean ±SE; means with same letter are not significantly different, $p < 0.05$).

Total phosphate available in the media was not exhausted after four days in weeks three and four of the experiment (Appendix VI; water samples from weeks one and two were not analyzed for phosphate concentration), with 93% and 92% uptake, respectively. Uptake of phosphate was not significantly different during week three ($p = 0.657$, F = 0.61) or week four ($p = 0.782$, F = 0.44) with mean uptake of 0.73 and 0.75 mg gDW^{-1} d^{-1}, respectively (Appendix V).

Chondrus crispus

Nitrogen source did not have a significant effect on specific growth rate ($p = 0.401$, F = 1.02) at any point of the four-week experiment. For all treatments, growth rate increased until week two, to a maximum of 6.0% d^{-1} (mean of all treatments). Growth rate decreased to an average of 3.7% d^{-1} during week four (Fig. 3.6). Growth rate of *P. palmata* was generally greater than that of *C. crispus* at all experimental time-points.

Like *P. palmata*, nitrogen uptake data is reported for the 24-hour sampling periods following medium change in week four. Eighty-nine to one-hundred per cent of ammonium was taken up by the plants, while 55-87% of nitrate was taken up from the media (Fig. 3.7). When uptake of nitrate- and ammonium-nitrogen were compared at similar concentrations of each nutrient, ammonium was taken up 0.7 to 1.8 times faster than nitrate (Fig. 3.8). Ammonium uptake was similar at treatments 2 and 5 with a mean value of 11.9 μmol NH_4^+ gDW^{-1} hr^{-1}. Nitrate uptake was similar at 300 μM NO^{3-}/0 μM NH_4^+, 150 μM NO^{3-}/150 μM NH_4^+, and 270 μM NO^{3-}/30 μM NH_4^+ with a mean uptake rate of 6.6 μmol NO_3^- gDW^{-1} hr^{-1}. There was only a marginal difference in uptake rate of

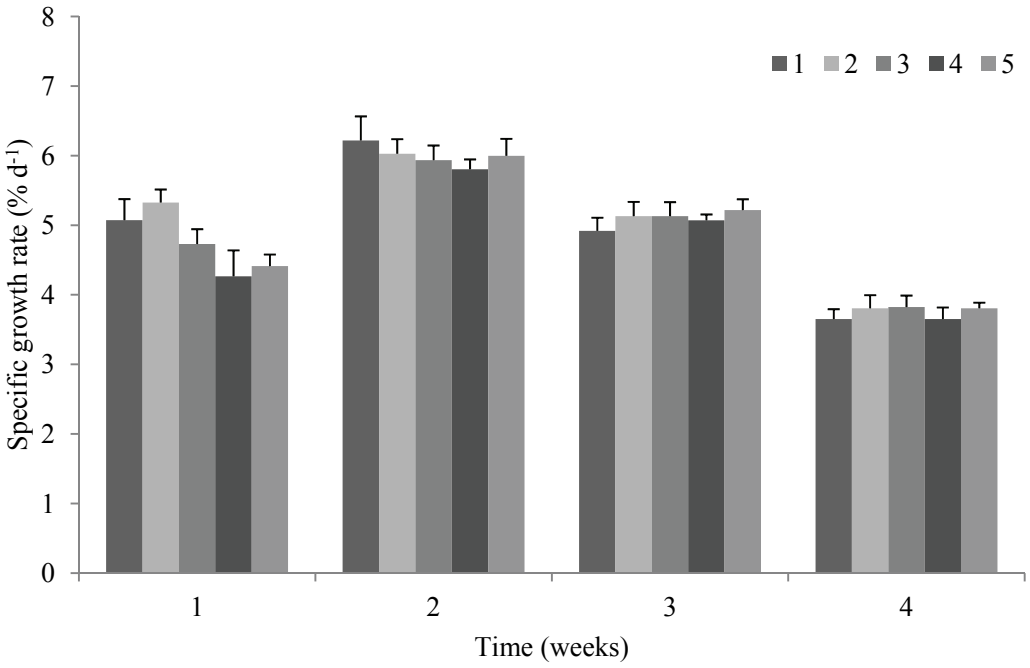

Figure 3.6 Specific growth rate (SGR) of *Chondrus crispus* in 1 L culture flasks at 1. 300 μM NO_3^-/0 μM NH_4^+, 2. 0 μM NO_3^-/300 μM NH_4^+, 3. 150 μM NO_3^-/150 μM NH_4^+, 4. 270 μM NO_3^-/30 μM NH_4^+, or 5. 30 μM NO_3^-/270 μM NH_4^+, over a four-week culture experiment (mean ±SE, n=6).

ammonium and nitrate when they were available to the plants at 30 μM, in combination with 270 μM of the alternate nitrogen source (Fig. 3.8).

Total N uptake was highest in the treatment enriched with equal amounts of ammonium and nitrate ($P < 0.001$, $F = 10.26$), though uptake at this treatment was not significantly different from 270 μM NO^{3-}/30 μM NH_4^+ and 0 μM NO^{3-}/300 μM NH_4^+ (Fig. 3.9). Mean nitrogen uptake rate for these three treatments was 4.9 mg N $gDW^{-1}d^{-1}$. Nitrogen uptake rate at 0 μM NO^{3-}/300 μM NH_4^+, 270 μM NO^{3-}/30 μM NH_4^+, and 30 μM NO^{3-}/270 μM NH_4^+ were also not significantly different, but at 300 μM NO^{3-}/0 μM NH_4^+, nitrogen uptake was significantly lower than all other treatments at a rate of 3.4 mg N gDW^{-1} d^{-1}.

Samples from the ammonium control treatments for each of the two water exchanges in week one were analyzed. Final concentrations of ammonium were not different from initial concentrations at 0 μM NO^{3-}/300 μM NH_4^+ and 30 μM NO^{3-}/270 μM NH_4^+ during either time period ($P > 0.05$, Appendix VII). During both time periods, final ammonium concentration was significantly higher than initial concentration at 150 μM NO^{3-}/150 μM NH_4^+ and 270 μM NO^{3-}/30 μM NH_4^+ ($P < 0.05$).

Tissue nitrogen was not significantly affected by nitrogen source in *Chondrus crispus* ($P = 0.228$, $F = 1.54$). Nitrogen source did have a significant effect on tissue carbon, but the difference between treatments was only marginal, ranging from 31.3% at 270 μM NO^{3-}/30 μM NH_4^+ to 32.0% at 300 μM NO^{3-}/0 μM NH_4^+ (Table 3.3) and no apparent relationship with nitrogen source. Initial and final tissue carbon was not significantly different for any of the treatments. Overall mean tissue N was 4.6%, a

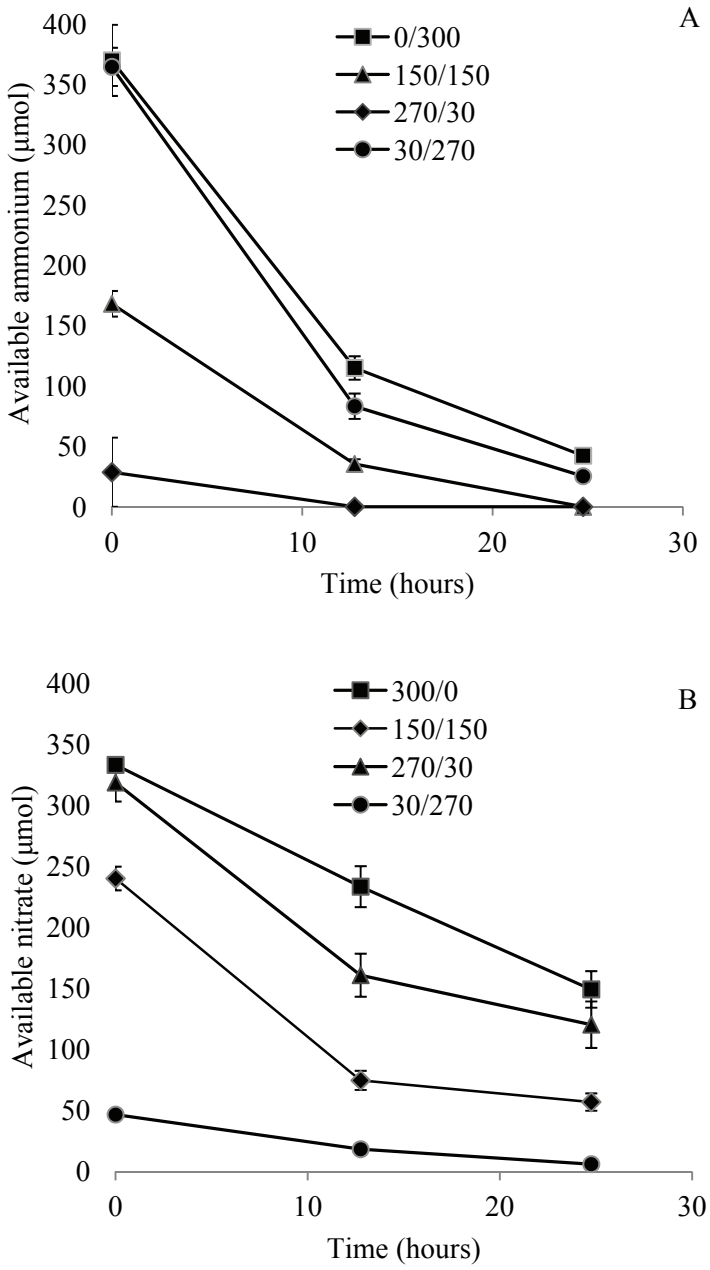

Figure 3.7 Depletion of ammonium (A) and nitrate (B) by 2 g FW *Chondrus crispus* in 1 L culture flasks at five combinations of nitrogen media (NO_3^-/NH_4^+). Water samples were collected over a 24-hour sampling period in week four of a four-week culture experiment (mean ±SE).

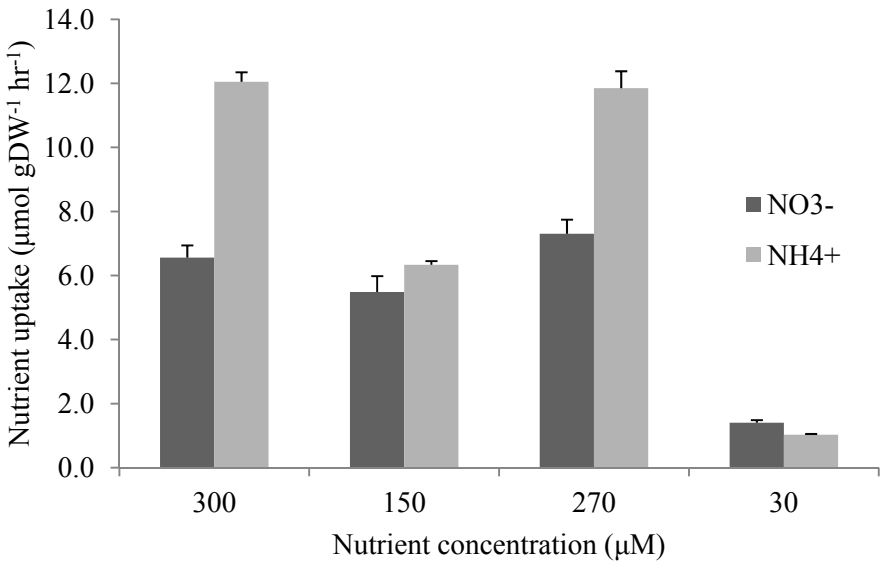

Figure 3.8 Nutrient uptake by *Chondrus crispus* over a 24-hour sampling period. Total nitrogen in media of all treatments was 300 µM as nitrate and/or ammonium. Mean of measurements from weeks three and four of a four-week culture experiment (mean ±SE).

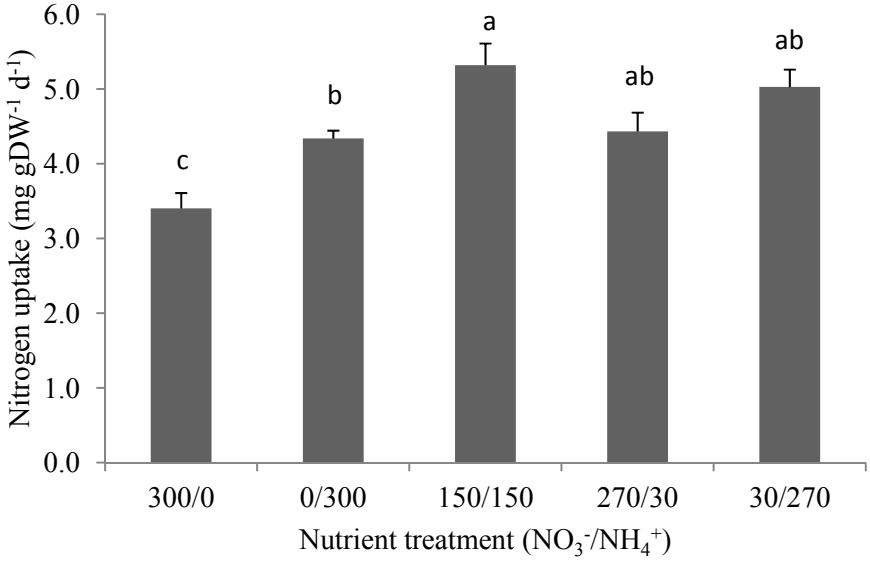

Figure 3.9 Nitrogen uptake by *Chondrus crispus* at five combinations of nitrogen media over a 24-hour sampling period. Mean of measurements from weeks three and four of a four-week culture experiment (mean ±SE).

reduction of 23.5% from initial tissue N of 6.0%. Given the marginal difference in tissue C and no difference in tissue N, C:N ratio was not significantly different among the treatments (P = 0.418, F = 1.03). Overall mean C:N ratio was 6.9.

Nitrogen source had a significant effect on nitrogen removal by *C. crispus* (P = 0.022, F = 3.65). Nitrogen removal was highest at 30 μM NO^{3-}/270 μM NH_4^+, 0.62 mgN gDW^{-1} d^{-1}, though not significantly different from other treatments using ammonium (Fig. 3.10.i). Nitrogen removal was lowest at 300 μM NO^{3-}/0 μM NH_4^+, with a value of 0.55 mgN gDW^{-1} d^{-1}. Nitrogen removal, therefore, was positively related to ammonium concentration in the culture media, though loosely according to statistical analysis. In general, nitrogen removal by *C. crispus* was higher than that of *P. palmata* at all treatments.

Carbon removal had a relationship to nitrogen media similar to that of nitrogen removal (Fig. 3.10.ii; P = 0.016, F = 3.93). Carbon removal was highest with 4.4 $mg \cdot gDW^{-1} \cdot d^{-1}$ at 30 μM NO^{3-}/270 μM NH_4^+ and lowest at 300 μM NO^{3-}/0 μM NH_4^+ with a value of 3.8 mg gDW^{-1} d^{-1}. Carbon removal by *C. crispus* was, in general, lower than removal by *P. palmata* at all treatments except at 30 μM NO^{3-}/270 μM NH_4^+ which had similar values for the two species. Carbon removal by *P. palmata* was positively related to nitrate concentration in the media, whereas carbon removal by *C. crispus* was higher, albeit marginally, at increasing concentrations of ammonium.

Table 3.3 Tissue carbon and nitrogen and C:N ratio of *Chondrus crispus* on dry weight basis (means ± SE, week four of four-week experiment; values with same letter are not significantly different ($p>0.05$) within that species).

Culture media		C (%)		N (%)	C:N
NO_3^- (µm)	NH_4^+ (µm)	Mean		Mean	Mean
initial *		31.7 ±0.16		6.0 ±0.11	5.3 ±0.11
300	0	32.0 ±0.11	a	4.6 ±0.04	6.9 ±0.06
0	300	32.0 ±0.09	a	4.8 ±0.12	6.7 ±0.16
150	150	31.8 ±0.15	ab	4.7 ±0.14	6.8 ±0.19
270	30	31.3 ±0.25	b	4.4 ±0.15	7.1 ±0.22
30	270	31.6 ±0.09	ab	4.5 ±0.04	7.1 ±0.08

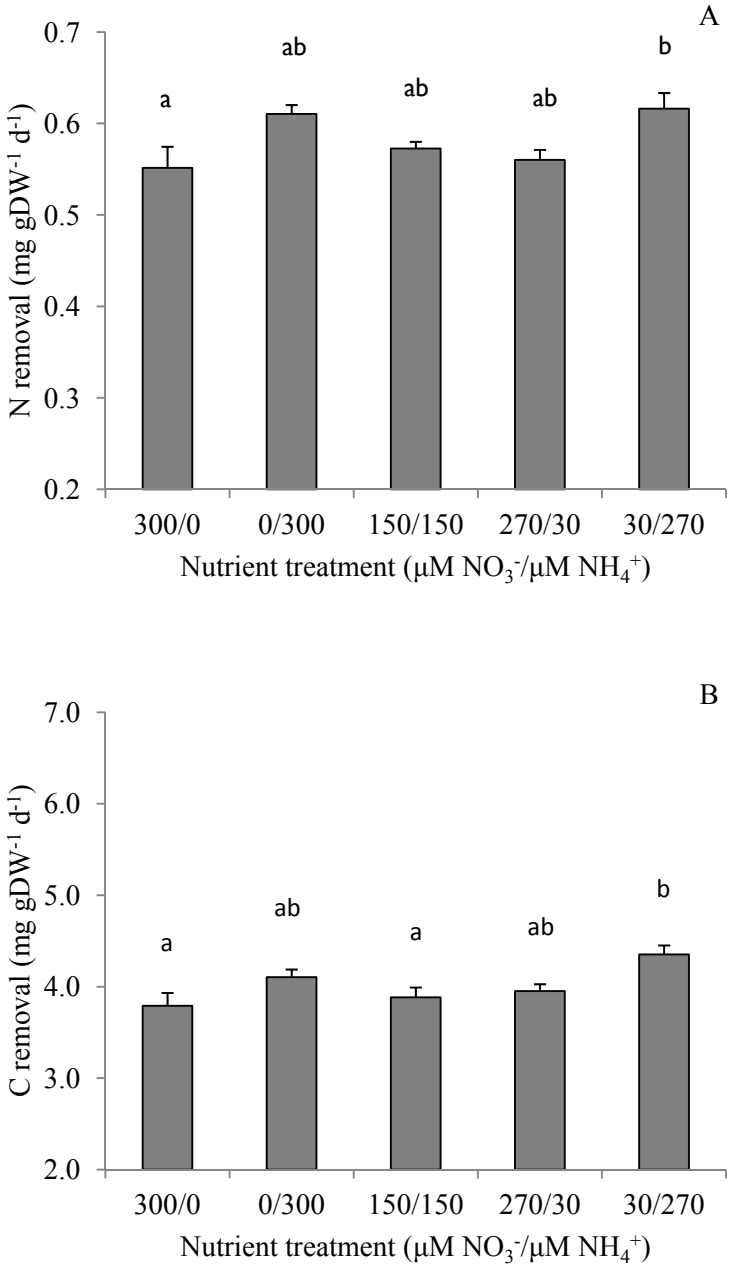

Figure 3.10 Nitrogen removal (A) and carbon removal (B) by *Chondrus crispus* at five combinations of nitrogen media (mean ±SE).

Total phosphate available in the media was not exhausted after three or four days at any period of the experiment and source of nitrogen did not have a significant effect on uptake of phosphate during six of the three- and four-day time periods ($P > 0.05$; Appendix VI). Overall mean phosphate uptake during these intervals ranged from 0.47 mgP gDW^{-1} d^{-1} to 0.51 mgP gDW^{-1} d^{-1}, with uptake efficiency of 71% (mean of the six intervals not significantly different). This is considerably less than uptake efficiency of 92-93% for *P. palmata*. For *C. crispus*, there was significant difference during period four (3 days; $P = 0.017$, $F = 3.90$) and period seven (4 days; $P = 0.030$, $F = 3.33$) with highest phosphate uptake occurring at highest inclusion of nitrate in the culture media, though only marginally (Fig. 3.11). Highest uptake rates were 0.52 mgP gDW^{-1} d^{-1} and 0.54 mgP gDW^{-1} d^{-1} for periods four and seven, respectively, both of these values occurring at 300 μM NO^{3-}/0 μM NH_4^+.

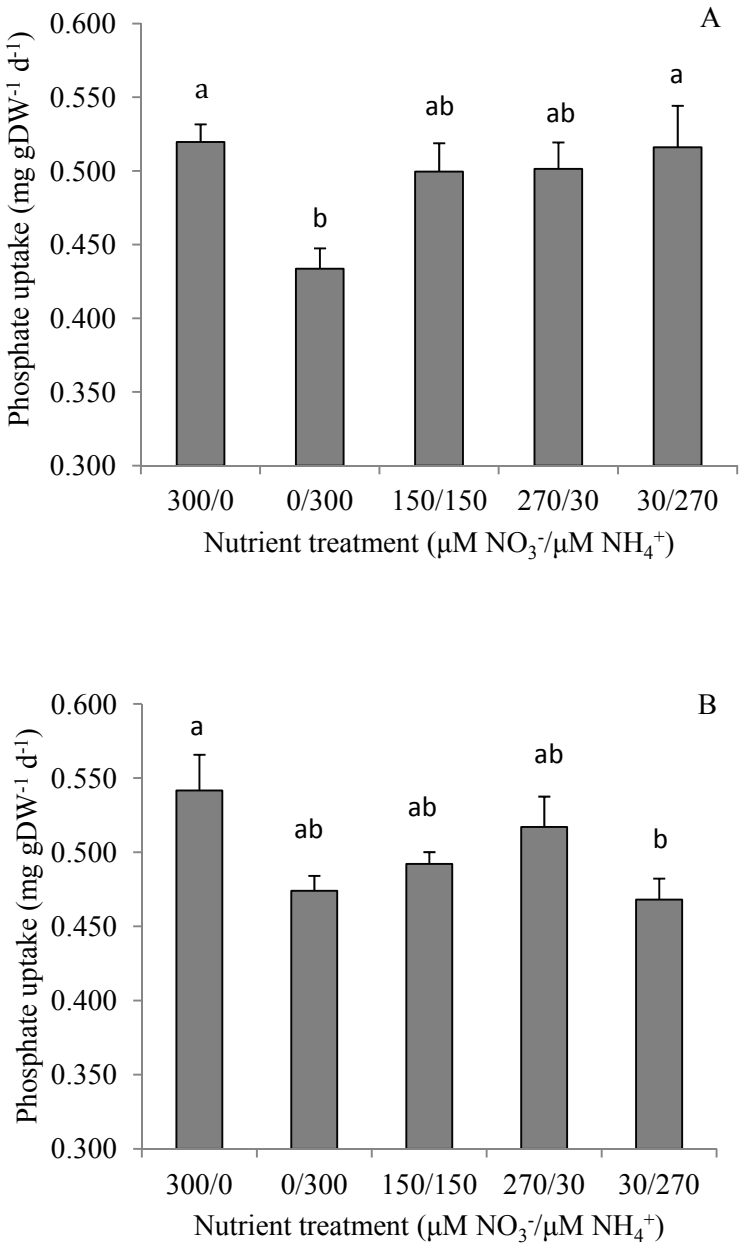

Figure 3.11 Phosphate uptake by *Chondrus crispus* at five combinations of nitrogen media during week 2 (A; time-period 4) and week 4 (B; time-period 7) of a four-week culture experiment (mean ±SE). Uptake was calculated as a function of change in media phosphate concentration.

Discussion

The results of this study clearly demonstrate that both *P. palmata* and *C. crispus* have greater affinity for ammonium than nitrate as a nitrogen source in terms of uptake. Morgan and Simpson (1981b) also came to this conclusion when cultivating *P. palmata* at 10°C in either nitrate or ammonium. When supplied with 500 μM ammonium or nitrate, the plants accumulated 397 μmol (79.4%) of ammonium within 8 hours post-inoculation compared to 229 μmol (45.8%) of nitrate. Overall, they concluded that ammonium was taken up three times faster than nitrate. In the current study, 67.5% of ammonium and 12.2% of nitrate at 300 μM nitrogen was accumulated by *P. palmata* within 12 hours of media inoculation. Under the same conditions, *C. crispus* accumulated 68.8% of ammonium and 29.9% of nitrate.

In contrast, Martinez and Rico (2004) determined that *P. palmata* had similar affinity for both sources of nitrogen. In their study, maximum nitrogen available to the plants was 21.5 μM and 65.2 μM as ammonium or nitrate, respectively. At similar concentrations in the current study, uptake of ammonium and nitrate by either *P. palmata* or *C. crispus* are also very similar (Figs. 3.3 and 3.8). Uptake of ammonium began to surpass that of nitrate uptake at 300 μM nitrogen in six species of *Porphyra* (Carmona et al. 2006), with nitrogen uptake significantly higher in ammonium than nitrate for *P. yezoensis*. The separation of uptake behaviour was also apparent when comparing greater inclusion of nitrate and ammonium in the mixed media of the current study (Fig. 3.3 and 3.8). Highest nitrogen uptake for *P. palmata* was > 150 μmol gFW^{-1} d^{-1} (5.0 mg gDW^{-1} d^{-1} equivalent) at 300 μM ammonium, which is a similar nitrogen uptake capability as *Porphyra amplissima, P. katadai, and P. yezoensis* at the same concentration (Carmona et

al. 2006). Highest nitrogen uptake by *Chondrus crispus* , at 150 μM nitrate/150 μM ammonium and 30 μM nitrate/270 μM ammonium, was similar to nitrogen uptake rates of *Porphyra haitanensis, P. purpurea, and P. umbilicalis* (Carmona et al. 2006).

Combined nitrogen source may have had a cumulative effect on nitrogen uptake in both species. Total nitrogen uptake by both species was higher in treatments containing ammonium than in the treatment containing only nitrate. However, when nitrogen was supplied at a rate of 270 μM nitrate and 30 μM ammonium, nitrate uptake was marginally higher than at 300 μM nitrate only. Total nitrogen uptake was also higher when 30 μM of ammonium was offered to the plants in combination with 270 μM of nitrate compared to nitrate only. Maestrini et al. (1982) suggested that uptake of nitrate by some microalgae may be suppressed by the presence of ammonium. This evidently is not the case for either of the species studied here.

Similar to nitrogen uptake as a measure of nutrient depletion, nitrogen removal as a function of tissue nitrogen and productivity was lowest for both species when only nitrate was supplied. Though statistically significant in *C. crispus*, this observation is considered of only marginal biological significance for two reasons: 1) nitrogen removal at 300 μM of either nitrate or ammonium only was not significantly different (Fig. 3.10), and 2) neither specific growth rate, nor tissue nitrogen, the only two factors used in calculation of nitrogen removal, were different across all treatments (Table 3.3). This was not the case for *P. palmata*. Though growth rate was not different within each of the final three weeks of culture, tissue nitrogen was significantly higher in treatments containing 300 μM and 270 μM ammonium. Thus, nitrogen removal was highest at 300 μM ammonium media, but nitrogen removal at 30 μM nitrate/270 μM ammonium was

not different from any of the other treatments. For *Gracilaria cornea*, a warm-water rhodophyte, nitrogen composition was 3.25% and 2.74% (assuming protein: nitrogen conversion factor of 4.92, Lourenco et al. 2002) at weekly inoculation of 824 μM ammonium or nitrate, respectively (Navarro-Angulo and Robledo 1999). These values were significantly different from each other, whereas tissue nitrogen at 824 μM combined ammonium and nitrate (NH_4NO_3) was the same as that of ammonium only (3.17%). Tissue nitrogen for *P. palmata* in the current study followed this same pattern only up to inclusion of 30 μM nitrate in the media. At equal inclusion of nitrate and ammonium, tissue nitrogen was the same as that of tissues cultured in nitrate only.

Tissue nitrogen content of *C. crispus* typically range from 0.8-4.5%DW for both wild and cultured specimens (Craigie 1990; Chopin and Wagey 1999). Tissue nitrogen of *C. crispus* at experiment end for the current study was at the higher limit of this range, suggesting that the level of nitrogen provided was not limiting growth at 10°C. Chopin et al. (1995) concluded that tissue nitrogen contents in excess of about 2.5%DW for this species resulted from luxury uptake and accumulation of nitrogen reserves, which can be used when nitrogen is not available in sufficient supply. In another study, C: N ratio decreased from 25 to 9.7 in *C. crispus* when cultured in continuous supply of 13 μM nitrogen (Magnusson et al. 1994). In *Gracilaria tikvahiae*, C: N ratio was inversely related to ammonium uptake rates (Friedlander and Dawes 1985), similar to *P. palmata* in the current study. C: N ratio of approximately 10 for macroalgae is considered optimal, with ratios less than 10 generally indicative of nitrogen surplus and/or light levels below saturation (Lobban and Harrison 1996). Since light was not a limiting factor, further evidenced by an increasing or stable tissue carbon (Tables 3.2 and 3.3; Kübler and Raven

1995), C: N ratios for *C. crispus* at all treatments and for *P. palmata* at treatments of highest ammonium inclusion indicated luxury uptake of nitrogen. At 3.1-3.3% DW in treatments of 150 μM nitrate or more, tissue nitrogen of *P. palmata* was well below the highest typical nitrogen content for this species of 3.8-5.0% (Morgan et al. 1980b, Appendices VIII and IX). Nitrogen may have been stored in the tissues when available to *P. palmata* as ammonium, whereas accumulation of nitrogen as nitrate was apparently impaired, though this phenomenon did not have an effect on growth of the species.

For the two species investigated, the only difference in growth rate caused by nitrogen source was in *P. palmata* during the first week of the experiment (Fig. 3.1). During this period, growth at high nitrate availability was greater than in treatments of high (≥150 μM) ammonium. Morgan and Simpson (1981b) found that *P. palmata* was twice as productive in five weeks under nitrate-only conditions than under ammonium-only conditions. *Palmaria mollis*, a North Pacific relative of *P. palmata*, has been assessed for co-culture with abalone. In this species, ammonium was more important than nitrate to growth on a short-term basis, but nitrate resulted in a higher growth rate over the long-term (Demetropoulos and Langdon 2004b).

Evidence of ammonium toxicity has been observed for *P. mollis* at >2000 μM N d^{-1} (Demetropoulos and Langdon 2004b) and *P. palmata* was assigned to the ammonium-sensitive group of macroalgae by Morgan and Simpson (1981b) when the plants were supplied with 2.0 mM nitrogen $week^{-1}$. In the current study, at nitrogen supply of 0.600 mM nitrogen $week^{-1}$, growth was not compromised by ammonium-only nitrogen. For *C. crispus*, when cultured at 8 and 15°C, growth rate was similar with either ammonium or

nitrate as the nitrogen source (Laycock et al. 1981; Neish et al. 1977). The current study confirms these results since nitrogen source did not have significant effect on growth.

Growth rates of both species declined during the final 2-3 weeks of cultivation, likely attributed to ageing of tissues. In another four-week study of *P. mollis*, specific growth rate was steady for the first two weeks, but then slowed during subsequent weeks (Demetropolous and Langdon 2004c). Young tissue has higher growth and nutrient uptake rate than older tissue (Harrison and Hurd 2001; Kim et al. 2007) and growth rate declines as plants become larger (Morgan et al. 1980b). At weekly biomass measurements and subsequent biomass reduction to initial stocking density, whole blades were removed whenever possible. The alternative method of biomass reduction by trimming basal portions of the fronds would have caused considerable stress to the plants and required recovery post-handling. The method chosen is more representative of the conditions under which plants would be cultured within IMTA.

If a species selected for integration with land-based recirculation technology has greater affinity for ammonium than nitrate, the seaweed biofilter should be located prior to the bacterial biofilter. Alternatively, if the species takes up nitrate faster than ammonium and is ammonium-sensitive, the seaweed biofilter should be located in the discharge of a bacterial biofilter and the bacterial component may be critical for the success of this system. The data presented here imply that, for integration of *P. palmata* and *C. crispus* with land-based finfish aquaculture, water treatment by these species should take place prior to bacterial biofiltration within a recirculating system, or directly in the effluent of a single-pass system. In this way, the plants would be exposed to the greatest concentration of ammonium-nitrogen. Results from integration of the alga *Ulva lactuca* and *Undaria pinnatifida* with abalone *Haliotis iris* led Cahill et al. (2010) to the

conclusion that seaweed filtration is superior to bacterial filtration. If seaweed can be maintained at sufficient biomass, the ideal solution is complete replacement of bacterial biofiltration by seaweed cultivation. This becomes, then, an issue of economics and commercial viability.

Chapter 4: Growth and Nutrient Uptake by *Palmaria palmata* Integrated with Atlantic halibut Recirculating Aquaculture

Abstract

For applied evaluation of its nutrient removal capabilities, *Palmaria palmata* was integrated with Atlantic halibut (*Hippoglossus hippoglossus*) in a commercial, land-based recirculating aquaculture system. Seaweed cages of extruded plastic mesh, 1.25 m^3 volume were suspended in the filtration sump of two recirculating systems receiving water from Atlantic halibut tanks, and a third, single-pass system receiving ambient seawater. *Palmaria palmata* was cultured for twelve months at an initial stocking density of 2.95 kg m^{-3} to 9.85 kg m^{-3}, with continuous aeration to ensure adequate tumbling, and photoperiod 16:8 (L:D). Temperature ranged from 0.4°C in winter to 19.1°C during summer. Specific growth rate of the macroalga was highest during spring when temperature was 8.0 to 9.0°C, 1.1% d^{-1} and 1.1% d^{-1} for halibut systems during April to June and 0.81% d^{-1} for the ambient system. Total tissue nitrogen of 4.2% DW and 4.4% DW in halibut systems was higher than that of the ambient system at 3.7% DW, though tissue carbon was unaffected by seawater source with mean 39.9% DW for the three systems. The area required for 50% removal of the nitrogen given off by 100 t of halibut during winter is 8 900 to 10 500 m^2, 2.5 to 3.5 times the area required for halibut culture. Fifty percent removal of carbon from the same system requires 2 200 to 3 000 m^2 cultivation area. Integration of *P. palmata* with Atlantic halibut during summer months is impractical, but may be highly effective at temperatures below 10°C.

Introduction

Atlantic halibut, a flatfish species of commercial aquaculture importance in Eastern Canada, Norway, Iceland, the UK, and the northeast US, is farmed using open-water technology similar to that of Atlantic salmon at the grow-out stage (Brown, 2002). Atlantic halibut are indigenous to the Atlantic coast of Canada and thrive at water temperatures of 10-14°C (Hallaråker et al. 1995; Jonassen et al. 2000). Farming of halibut is as yet still in its infancy in Atlantic Canada with a total production of around 50 t in 2010, approximately 50% of which was produced in land-based farms (B. Blanchard, Scotian Halibut Ltd., pers comm).

Land-based recirculation, a form of closed-contained aquaculture, facilitates greater control over culture water and waste discharge (Blancheton et al. 2009). Closed aquaculture systems further contribute to aquaculture development by providing some flexibility in site selection, and are less dependent on vast water resources than are flow-through designs (Metaxa et al. 2006). By reusing the water, temperature fluctuations of ambient supply may be mitigated, waste streams can be directly filtered, disease treatment is more specific, and escape of stock is non-existent. As mentioned in an earlier chapter, these systems are more capital-intensive than open-water methods and have higher operating costs (Neori et al. 2004). Farmers must balance these costs with optimized water quality and rates of productivity superior to those of traditional farming methods.

Integration of seaweed and land-based finfish aquaculture is a possible method of accomplishing this balance. Seaweed and finfish do not compete for farming resources, but rather complement the other in efficient use of nutrient and water cycles. Integration of these forms of aquaculture can yield an overall net increase in productivity within a land-based farming system. Some marine aquaculture enterprise (e.g. Scotian Halibut

Ltd., based in Nova Scotia, Canada), believe that development of these practices can have immediate impacts for existing (and planned expansions to) land-based marine farms in Atlantic Canada, and ongoing benefits to land-based mariculture in the region as the industry makes advances in culture of high-value species such as halibut.

Integration of seaweeds with heterotrophic aquaculture species has been widely studied, particularly since the early 1990s. Studies have included *Gracilaria* sp. and Atlantic salmon (Troell et al. 1999); *Ulva lactuca, Undaria pinnatifida*, and abalone (Cahill et al. 2010); *Chondrus crispus, Palmaria palmata*, and *Gracilaria bursa pastoris* with turbot and sea bass (Matos et al. 2006); and *Palmaria mollis* with abalone (Demetropoulos and Langdon 2004a). Investigation into the effectiveness of seaweed-halibut integration has not been previously documented.

Scotian Halibut Ltd. is a research-intensive, commercial producer of Atlantic halibut (*Hippoglossus hippoglossus*) juveniles. The company is intent on not only maximizing productivity and profitability, but also accomplishing these using environmentally responsible practices. Efficient use of energy (e.g. pumping of water) and natural resources (ambient water and waste streams) are key elements in this approach. Therefore, integration of *Palmaria palmata* and Atlantic halibut within a land-based recirculating aquaculture facility was established for one year to evaluate the growth and nutrient uptake characteristics of the seaweed in commercial application.

Materials and methods

Palmaria palmata was collected in June 2009 from natural populations in the low-intertidal zone of the Bay of Fundy, at Point Prim, Digby County, Nova Scotia (44°69'15.7"N, 65°78'5.4"W). Algal material was stocked to culturing units at the land-

based Atlantic halibut nursery site of Scotian Halibut Ltd., Wood's Harbour, Nova Scotia (43°55'80.6"N, 65°73'61.1"W). Three independent seaweed culturing areas were established, two mid-stream of an Atlantic halibut recirculating system (Figure 4.1) and the third receiving sand-filtered (50-80 μm) seawater only. Data were collected from November 2009 until October 2010.

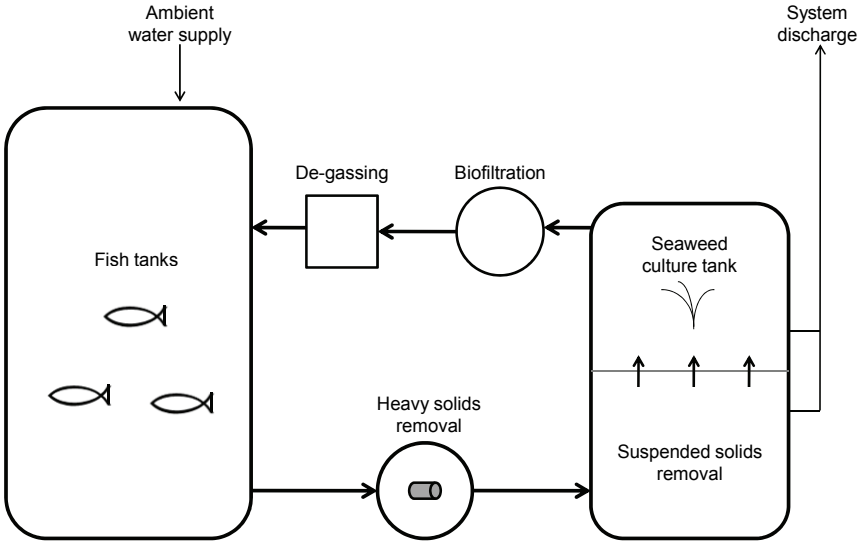

Figure 4.1 Schematic diagram of integrated aquaculture system, Scotian Halibut Ltd., Wood's Harbour, Nova Scotia. Total system volume 500 m^3.

Culturing system Total volume of each commercial aquaculture system is approximately 500 m^3 with holding capacity of 10–14 t of Atlantic halibut. Solids filtration is accomplished by swirl separator for removal of heavy waste, followed by diffused air flotation for removal of suspended solids. The seaweed was held in cages that were 1.2 m long, 1.1 m wide, and 0.95 m deep, surface area of 1.32 m^2, and volume

1.25 m^3 constructed of extruded plastic mesh (7 mm x 7 mm mesh size, Vexar®, DuPont). The cages were suspended at the water surface within the wastewater collection sump of the recirculating systems. Nine of these cages were used in each of the three culture systems. Combined fish tank and filtration sump volume is approximately 500 m^3 with turnover by sand-filtered make-up water at a rate of 25-100% per day according to season. The sumps where seaweed was cultivated were 64.7, 38.8, and 50.0 m^3 for Aquaculture systems 1 and 2, and the Ambient system, respectively. Total water depth was between 1.5 and 2.0 m, with water turnover rate of 24 to 28 minutes.

Air was supplied beneath the cages continuously such that two rows of air bubbles, approximately 0.6 m apart rose through the cages, keeping the seaweed in suspension. Light was supplied by natural sunlight through a translucent tarp structure, supplemented with 120 cm, 32 W, 5000 K white T8 fluorescent lamps (six bulbs per cage; Philips, Somerset, NJ). Irradiance was measured using a light meter (Li-Cor Li-1400 with Li-193SA spherical sensor, Lincoln, NE). Minimum irradiance of 100 μmol photons m^{-2} s^{-1} was available to the plants. A 16:8 L:D (Sagert and Schubert 2000) photoperiod was maintained throughout the year using the fluorescent lamps controlled by an automatic timer. Salinity was around 30 psu.

Temperatures in the sumps were seasonally variable. Minimum and maximum temperatures for Effluent 1 were 1.2° and 19.1°C, respectively, and those of Effluent 2 were 0.4° and 18.5°C (Fig. 4.2). Temperatures of Effluents 1 and 2 were similar, though a difference of as much as 2.3°C between these two water systems occurred from June to August,. On average, Effluent 1 was 1.0°C warmer than Effluent 2 over the entire year. Water temperature reached 6°C early April for all systems. Effluent 1 temperature was

14°C early June, whereas Effluent 2 did not reach this temperature until two weeks later, and Ambient was cooler than 14°C until late August. Temperature differences between the effluent systems are a result of difference in rate of clean seawater entering the systems. Rate of seawater inflow was governed according to fish biomass and feed loading of the system.

Temperature of inflow seawater was maintained at a minimum temperature slightly above 0°C during winter months. This affected the minimum temperature of all systems, including that of Ambient. As such, the latter is not a direct representation of natural seawater temperature in Wood's Harbour, Nova Scotia during this period. Minimum and maximum temperatures of the Ambient system were 0.5° and 17.2°C, respectively. Water temperature in this system was 1.4°C lower on average than mean temperature of the two effluent systems. The difference was most profound during June to August when Ambient temperature was as much as 3.6°C cooler than the effluent systems. Carbon dioxide de-gassing (Fig. 4.1) by spilling over dispersal plates to maximize water surface exposed to the air in the effluent systems is the primary site of thermal losses and gains.

Palmaria palmata was stocked at an initial stocking density of 2.95 kg FW m^{-3} in November 2009. Biomass in each cage was measured bi-weekly. FW was obtained after removal of excess water by spinning in a residential washing machine. Seaweed biomass in each cage was reduced to initial stocking density at each weighing, and samples were taken for dry weight. Surplus seaweed material was stored in a separate culture vessel receiving aquaculture effluent. As excess material was available, the stocking density of

the cages was increased incrementally to a maximum stocking density of 9.85 kg FW m^{-3} in May 2010 (Table 4.1).

Dry weight (DW) was determined by drying tissue samples to constant weight at 60°C. Using the biomass data collected bi-weekly, specific growth rate (SGR, expressed as % d^{-1}) was calculated weekly using Equation 2.1.

Table 4.1 Stocking density of *Palmaria palmata* in seaweed culturing units at the land-based recirculating aquaculture facility of Scotian Halibut Ltd., Wood's Harbour, NS.

Initial biomass (kg FW per cage)	Time period	Stocking density (kg FW·m^{-3})	Stocking density (kg FW·m^{-2})
3.9	Nov - Dec 2009	2.95	3.12
5.0	Dec 2009 – Feb 2010	3.79	4.00
7.3	Feb – Apr 2010	5.53	5.84
11.0	Apr – May 2010	8.33	8.80
13.0	May – Oct 2010	9.85	10.40

Water samples were collected at 8:00 am, 2:00 pm, and 8:00 pm the day following each biomass survey. Nitrate concentration was determined by nitrite cadmium reduction modified from Jones (1984). The method of Liddicoat et al. (1975) was followed for analysis of ammonium concentration, and phosphate concentration was determined following Parsons et al. (1984). Availability of each nutrient was calculated as the mean of the three samples collected for any particular sample day. This data was used to

estimate total nitrogen concentration of the water and occurrence of nitrogen as nitrate or ammonium.

Dried tissues were ground to powder with an MM301 ball mill (Retsch, Newtown, PA) and then analyzed for total tissue N and carbon (C) using a CNS-1000 Carbon, Sulphur and Nitrogen Analyzer (Leco, St. Joseph, MI). Total tissue N and C were determined for each biomass survey. N removal was calculated using Equation 2.2 (Kim et al. 2007). The same calculation was used for C removal, substituting *Tissue C* for *Tissue N*.

Results were presented with respect to two seasons extending from November to April and may until October. These two seasons encompassed temperature ranges ≤10°C and >10°C, representing temperature conditions preferable and sub-optimal for growth of *P. palmata*, respectively.

Results

System pH was similar for the effluent systems. Effluent 1 pH was, on average, 7.5 for the duration of the experiment, with minimum and maximum pH 7.1 and 8.0, respectively. For Effluent 2, mean pH was 7.4, with minimum and maximum pH 7.0 and 8.0. Ambient pH was consistently higher than that of either effluent system, with mean difference of pH 0.6 between Ambient pH and mean of both effluent systems. Mean Ambient pH was 8.0 with minimum and maximum pH 7.7 and 8.5, respectively.
Total nitrogen concentrations of all systems were variable with season. When water temperatures were sub-optimal for culture of Atlantic halibut (e.g. January to March), feed loading was low, leading to total nitrogen concentrations as low as 13 μM in Effluent

1. The concentration of nitrate as a proportion of total nitrogen present in the system was on average 81 to 88% for all systems, owing to the occurrence of nitrogen primarily as nitrate in the natural environment, and the function of bacterial nitrification in oxidation of ammonium. Mean nitrogen: phosphorus ratio in the water was 19.8, 23.4, and 24.9 μM for Effluent 1, Effluent 2, and Ambient, respectively. Phosphorus concentration ranged from 0.3 μM to 23.3 μM PO_4^{3+} and 0.3 to 24.1 μM PO_4^{3+} for Effluents 1 and 2, respectively. Ambient phosphate ranged from 0.1 to 8.3 μM PO_4^{3+}.

Growth rate of *P. palmata* was seasonally variable (Fig. 4.2). Highest growth rate of 1.1% d^{-1} in Effluent system 1 occurred at the end of April when the water temperature was 8.1° to 8.5°C. Growth was most vigorous at this point, older tissues were generating new plantlets at all margins, and a single plant had a length of 80 cm on 31 May 2010. Around this time, the biomass was increased to 8.33 kg m^{-3}, which caused the growth rate to decline. A brief period of recovery after biomass increase to 8.33 kg m^{-3} was followed by drastic decline in growth rate when temperatures approached 14°C. The growth rate declined drastically above 14°C to a point of negative productivity. A similar pattern was observed in Effluent 2. Highest growth rates of 0.93% d^{-1} and 1.08% d^{-1} occurred at end of April and another in June, respectively. Growth rate continued to improve even following stocking density increase to 9.85 kg m^{-3}, until water temperature reached 14°C.

Seaweed grown in the Ambient system had highest growth rate of 0.8% d^{-1} when water temperature was around 9°C (Fig. 4.2). Growth rate was steady at roughly 0.3% d^{-1} from mid-December until April-end, a period in which temperature fluctuated from a high of 9°C to as low as 0.5°C. When stocking density was increased to 8.33 kg m^{-3} in April, growth rate decreased to a minimum of -1.1% d^{-1} over two weeks. This drop in growth

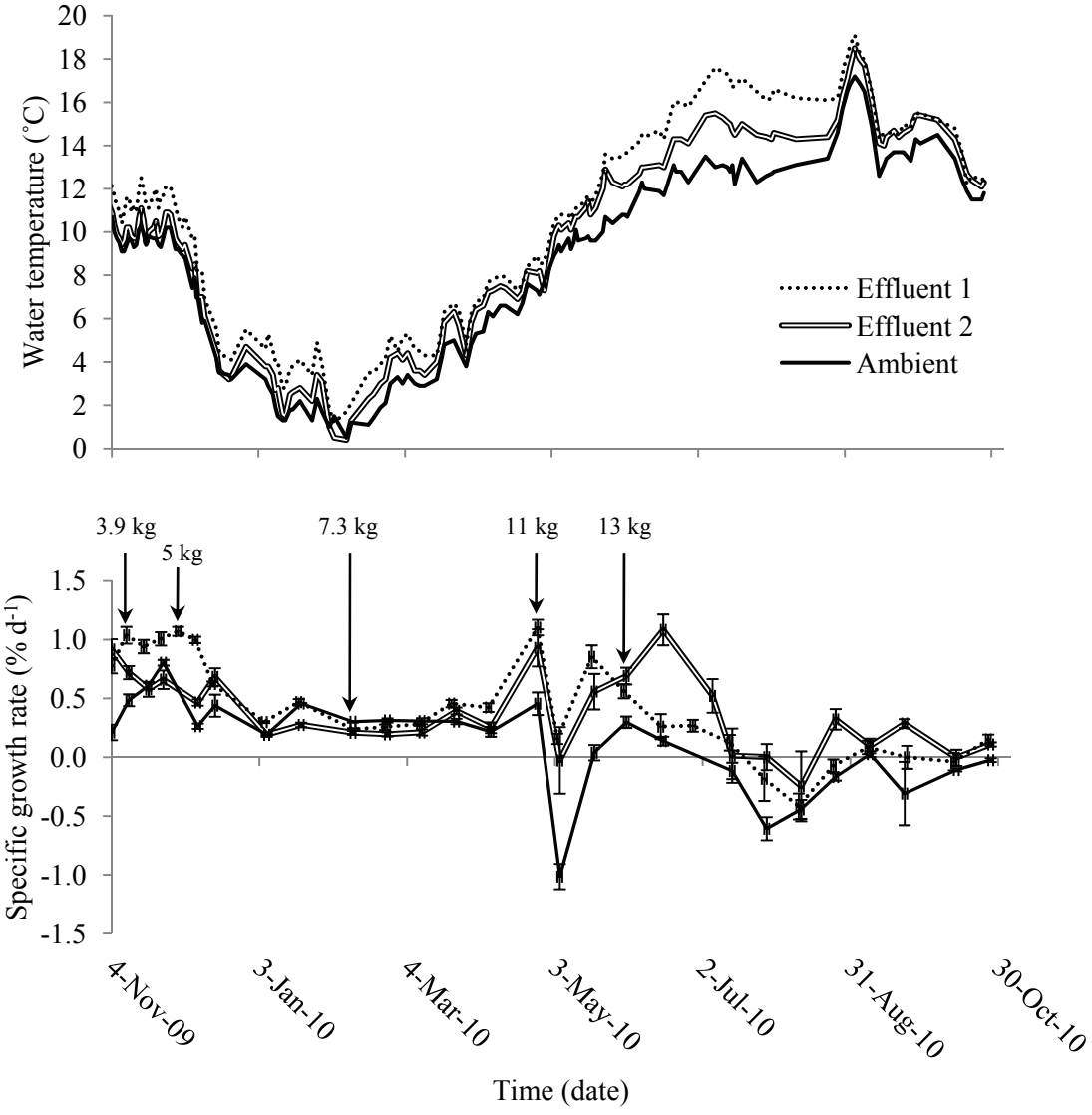

Figure 4.2 Water temperature of seaweed cultivation areas and specific growth rate of *Palmaria palmata* in these systems. Effluent 1 and 2 are systems using recirculation water of land-based Atlantic halibut aquaculture; Ambient is filtered, natural seawater. Values and arrows above figure for growth rate indicate incremental biomass additions. (Growth rate mean ± SE; n=9).

rate was caused by the introduction of wild materials collected from Digby, Nova Scotia. The seaweed recovered to positive growth until the biomass was increased again to 9.85 kg m^{-3}. Growth rate then ceased in early July and remained at or below zero growth rate until the end of the experiment. Leading up to stocking density increases to 8.33 and 9.85 kg m^{-3}, growth rate in Effluent systems was twice that of Ambient , which was consistently lower than that of either Effluent system from late-April until experiment-end.

Source of seawater (ambient or recirculating aquaculture water) had an effect on tissue nitrogen content. Mean tissue nitrogen of *P. palmata* cultured in Effluents 1 and 2 was 4.2 and 4.4% DW, respectively, while that of Ambient was 3.7% DW (Appendix 8). Tissue carbon contents were not so clearly affected, with mean tissue carbon of 40.2, 39.9, and 39.6% DW for Effluents 1 and 2 and Ambient. C: N ratios for Effluents 1 and 2 were 9.6–11.9 and 8.8–11.8, respectively, from November to late March. For the remainder of the culture experiment, April to October, C: N ratio ranged 8.4–10.0 and 8.0–9.1, for Effluents 1 and 2, respectively. Ambient C: N ratio was 8.7–12.3 November to early April and 10.5–13.2 from April to October. Mean C: N ratios for the entire year-long experiment were 9.8, 9.2, and 11.0 for Effluents 1 and 2 and Ambient, respectively.

Nitrogen and carbon removal followed the same general pattern as growth rate (Fig. 4.3). Nitrogen removal remained stable from experiment-start in November until late March within a range of 1.4–4.2, 1.2–3.4, and 0.7–2.8 g d^{-1} in Effluent 1, Effluent 2, and Ambient, respectively. This consistency was also observed for carbon removal over the same time period with ranges of 17.0–44.0, 11.8–37.9, and 8.6–31.0 g d^{-1} for Effluent 1, Effluent 2, and Ambient, respectively.

Nitrogen and carbon removal increased sharply near the end of April when water temperature was around 8°C in all systems, corresponding to a similar spike in growth rate at this time. A decline in both parameters followed stocking density increase to 8.33 kg m^{-3}, but recovery of growth rate resulted in recovery of both nitrogen and carbon removal in June. Highest nitrogen removal rates of 10.5, 14.5, and 2.8 g d^{-1} for Effluent 1, Effluent 2, and Ambient occurred at this time. Simultaneous high carbon removal was 95.7, 129.7, and 30.8 g d^{-1} for Effluent 1, Effluent 2, and Ambient, respectively.

Overall, during winter, mean seaweed system biomass of 52.5 kg removed 1.1% of nitrogen released by feeding and fish metabolism in Effluent system 1 (Table 4.2). The same result for Effluent system 2 was 0.7%. During the same period, carbon removal was 4.7 and 2.6% of that produced by the fish in Effluent 1 and 2, respectively. Nitrogen and carbon removal efficiencies were lower during summer months for both systems.

Incidence of epiphytes on *P. palmata* was very low in all systems for the duration of the experiment. Plants in recirculating aquaculture water maintained deep red-purple colour and pliable texture throughout the experiment. Plants in the Ambient system became noticeably paler than those grown in aquaculture water from middle to end of summer and into the fall, and the texture of these plants was similar to that of paper The colour resembled samples of wild *P. palmata* from south-west Nova Scotia,. All plants in this system also developed perforations throughout the entire blade, likely a result of green-spot disease, which caused localized necrosis and senescence of tissues in between biomass surveys.

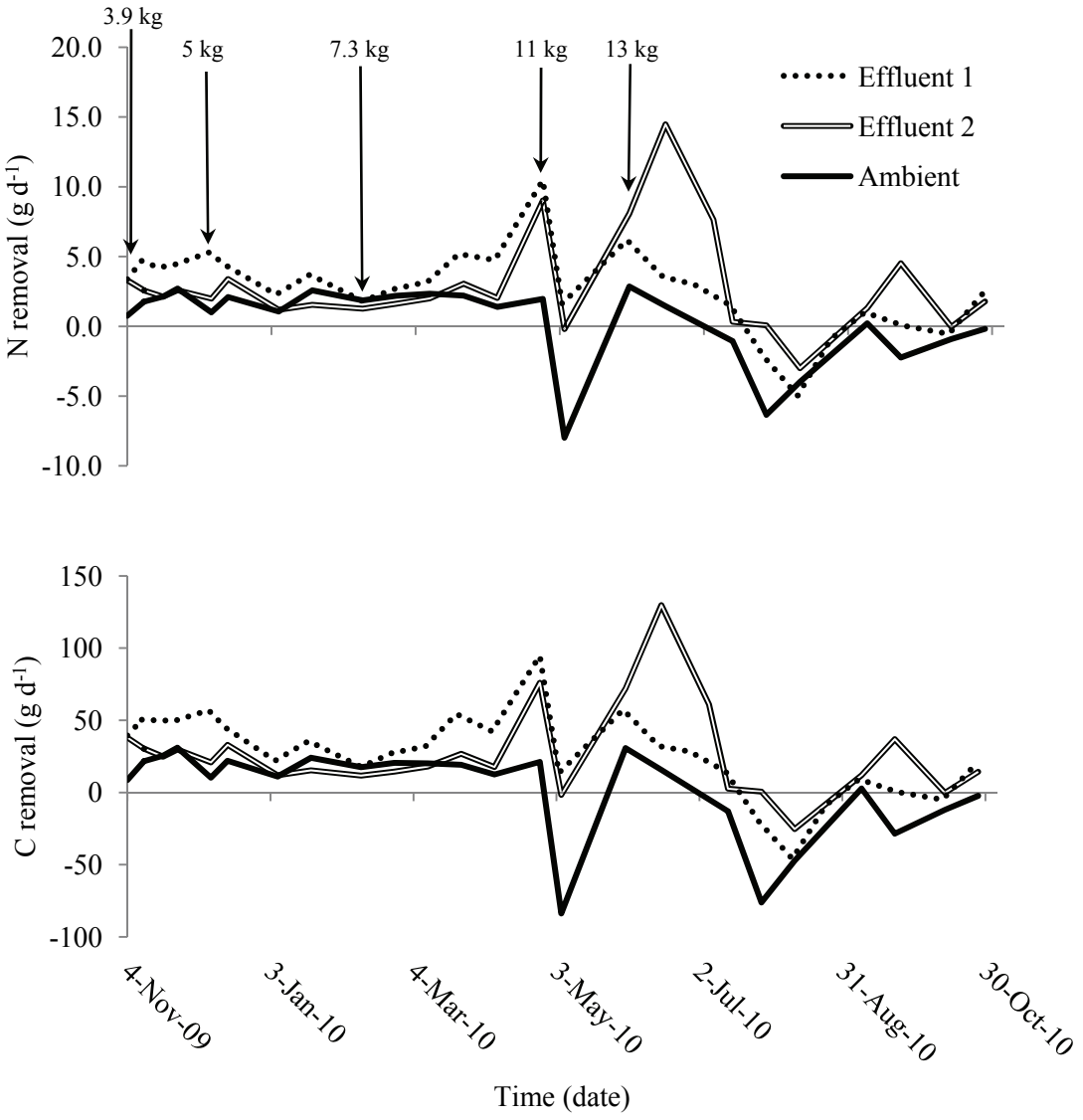

Figure 4.3 Total nitrogen and carbon removal per culture system by *Palmaria palmata* grown in recirculating Atlantic halibut water (Effluent 1 and Effluent 2) and filtered, natural seawater (Ambient).

Table 4.2 Nitrogen (N) and carbon (C) removal by *Palmaria palmata* relative to amount of feed available to Atlantic halibut in an integrated recirculating aquaculture system.

	Feed input (kg)	N release (kg) [a]	C release (kg) [b]	*P. palmata* produced (kg)	N removal		C removal	
					(kg)	% of release	(kg)	% of release
Recirculation system 1								
Nov '09 - Apr '10	1 044	56.1	134.6	57.7	0.637	1.1	6.261	4.7
May '10 - Oct '10	1 820	97.9	234.6	25.5	0.133	0.1	1.190	0.5
Total	2 865	154.0	369.3	83.2	0.770	0.5	7.451	2.0
Recirculation system 2								
Nov '09 - Apr '10	1 351	66.4	174.2	40.8	0.491	0.7	4.609	2.6
May '10 - Oct '10	2 161	106.2	278.6	61.5	0.541	0.5	4.644	1.7
Total	3 512	172.6	452.7	102.3	1.032	0.6	9.253	2.0

[a] Average protein content of feed 48%; N content 6.25% of protein; 70% of fed N is released (Davies and Slaski 2003)
[b] C production based on 1.375 grams CO_2 produced for each gram of O_2 consumed (Timmons et al. 2002)

Discussion

Productivity of *P. palmata* was highly dependent on season within Atlantic halibut recirculating aquaculture. Previous work demonstrated that growth of this species is optimal at temperatures of 6 to 10°C (Chapter 2 of this document). It is also clear that productivity was compromised with stocking density increase to 8.33 kg m^{-3} or more per cage. Therefore, during periods when environmental conditions were favourable—e.g. April-May—growth was hindered by a recovery period of as much as one month.

Matos et al. (2006) reported yield of 40.2 g DW m^{-2} d^{-1} at 17-21°C for *P. palmata*. Highest yield in the current study was 28.4 g DW m^{-2} d^{-1} during the first two weeks of June 2010. This level of productivity, however, occurred at stocking density of 5.5 kg m^{-2} while that of Matos et al. (2006) was accomplished at 3 kg m^{-2}. Morgan and Simpson (1981a) reported growth rates of 7% d^{-1} under fluorescent light and 6-14°C water temperature, much greater than the highest growth rate of 1.1% d^{-1} in the current study. The afore-mentioned study cultivated 50 g FW of apical segments at a stocking density of 1 kg FW m^{-2}. At these stocking densities, *P. palmata* has grown at 7% d^{-1} in halibut effluent and 5°C, declining dramatically to 1% d^{-1} at 10 kg m^{-2} (J. Kim, NS Agricultural College, unpubl data).

Nitrogen removal by *P. palmata* in integration with Atlantic halibut during winter was higher than that of *P. palmata* and *C. crispus* in a cascade system. At 17-21°C, these two rhodophytes removed 1.35 g N m^{-2} d^{-1} (Matos et al. 2006) while highest nitrogen removal in the current study was 2.3 g N m^{-2} d^{-1}. Matos et al. (2006) used more than two times more *C. crispus* than *P. palmata*, adding to system productivity at these warmer temperatures, since productivity of *P. palmata* declines dramatically at temperatures

above 14°C and ceases at temperatures exceeding 20°C (Matos et al. 2006), but most likely even lower.

Nitrogen removal may have been greater if more nitrogen had been available as ammonium. On average, 80% of the available nitrogen was in the form of nitrate (unpubl data). Nitrogen uptake can be as much as 50% faster in *P. palmata* when 80% of nitrogen is present as ammonium instead of nitrate (Fig. 3.3). Morgan and Simpson (1981b) concluded that ammonium was taken up three times faster than nitrate, which is produced through nitrification of ammonium within bacterial bioreactors (Timmons et al. 2002). Rate of nitrate production from ammonium is determined by biofilter capacity, nutrient (feed) load, and water temperature (Wortman and Wheaton 1991; Jones and Morita 1985). The biofiltration equipment of the systems used in this study was originally sized for a maximum of 10 t of finfish biomass. Normal biomass is currently around 5 t. This reduction in nutrient load results in greater proportion of the ammonium being oxidized to nitrate with each pass of the water.

In experiments of stocking density with *P. palmata*, the optimal stocking density for nitrogen removal was 10 kg m^{-3} at a water depth of 0.2 m with growth rate of 1.0 and 0.5% d^{-1} at 5 and 15°C, respectively (J. Kim, NS Agricultural College, unpubl data). Similar growth rate occurred in the current study from November to April at water depth of 0.95 m (Fig. 4.2). By estimating mean finfish biomass for two seasonal periods of the experiment, it is possible to predict seaweed biomass required for bioremediation of the effluent. For removal of 50% of the nitrogen produced by 100 t of halibut during winter, assuming seaweed stocking density of 4.4 kg m^{-2} and growth rate 0.5 to 1.0% d^{-1}, 20 000 to 23 000 m^2 of cultivation area for *P. palmata* would be required. A 100 t land-based

Table 4.3.i Required biomass and cultivation area of *Palmaria palmata* for nitrogen removal of integrated seaweed-halibut aquaculture.

		seaweed biomass			seaweed
	finfish biomass (t) [a]	system total (kg) [b]	50% N removal (t)	50% N removal for 100 t fish (t)	cultivation area [c] (m^2) [b]
Recirculation system 1					
Nov. '09 - Apr. '10	2.6	52.5	2.3	88.6	20043
May '10 - Oct. '10	4.6	113.5	41.9	920.9	96361
Recirculation system 2					
Nov. '09 - Apr. '10	3.4	52.5	3.5	105.1	23767
May '10 - Oct. '10	5.4	113.5	11.2	206.4	21598
Mean					
Nov. '09 - Apr. '10	3.0	52.5	2.9	96.8	21905
May '10 - Oct. '10	5.0	113.5	26.5	563.7	58979

Table 4.3.ii Required biomass and cultivation area of *Palmaria palmata* for carbon removal of integrated seaweed-halibut aquaculture.

		seaweed biomass			seaweed
	finfish biomass (t) [a]	system total (kg) [b]	50% C removal (t)	50% C removal for 100 t fish (t)	cultivation area [c] (m^2) [b]
Recirculation system 1					
Nov. '09 - Apr. '10	2.6	52.5	0.6	21.6	4892
May '10 - Oct. '10	4.6	113.5	11.2	246.0	25741
Recirculation system 2					
Nov. '09 - Apr. '10	3.4	52.5	1.0	29.4	6645
May '10 - Oct. '10	5.4	113.5	3.4	63.0	6596
Mean					
Nov. '09 - Apr. '10	3.0	52.5	0.8	25.5	5769
May '10 - Oct. '10	5.0	113.5	7.3	154.5	16168

[a] Estimate based on feeding rate of 0.4% body weight during winter; 0.7% body weight during summer (Davies and Slaski 2003)

[b] Mean stocking density Nov-Apr = 5.836 kg per cage (4.421 kg m^{-2}; 4.669 kg m^{-3}); mean stocking density May-Oct = 12.615 kg per cage (9.557 kg m^{-2}; 10.092 kg m^{-3})

[c] Seaweed cultivation area required for 50% nutrient removal of 100 t fish

halibut farm has a tank surface area of at least 3 000 m^2 (pers obs, Scotian Halibut Ltd., CanAqua Ltd.). Therefore, seaweed cultivation area would be 6.5 to 7.5 times larger than halibut cultivation area for just 50% nitrogen mitigation. This area would, of course be greatly reduced at stocking density of 10 kg m^{-2}, though further investigation is needed to verify that N removal is not compromised at these high stocking densities. According to data for *Gracilaria* + *Chondrus* in a cascade system with turbot and seabass, 8 200 m^2 of seaweed cultivation area were required for 50% removal of 54.0 kgN d^{-1} at 17 to 21°C (Matos et al. 2006). Pagand et al. (2000) demonstrated that 15 000 m^2 of high rate algal pond using *Ulva, Enteromorpha*, and *Cladophora* spp. during temperate periods of 16 to 26.5°C were required to remove the nutrients produced by 100 t of sea bass. The biomass of *P. palmata* required for 50% nitrogen removal approaches a 1:1 ratio seaweed:finfish. Haglund and Pedersén (1993), in co-cultivation of *Gracilaria tenuistipitata* and rainbow trout came to a similar conclusion of 1: 1 seaweed: finfish for efficient removal of inorganic nitrogen and phosphorus while maintaining high growth rates. Yet another example was reported by Abreu et al. (2011) in which 4 500 m^2 of *G. vermiculophylla* were required for 50% removal of the nitrogen produced by 100 t of fish.

Where such large area is required for seaweed cultivation, two primary concerns arise: 1. Land allocation and capital investment and, 2. Energy required for tumble culture. Small-scale experiments at Scotian Halibut Ltd., Wood's Harbour, including the study reported here, had a total cultivation area of 25 to 40 m^2. Energy required for agitation of this cultivation area is 0.29 to 0.34 hp m^{-2}. At an average energy cost of \$0.0825 kWh^{-1}, this is equivalent to \$12.92-15.15 $month^{-1}$. Agitation of 20,000 m^2 of cultivation area, therefore, would cost \$258,400-303,000 $month^{-1}$. A commercial, land-

based seaweed cultivation facility in Charlesville, Nova Scotia cultivates *Chondrus* species in ponds 1 m deep. Energy required for agitation of these ponds is 0.025 horsepower m^{-2} (Allan Archibald, Acadian Seaplants Ltd., pers comm), a magnitude lower than the energy required for agitation of my small-scale experiments. At this rate of energy consumption, cost of agitation would be \$22,275 $month^{-1}$. Assuming a holding biomass of 88 000 kg of *P. palmata* (Table 4.3.i), a growth rate of 0.5-1.0% d^{-1} would yield 440 to 880 kg FW of *P. palmata* per day at a potential value of \$1.00 kg^{-1} FW (Wanda van Tassel, Fundy Dulse Ltd., pers comm). This represents \$13,200-26,400 $month^{-1}$, sufficient revenue to offset cost of energy under optimal conditions, but does nothing to address capital investment, land-use costs, and labour.

Seaweed cultivation area required for 50% carbon removal is 24 to 28% of the area required for the same removal of nitrogen (Table 4.3). Extent of integrated seaweed cultivation, then, may depend on the desired objectives. Carbon dioxide is the carbon form most detrimental to fish health and can have such long-term effects as decreased growth and reproduction and calcification (Good et al. 2010). By binding to haemoglobin at concentrations greater than ambient, carbon dioxide can impair the blood's ability to bind oxygen (Landau 1992). Carbon dioxide is most commonly removed from recirculating aquaculture systems by gas exchange through a packed column and gravity flow of water, but high solubility of CO_2 requires gas: liquid ratios of 5: 1 to 10:1 for effective removal (Barsanti and Bualtieri 2006).

Carbon occurs in equilibrium as carbon dioxide (CO_2) and bicarbonate (HCO_3^-) (Lobban and Harrison 1996), CO_2 readily dissociating to HCO_3^-, causing release of hydrogen ions (H+) to the seawater (Shulz et al. 2009) and subsequent decline in pH (Barsanti and Bualtieri 2006). As CO_2 is removed from solution, HCO_3^- acts as a

reservoir of CO_2, replenishing what is removed (Timmons et al. 2002). All algal growth removes CO_2 into organic matter by means of photosynthesis (Barsanti and Bualtieri 2006), though *P. palmata* is able to use carbon as both HCO_3^- and CO_2 (Kübler and Raven 1995). Thus, carbon removal as a function of productivity and tissue carbon is a valid estimate of total carbon dioxide removal. Carbon dioxide fixation is greater at pH 7 than at 8 or 9 (Smith and Bidwell 1989), so a balance of 50% carbon removal may ensure that pH remains around 7.4-7.6 for more efficient removal of CO_2. In addition, as pH increases, a higher proportion of ammonia is present in the un-ionized, and more toxic, form (Bower and Bidwell 1978).

Few studies have considered the carbon removal potential of seaweed species in seaweed-fish integration. In 2007, mariculture of seaweed reached 1.4 million t in China, representing an estimated 0.34 million t of carbon removed from the marine coastal environment by seaweed harvesting (Tang et al. 2011). Investigating carbon dioxide fixation by microalgae in a photobioreactor, Otsuki (2001) found carbon removal rates of 13.75 g C m^{-2} d^{-1}. Highest carbon removal rates in the current study were 8.0 and 10.9 g C m^{-2} d^{-1} for Effluent 1 and Effluent 2, respectively, similar to 11 g C removal m^{-2} d^{-1} by *Gracilaria vermiculophylla* in effluent of turbot, sea bass, and Senegalese sole aquaculture (Abreu et al. 2011). Marine algal species representative of Rhodophyta, Chlorophyta, and Phaeophyta in cultivation have tissue carbon contents ranging from 20 to 35% DW (Tang et al. 2011), lower than that of *P. palmata* integrated with halibut. In samples of *P. palmata* from the wild, tissue carbon contents were 40 to 43% AFDW during summer (13.5-20°C) and 43 to 45% AFDW during winter (12-16°C) (Martinez

and Rico 2002). Tissue carbon of this study was likely higher than in *P. palmata* cultured in the lab under continuous aeration (Table 3.2).

From the data presented here, it is clear that *P. palmata* is not suitable for bioremediation during the summer season. On average, both nitrogen and carbon removal were six times more efficient from November to April than during summer months (Table 4.3). Strategic integration of multiple species may be the appropriate application since *P. palmata* has greater value ($1.00 kg^{-1} FW) and potential versatility as fodder for certain commercially valuable marine heterotrophs such as abalone (Demetropoulos and Langdon 2004a) than, for example, *C. crispus* ($0.42 kg^{-1} FW, Wayne Nickerson, Irish moss harvester, pers comm). For these reasons, *P. palmata* may be the superior species of cold-water seaweed for integration with marine, land-based halibut farming during winter.

Chapter 5: Conclusion

The use of seaweed in both land-based and open-water integrated multi-trophic aquaculture (IMTA) has been considered by many studies over the past several years (Table 1.1; Chopin et al. 1999a; Soto 2009; Troell et al. 2009). While both applications have great merit in reducing ecological impacts and exploiting an otherwise unused resource, IMTA on land represents much higher capital investment and on-going costs of operating than does open-water IMTA. Seaweed cultivation in open-water is most often conducted on long-lines requiring relatively little investment of equipment and energy (Troell et al. 1999; Chopin et al. 2001). On-land tumble culture of seaweed is capital-intensive in set-up of tanks and other equipment, and high costs of aeration, as discussed in Chapter 4, can be prohibitive to new investment. Therefore, implementation of land-based IMTA requires rigorous strategic planning to ensure its financial viability and longevity.

There is little doubt that both *P. palmata* and *C. crispus* are effective as nutrient-scrubbers in bioremediation of aquaculture effluent and recirculating water. As discussed in previous chapters and presented in Table 1.1, nutrient removal by these species is on a similar level to that of other species used in IMTA. However, given variable water temperatures, tank design, nutrient sources, and other conditions, it is difficult to make direct comparison. The variability caused by environmental and physiological factors is even apparent between the two species in this study. Therefore, use of *P. palmata* and *C. crispus* in alternating seasons within the culture system is likely to be the most effective application of these species in land-based IMTA. An example of this approach would be cultivation of *P. palmata* from late autumn to early spring and *C. crispus* from late spring

to early autumn. Inclusion of multiple seaweed species into an integrated system affords greater flexibility of the function and output of that system on several fronts:

1. Bioremediation potential over broader range of temperature. In the case of Scotian Halibut Ltd., water temperature of recirculating aquaculture systems ranges from 0.5 to 17°C or higher (unpubl data).

2. Effective nutrient removal in both effluent and recirculating streams (ammonium- and/or nitrate-nitrogen).

3. Diversity of products available for use or sale. *P. palmata* and *C. crispus* are species with economic history within Atlantic Canada, both having some market value.

One possible challenge of seasonal cultivation is the maintenance or accumulation of sufficient biomass for effective bioremediation by that species early in its growing season. This requires substantial allocation of space and technical resources to ensure that waste mitigation is not compromised in the transition between species. Nonetheless, these are simply operational and management constraints which can be overcome. The greater challenge remains economic viability of land-based integrated aquaculture. Species must integrate well and be of sufficient value to warrant investment (Bostock et al. 2010). The economic outcome (product sale) resulting from integration must, at the very least, offset operating costs of that product (Barrington et al. 2009). There are few seaweed species that have yet been found to meet this latter criterion adequately.

The value of integration is not only tangible, but can also be considered in the improvement of environmentally responsible practices, which relieve pressure on surrounding ecosystems and may improve social perception concerning aquaculture (Barrington et al. 2010). Regardless of the level of seaweed integration and the biomass

of seaweed produced, the benefits to the environment are measureable in terms of nitrogen and phosphorus removal. This may, in turn, carry economic value through internalization of ecological costs. Barrington et al. (2009) considered costs of nitrogen and phosphorus mitigation of sewage in Sweden, associating this with profit analysis of salmon/seaweed integrated farming. If waste discharge regulations and fees were assigned to aquaculture operations, feasibility of seaweed integration would quickly become economically viable. Additional value may be added to an integrated system in the potential eco-labelling of its products (Barrington et al. 2009). This would allow producers to apply higher prices to all outputs (fish and seaweed) of the system. This may, however, subsequently render aquaculture products inaccessible to some consumers due to elevated prices.

Economic value is also associated with the waste resource itself. In 2008, global production of aquatic plants reached an estimated 15.8 million tonnes, 93.8% of total world-wide production (FAO 2010). Hall et al. (2011) reported that 99.5% of world seaweed production currently originates from aquaculture. Though the majority of this production occurs in open-water systems, land-based cultivation is typically dependent on artificially-available nitrogen, phosphorus, and dissolved inorganic carbon as CO_2 to maintain commercial yields (Schuenhoff et al. 2006; Acadian Seaplants Ltd., pers comm). Even seaweeds cultivated in open-water systems are dependent on, or productivity is increased by, the presence of nutrients in excess of ambient levels. These inputs are available at virtually no cost within aquaculture systems.

The optimization of an integrated aquaculture system will depend on the objectives to achieve, be they biofiltration or biomass yield (Schuenhoff et al. 2006). Buschmann et al. (1994) concluded that optimizing both seaweed growth and nutrient

removal cannot be done simultaneously, since productivity decreases as nutrients are depleted from the water through uptake by seaweeds. Optimal integration may also depend on strategic uptake of specific nutrients. In Chapter 4, it was demonstrated that the biomass required to remove 50% of the nitrogen released in a recirculating aquaculture system might remove 100% of the carbon dioxide. The surface area required to accomplish this would be 2.5 to 3.5 times that required for finfish culture. However, if this level of investment is not practical based on budget and land, carbon dioxide removal to 50% of that released may be more efficient.

Clearly, land-based integration is far more complex than simple, extensive cultivation of seaweeds, expecting these materials to soak up the waste nutrients of a particular finfish system. Integrated aquaculture is a potential solution to an expanding aquaculture industry faced with occasional challenges of narrow profit margins and low public opinion, and the on-going issue of waste mitigation (Barrington et al. 2009). Challenges of economics and space requirements may be met through improved efficiencies. Careful planning and strategic implementation will be key to the success of IMTA on land and its widespread commercial implementation.

References

Abreu MH, Pereira R, Yarish C, Buschmann AH, Sousa-Pinto I (2011) IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. Aquaculture 312: 77-87

Amat MA, Braud JP (1990) Ammonium uptake by *Chondrus crispus* Stackhouse (Gigartinales, Rhodophyta) in culture. Hydrobiologia 204/205: 467-471

Asare SO, Harlin MM (1983) Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode Island Sound. J Phycol 19: 254-257

Barrington K, Chopin T, Robinson S (2009) Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. *In* Soto D (2009) Integrated mariculture: A global review. FAO Fisheries and Aquaculture Technical Paper 529. Food and Agriculture Organization of the United Nations, Rome, pp. 7-46

Barrington K, Ridler N, Chopin T, Robinson S, Robinson B (2010) Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquacult Int 18: 201-211

Barsanti L, Bualtieri P (2006) Algae: Anatomy, Biochemistry, and Biotechnology. CRC Press, Boca Raton, Fl.

Beer S, Eshel A (1985) Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Aust J Mar Freshw Res 36: 785-792

Bidwell RGS, McLachlan, J, Lloyd, NDH (1985) Tank cultivation of Irish moss, *Chondrus crispus* Stackhouse. Bot Mar 28: 87-97

Bird C J, McLachlan, JL (1992) Seaweed flora of the Maritimes. 1. Rhodophyta – the red algae. Biopress Ltd., Bristol, England. 177 p

Blancheton JP, Bosc P, Hussenot JME, d'Orbcastel ER, Romain, D (2009) The 'new' European fish culture systems: Recirculating systems, offshore cages, integrated systems. Cah Agricult 18: 227-234 [in French, English abstract]

Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I, Corner R (2010) Aquaculture: global status and trends. Phil Trans R Soc B 365: 2897-2912

Bower CE, Bidwell JP (1978) Ionization of ammonia in seawater: effects of temperature, pH, and salinity. J Fish Res Board Can 35: 1012-1016

Boyd CE, Tucker C, McNevin A, Bostick K, Clay J (2007) Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. Rev Fish Sci 15: 327-360

Brown N (2002) Flatfish farming systems in the Atlantic region. Rev Fish Sci 10(3/4): 403-419

Buschmann AH, Mora OA, Gomez P, Bottger M, Buitano S, Retamaleo C, Vergara PA, Gutierrez A (1994) *Gracilaria chilensis* outdoor tank cultivation in Chile: Use of land-based salmon culture effluents. Aquacult Eng 13: 283-300

Buschmann AH, Troell M, Kautsky N, Kautsky L (1996) Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta). Hydrobiologia 326/327: 75-82

Cahill PI, Hurd CL, Lokman M (2010) Keeping the water clean—seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. Aquaculture 306: 153-159

Carmona R, Kraemer GP, Yarish C (2006) Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish-algal aquaculture system. Aquaculture 252: 54-65

Chen LCM, McLachlan J (1971) The life history of *Chondrus crispus* in culture. Can J Bot 50: 1055-1060

Chopin T, Gallant T, Davison I (1995) Phosphorus and nitrogen nutrition in *Chondrus crispus* (Rhodophyta): effects on total phosphorus and nitrogen content, carrageenan production, and photosynthetic pigments and metabolism. J Phycol 31: 283-293

Chopin T, Wagey BT (1999) Factorial study of the effects of phosphorus and nitrogen enrichments on nutrient and carrageenan content in *Chondrus crispus* (Rhodophyceae) and on residual nutrient concentration in seawater. Bot Mar 42: 23-31

Chopin T, Yarish C, Wilkes R, Belyea E, Lu S, Mathieson A (1999a) Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. J Appl Phycol 11: 463-472

Chopin T, Sharp G, Belyea E, Semple R, Jones D (1999b) Open-water aquaculture of the red alga *Chondrus crispus* in Prince Edward Island, Canada. Hydrobiologia 398/399: 417-425

Chopin T, Buschmann A H, Halling C, Troell M, Kautsky N, Neori A, Kraemer G P, Zertuche-Gonzalez JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J Phycol 37: 975-986

Copertino MS, Tormena T, Seeliger U (2009) Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Chlorophyceae) cultivated in shrimp aquaculture waste water. J Appl Phycol 21: 31-45

Craigie JS (1990) Irish moss cultivation: some reflections. In Economically important plants of the Atlantic: their biology and cultivation. *Eds* Yarish C, Penniman C A, Van Patten P. Connecticut Sea Grant College Program, Marine Sciences Inst, Groton. pp. 37-52

Davies IM, Slaski RJ (2003) Waste production by farmed Atlantic halibut (*Hippoglossus hippoglossus* L.) Aquaculture 219: 495-502

Dean PR, Hurd CL (2007) Seasonal growth, erosion rates, and nitrogen and photosynthetic ecophysiology of *Undaria pinnatifida* (Heterokontophyta) in southern New Zealand. J Phycol 43: 1138-1148

Demetropoulos CL, Langdon CJ (2004a) Pacific dulse (*Palmaria mollis*) as a food and biofilter in recirculated, land-based abalone culture systems. Aquacult Eng 32: 57-75

Demetropoulos CL, Langdon CJ (2004b) Enhanced production of Pacific dulse (*Palmaria mollis*) for co-culture with abalone in a land-based system: nitrogen, phosphorus, and trace metal nutrition. Aquaculture 235: 433-455

FAO (2011) World aquaculture 2010: FAO fisheries and aquaculture technical paper 500/1. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgol Meeres 34: 15-29

Foss A, Imsland AK, Roth B, Schram E, Stefansson SO (2009) Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). Aquaculture 296: 45-50

Friedlander M, Dawes CJ (1985) In situ uptake kinetics of ammonium and phosphate and chemical composition of the red seaweed *Gracilaria tikvahiae*. J Phycol 21: 448-453

Garbary DJ, Tompkins E, White K, Corey P, Kim JK (2011) Temporal and spatial variation in the distribution of life history phases of *Chondrus crispus* (Gigartinales, Rhodophyta). Algae 26: 66-71

Gerard VA, Mann KH (1979) Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. J Phycol 15: 33-41

Good C, Davidson J, Welsh C, Snekvik K, Summerfelt S (2010) The effects of carbon dioxide on performance and histopathology of rainbow trout *Onchorynchus mykiss* in water recirculation aquaculture systems. Aquacult Eng 42: 51-56

Guiry MD, Cunningham EM (1984) Photoperiodic and temperature responses in the reproduction of north-eastern Atlantic *Gigartina acicularis* (Rhodophyta: Gigartinales). Phycologia 23: 357-367

Haglund K, Pedersén M (1993) Outdoor pond cultivation of the subtropical marine red alga *Gracilaria tenuistipitata* in brackish water in Sweden. Growth, nutrient uptake, co-cultivation with rainbow trout and epiphyte control. J Appl Phycol 5: 271-284

Hall SJ, Delaporte A, Phillips MJ, Beveridge M, O'Keefe M (2011) Blue frontiers: managing the environmental costs of aquaculture. The WorldFish Center, Penang, Malaysia

Hallaråker H, Foklvord A, Stefansson SO (1995) Growth of juvenile halibut (*Hippoglossus hippoglossus*) related to temperature, day length and feeding regime. Neth J Sea Res 34: 139-147

Harlin MM (1978) Nitrate uptake by *Enteromorpha* spp. (Chlorophyceae): applications to aquaculture systems. Aquaculture 15: 373-376

Harrison PJ, Hurd CL (2001) Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah Biol Mar 42: 71-82

Hayashi L, Yokoya NS, Ostini S, Pereira RTL, Braga ES, Oliveira EC (2008) Nutrients removed by *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in integrated cultivation with fishes in re-circulating water. Aquaculture 277: 185-191

Hernández I, Pérez-Pastor A, Vergara JJ, Martinez-Aragón JF, Fernández-Engo MA, Pérez-Lloréns JL (2006) Studies on the biofiltration capacity of *Gracilariopsis longissima*: from microscale to macroscale. Aquaculture 252: 43-53

Jones MN (1984) Nitrate reduction by shaking with cadmium: alternative to cadmium columns. Water Res 18(5): 643-646

Jones RD, Morita RY (1985) Low temperature growth and whole cell kinetics of marine ammonium oxidizer. Mar Eco Prog Ser 21: 239-243

Jonassen TM, Imsland AK, Kadowaki S, Stefansson SO (2000) Interaction of temperature and photoperiod on growth of Atlantic halibut *Hippoglossus hippoglossus* L. Aquacult Res 31: 219-227

Kim JK, Kraemer GP, Neefus CD, Chung IK, Yarish C (2007) Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of *Porphyra* (Bangiales, Rhodophyta) native to the New England coast. J Appl Phycol 19: 431-440

Kübler JE, Dudgeon SR (1996) Temperature dependent change in the complexity of form of *Chondrus crispus* fronds. J Exp Mar Biol Ecol 207: 15-24

Kübler JE, Raven JA (1995) The interaction between inorganic carbon acquisition and light supply in *Palmaria palmata* (Rhodophyta). J Phycol 31: 369-375

Landau M (1992) Introduction to aquaculture. John Wiley & Sons, Inc., NY. 440 p

Langton RW, Haines KC, Lyon RE (1977) Ammonia nitrogen production be the bivalve mollusc *Tapes japonica* and its recovery by the red seaweed *Hypnea musciformis* in a tropical mariculture system. Helgol Wiss Meeresunters 30: 217-229

Laycock MV, Morgan KC, Craigie JS (1981) Physiological factors affecting the accumulation of L-citrullinyl-L-arginine in *Chondrus crispus*. Can J Bot 59: 522-527

Liddicoat MI, Tibbits S, Butler EI (1975) The determination of ammonia in seawater. Limnol Oceanogr 20: 131-132

Lobban CS, Harrison PJ (1996) Seaweed ecology and physiology. Cambridge University Press, New York, NY. 366 p

Maestrini SY, Robert JM, Truquet I (1982) Simultaneous uptake of ammonium and nitrate by oyster-pond algae. Mar Bio Lett 3(3): 143-153

Magnusson G, Axelsson L, Larsson C, Ryberg H (1994) A model for calculation of nutrient uptake capacity of seaweed in cultivation. Bot Mar 37: 115-123

Martinez B, Rico JM (2002) Seasonal variation of P content and major N pools in *Palmaria palmata* (Rhodophyta). J Phycol 38: 1082-1089

Martinez B, Rico JM (2004) Inorganic nitrogen and phosphorous uptake kinetics in *Palmaria palmata* and *Chondrus crispus* (Rhodophyta). J Phycol 40: 642-650

Mata L, Schuenhoff A, Santos R (2010) A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. J Appl Phycol 22: 639-644

Matos J, Costa S, Rodrigues A, Pereira R, Pinto IS (2006) Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquaculture 252: 31-42

McCurdy P (1980) Investigation of a unique population of *Chondrus crispus* in Basin Head Harbour, Prince Edward Island. Department of Fisheries and Oceans Canada, CS155-0-6302, 060-005

Menéndez M, Herrera J, Comin FA (2002) Effect of nitrogen and phosphorus supply on growth, chlorophyll content and tissue composition of the macroalga *Chaetomorpha linum* (O.F. Müll.) Kütz in a Mediterranean coastal lagoon. Sci Mar 66 (4): 355-364

Metaxa E, Deviller G, Pagand P, Alliaume C, Casellas C, Blancheton JP (2006) High rate algal pond treatment for water reuse in a marine fish recirculation system: Water purification and fish health. Aquaculture 252: 92-101

Morgan KC, Shacklock PF, Simpson FJ (1980a) Some aspects of the culture of *Palmaria palmata* in greenhouse tanks. Bot Mar 23: 765-770

Morgan KC, Wright JLC, Simpson FJ (1980b) Review of chemical constituents of the red alga *Palmaria palmata* (Dulse). Econ Bot 34(1): 27-50

Morgan KC, Simpson FJ (1981a) The cultivation of *Palmaria palmata*. Effect of light intensity and temperature on growth and chemical composition. Bot Mar 24: 547-552

Morgan KC, Simpson FJ (1981b) Cultivation of *Palmaria* (*Rhodymenia*) *palmata*: effect of high concentrations of nitrate and ammonium on growth and nitrogen uptake. Aquat Bot 11: 167-171

Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N:P ratio and N-pulse concentration and frequency on the growth of *Gracilaria cornea* (Gracilariales, Rhodophyta) in culture. Hydrobiologia 398/399: 315-320

Navarro NP, Palacios M, Mansilla A, Jofre J (2010) Morphological and growth alterations on early development stages of *Iridaea cordata* (Rhodophyta) under different intensities of UVB radiation. Rev Biol Mar Oceanog 45: 255-265

Neish AC, Shacklock PF, Fox CH, Simpson FJ (1977) The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. Can J Bot 55: 2263-2271

Neori A, Krom MD, Ellner SP, Boyd CE, Popper D, Rabinovitch R, Davison PJ, Dvir O, Zuber D, Ucko M, Angel D, Gordin H (1996) Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. Aquaculture 141: 183-199

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231: 361-391

Nobre, A.M., Roberston-Andersson, D., Neori, A., and Sankar, K (2010) Ecological-economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture 306: 116-126

Otsuki T (2001) A study for the biological CO_2 fixation and utilization system. Sci Total Environ 277: 21-25

Pagand P, Blancheton JP, Lemoalle J, Casellas C (2000) The use of high rate algal ponds for the treatment of marine effluent from a recirculating fish rearing system. Aquacult Res 31(10): 729-736

Pang S, Lüning K (2004) Tank cultivation of the red alga *Palmaria palmata*: Effects of intermittent light on growth rate, yield and growth kinetics. J Appl Phycol 16: 93-99

Pang SJ, Lüning K (2006) Tank cultivation of the red alga *Palmaria palmata*: Year-round induction of tetrasporangia, tetraspore release in darkness and mass cultivation of vegetative thalli. Aquaculture 252: 20-30

Pang S, Shan T (2008) Temperature and light tolerance of representative brown, green and red algae in tumble culture revealed by chlorophyll fluorescence measurements. Acta Oceanol Sin 27: 137-146

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK

Person-Le Ruyet J, Galland R, Le Roux A, Chartois H (1997) Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). Aquaculture 154: 155-171

Ryther JH (1977) Preliminary results with a pilot plant waste recycling-marine aquaculture system. In: Wastewater Renovation and Reuse. D'Itri (Eds.) Marcel Dekker, Inc., New York. pp. 89-132

Ryther JH, DeBoer JA, Lapointe BE (1979) Cultivation of seaweeds for hydrocolloids, waste treatment, and biomass for energy conservation. Proc Int Seaweed Symp 9: 1-17

Sagert S, Schubert H (2000) Acclimation of *Palmaria palmata* (Rhodophyta) to light intensity: comparison between artificial and natural light fields. J Phycol 36: 1119-1128

Schuenhoff A, Mata L, Santos R (2006) The tetrasporophyte of *Asparagopsis armata* as a novel seaweed biofilter. Aquaculture 252: 3-11

Schulz KG, Barcelos e Ramos J, Zeebe RE, Riebesell U (2009) CO_2 perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity. Biogeosciences 6: 2145-2153

Shacklock PF, Craigie JS (1986) Winter survival and growth of *Chondrus crispus* in onshore culture tanks. Can J Fish Aquat Sci 43: 263-268

Sharp G, Semple R, Vandermeulen H, Wilson M, LaRocque C, Nebel S (2010) The Basin Head Irish Moss (*Chondrus crispus*) population abundance and distribution 1980 to 2008. DFO Can Sci Advis Sec Res Doc 2010/054. vi + 32 p

Simpson FJ, Shacklock PF (1979) The cultivation of *Chondrus crispus*: effect of temperature on growth and carrageenan production. Bot Mar 22: 295-298

Smith RG, Bidwell RGS (1989) Mechanism of photosynthetic carbon dioxide uptake by the red macroalga, *Chondrus crispus*. Plant Physiol 89: 93-99

Soto D (2009) Integrated mariculture: A global review. FAO Fisheries and Aquaculture Technical Paper 529. Food and Agriculture Organization of the United Nations, Rome

Tang Q, Zhang J, Fang J (2011) Shellfish and seaweed mariculture increase atmospheric CO_2 absorption by coastal ecosystems. Mar Ecol Prog Ser 424: 97-104

Timmons MB, Ebeling JM, Wheaton FW, Summerfelt ST, Vinci BJ (2002) Recirculating aquaculture systems. 2nd edition. Cayuga Aqua Ventures, Ithaca, NY. 769 p

Troell M, Ronnback P, Halling C, Kautsky N, Buschmann A (1999) Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. J Appl Phycol 11: 89-97

Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang, J (2009) Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297: 1-4

van der Meer JP, Todd ER (1980) The life history of *Palmaria palmata* in culture. A new type for the Rhodophyta. Can J Bot 58: 1250-1256

White KL, Kim, JK, Garbary DJ (2011) Effects of land-based fish farm effluent on the morphology and growth of *Ascophyllum nodosum* (Fucales, Phaeophyceae) in southwestern Nova Scotia. Algae 26: 253-263

Wortman B, Wheaton FW (1991) Temperature effects on biodrum nitrification. Aquacult Eng 10: 183-205

Xu Y, Fang J, Wei W (2008) Application of *Gracilaria lichenoides* (Rhodophyta) for alleviating excess nutrients in aquaculture. J Appl Phycol 20: 199-203

Appendix I: Dry Weight and Phycoerytherin Data, Experiment 1 (Chapter 2)

Table I.i Dry weights (%DW) of *Palmaria palmata* and *Chondrus crispus* during four-week culture experiment at difference levels of nitrate enrichment and water temperature (Mean±SD; *collected from tissues acclimated at 10˚C ambient seawater for 4 days).

	Week 1		Week 2		Week 3		Week 4	
	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-
Palmaria palmata								
Initial *	22.6 ±0.9							
6˚C	24.23±3.9	30.90±0.8	23.43±2.9	24.86±5.5	24.91±4.3	21.34±2.6	21.81±2.6	21.74±6.5
10˚C	23.92±5.9	24.31±2.5	23.40±7.1	21.00±2.4	27.44±0.7	24.27±2.0	23.84±1.3	22.63±2.9
17˚C	22.41±4.4	26.93±1.2	22.89±1.8	19.07±2.3	24.06±3.6	23.03±3.4	24.46±1.5	24.01±1.4
Chondrus crispus								
Initial *	20.10 ±1.1							
6˚C	26.34±1.9	26.22±3.8	27.74±1.8	26.67±4.3	30.44±2.3	24.48±0.6	30.25±0.8	27.06±1.2
10˚C	28.05±2.5	26.93±1.8	27.47±3.6	25.10±2.9	29.78±0.5	23.82±0.7	29.86±1.1	24.97±0.2
17˚C	25.78±0.1	24.29±2.4	28.81±0.4	24.71±1.5	31.15±1.4	23.40±0.1	29.78±2.6	23.18±0.8

Table I.ii Phycoerythrin contents (mg.gFW-1) of *Palmaria palmata* and *Chondrus crispus* during four-week culture experiment at difference levels of nitrate enrichment and water temperature (Mean±SD; *collected from tissues acclimated at 10˚C ambient seawater for 4 days).

	Week 1		Week 2		Week 3		Week 4	
	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-	30 µm NO_3^-	300 µm NO_3^-
Palmaria palmata								
Initial	9.37							
6˚C	5.17±3.77	26.92±9.89	0.64±1.05	9.50±2.60	3.39±2.78	11.72±5.88	3.04±1.47	18.36±5.81
10˚C	8.01±1.46	17.20±17.51	1.99±2.24	5.19±2.60	5.59±6.20	21.96±10.09	4.13±4.84	24.08±5.30
17˚C	2.57±0.58	9.31±2.67	2.20±0.71	8.38±6.11	4.09±0.44	16.62±5.0	2.32±0.51	28.27±5.40
Chondrus crispus								
Initial	4.83							
6˚C	11.77±2.6	11.73±5.00	2.52±0.88	7.08±1.75	1.76±1.06	6.45±1.92	1.37±0.24	8.95±1.51
10˚C	6.88±4.17	5.73±11.29	1.12±0.63	2.95±0.48	0.74±0.51	8.13±5.07	0.43±0.10	9.47±5.77
17˚C	3.71±2.17	11.29±3.96	0.70±0.38	4.97±3.25	0.95±0.34	16.22±3.09	0.54±0.22	12.57±0.69

Appendix II: Specific Growth Rate Regression for Experiment 1 (Chapter 2)

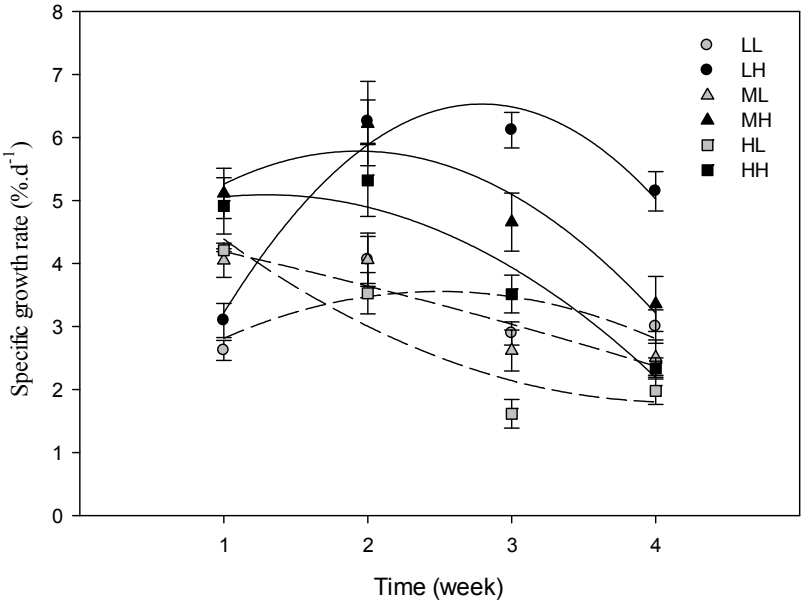

Figure II.i Specific growth rate (SGR) of *Palmaria palmata* at 6°C (L), 10°C (M), and 17°C (H) and 30 µM (L) and 300 µM (H) nitrate (mean ±SE).

Table II.i Regression formulas for SGR of *Palmaria palmata*, Experiment 1.

Treatment			
Temp (°C)	Nitrate (µM)	Regression formula	r^2-value
6	30	$y = 2.813(x^2) + 0.997(x) - 0.334$	0.245
6	300	$y = 3.219(x^2) + 3.697(x) - 1.032$	0.854
10	30	$y = 4.191(x^2) - 0.519(x) - 0.030$	0.612
10	300	$y = 5.261(x^2) + 1.123(x) - 0.602$	0.607
17	30	$y = 4.388(x^2) - 1.648(x) - 0.262$	0.794
17	300	$y = 5.058(x^2) + 0.232(x) - 0.396$	0.755

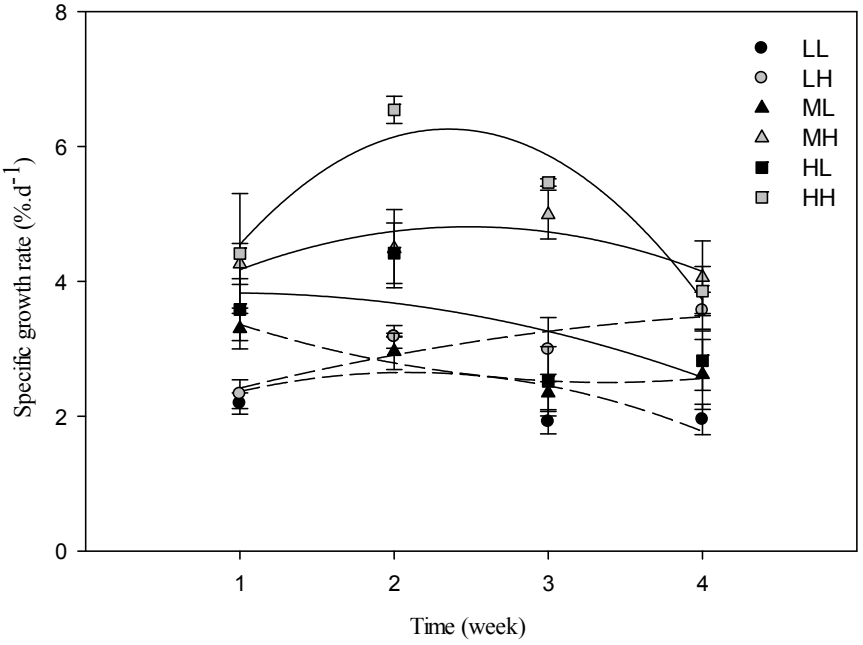

Figure II.ii Specific growth rate (SGR) of *Chondrus crispus* at 6°C (L), 10°C (M), and 17°C (H) and 30 µM (L) and 300 µM (H) nitrate (mean ±SE).

Table II.ii Regression formulas for SGR of *Chondrus crispus*, Experiment 1.

Treatment			
Temp (°C)	Nitrate (µM)	Regression formula	r^2-value
6	30	$y = 2.365(x^2) + 0.521(x) - 0.240$	0.335
6	300	$y = 2.418(x^2) + 0.555(x) - 0.068$	0.412
10	30	$y = 3.358(x^2) - 0.724(x) + 0.153$	0.291
10	300	$y = 4.174(x^2) + 0.858(x) - 0.289$	0.154
17	30	$y = 3.829(x^2) - 0.019(x) - 0.133$	0.242
17	300	$y = 4.548(x^2) + 2.530(x) - 0.935$	0.628

Appendix III: Statistical Data, Experiment 1 (Chapter 2)

Temp ≡ water temperature (6, 10, 17°C); Nitrogen ≡ media nitrate (30 and 300 μM)

Table III.i.a Analysis of variance (ANOVA) tables for tissue nitrogen (N) and carbon (C) in *Palmaria palmata*.

ANOVA for %C, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	1.4647	1.1964	0.5982	1.13	0.352
Nitrogen	1	3.6214	3.6214	3.6214	6.86	0.021
Error	13	6.8586	6.8586	0.5276		
Total	16	11.9447				

ANOVA for %N, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	0.0776	0.0449	0.0225	0.29	0.753
Nitrogen	1	11.6400	11.6400	11.6400	150.32	0.000
Error	13	1.0067	1.0067	0.0774		
Total	16	12.7243				

ANOVA for C:N ratio, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	0.21	9.08	4.54	0.59	0.567
Nitrogen	1	850.91	850.91	850.91	111.09	0.000
Error	13	99.58	99.58	7.66		
Total	16	950.69				

Table III.i.b Analysis of variance (ANOVA) tables for tissue nitrogen (N) and carbon (C) in *Chondrus crispus*.

ANOVA for %C, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	13.9927	15.4129	7.7065	8.11	0.005
Nitrogen	1	6.9944	6.9944	6.9944	7.36	0.018
Error	13	12.3576	12.3576	0.9506		
Total	16	33.3447				

ANOVA for %N, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	2.767	1.720	0.860	14.77	0.000
Nitrogen	1	30.927	30.927	30.927	531.41	0.000
Error	13	0.757	0.757	0.058		
Total	16	34.451				

ANOVA for C:N ratio, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	81.80	42.15	21.07	8.77	0.004
Nitrogen	1	1022.68	1022.68	1022.68	425.73	0.000
Error	13	31.23	31.23	2.40		
Total	16	1135.71				

Table III.ii.a ANOVA tables for nitrogen (N) and carbon (C) removal in *Palmaria palmata*.

Analysis of Variance for N-removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	0.07051	0.07051	0.03526	14.41	0.000
Nitrogen	1	0.28009	0.28009	0.28009	114.46	0.000
Error	14	0.03426	0.03426	0.00245		
Total	17	0.38486				

Analysis of Variance for C removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	16.583	16.583	8.292	12.57	0.001
Nitrogen	1	14.908	14.908	14.908	22.59	0.000
Error	14	9.239	9.239	0.660		
Total	17	40.730				

Table III.ii.b ANOVA tables for nitrogen (N) and carbon (C) removal in *Chondrus crispus*.

Analysis of Variance for Nremoval, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	0.02339	0.01065	0.00532	0.66	0.533
Nitrogen	1	2.49665	2.49665	2.49665	309.60	0.000
Error	13	0.10483	0.10483	0.00806		
Total	16	2.62488				

Analysis of Variance for Cremoval, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	2.7184	2.7184	1.3592	3.45	0.060
Nitrogen	1	8.9548	8.9548	8.9548	22.74	0.000
Error	14	5.5139	5.5139	0.3939		
Total	17	17.1871				

Table III.iii ANOVA tables for specific growth rate (SGR).

Palmaria palmata

Analysis of Variance for SGR, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	21.153	21.153	10.577	8.96	0.000
nit	1	46.325	46.325	46.325	39.26	0.000
Error	50	58.994	58.994	1.180		
Total	53	126.472				

Chondrus crispus

Analysis of Variance for SGR, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	19.688	15.651	7.825	9.23	0.000
nitrogen	1	34.720	34.720	34.720	40.96	0.000
Error	47	39.838	39.838	0.848		

Table III.iv ANOVA tables for phosphate uptake.

Palmaria palmata

Analysis of Variance for Puptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	6084	6084	3042	0.39	0.677
Nit	1	1763013	1763013	1763013	228.49	0.000
Error	32	246910	246910	7716		
Total	35	2016007				

Chondrus crispus

Analysis of Variance for Puptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	238219	238219	119110	6.90	0.003
Nit	1	1795014	1795014	1795014	104.05	0.000
Error	32	552044	552044	17251		
Total	35	2585277				

Table III.v ANOVA tables for pigment content.

Palmaria palmata

Effect	Num DF	Den DF	F Value	Pr > F
week	3	46	9.97	<.0001
temp*nit	2	46	0.09	0.9122
week*temp*nit	15	46	3.17	0.0013
temp	2	46	0.64	0.5337
nit	1	46	95.01	<.0001

Chondrus crispus

Effect	Num DF	Den DF	F Value	Pr > F
week	3	46	11.51	<.0001
temp*nit	2	46	12.46	<.0001
week*temp*nit	15	46	4.45	<.0001
temp	2	46	6.36	0.0036
nit	1	46	134.84	<.0001

Table III.vi ANOVA tables for dry weight ratio.

Palmaria palmata

Effect	Num DF	Den DF	F Value	Pr > F
week	3	46	2.48	0.0725
temp*nit	2	46	1.30	0.2833
week*temp*nit	15	46	1.61	0.1084
temp	2	46	0.47	0.6253
nit	1	46	0.07	0.7982

Chondrus crispus

Effect	Num DF	Den DF	F Value	Pr > F
week	3	46	1.44	0.2438
temp*nit	2	46	2.72	0.0763
week*temp*nit	15	46	1.94	0.0432
temp	2	46	1.95	0.1537
nit	1	46	77.74	<.0001

Appendix IV: Dry Weight Data, Experiments 2 and 3 (Chapter 3)

Table IV.i Dry weights of *Palmaria palmata* during a four-week culture experiment at 10˚C and various levels of nitrate and ammonium enrichment (mean±SD; n=6; *collected from tissues acclimated to 10˚C ambient seawater over 4 days).

Nitrate (μM)	Ammonium (μM)	%DW Week 1	Week 2	Week 3	Week 4
Initial *		14.30 (±2.34)			
300	0	18.58 (±1.60)	19.75 (±2.81)	19.39 (±2.24)	20.95 (±1.26)
0	300	22.22 (±9.02)	21.41 (±2.50)	20.45 (±1.89)	20.18 (±0.68)
150	150	18.38 (±3.84)	19.77 (±2.01)	20.60 (±2.31)	20.21 (±1.18)
270	30	19.18 (±2.34)	19.21 (±2.04)	19.96 (±1.38)	20.82 (±1.00)
30	270	19.00 (±2.79)	21.00 (±2.04)	20.56 (±3.14)	20.60 (±2.24)

Table IV.ii Dry weights of *Chondrus crispus* during a four-week culture experiment at 10˚C and various levels of nitrate and ammonium enrichment (mean±SD; n=6; *collected from tissues acclimated to 10°C ambient seawater over 3 days).

Nitrate (μM)	Ammonium (μM)	%DW Week 1	Week 2	Week 3	Week 4
Initial *		23.10 (±0.34)			
300	0	24.47 (±0.43)	23.46 (±0.26)	24.34 (±0.37)	23.46 (±0.18)
0	300	24.46 (±0.61)	23.34 (±0.16)	23.52 (±0.42)	23.43 (±0.37)
150	150	24.33 (±0.65)	23.64 (±0.51)	23.60 (±0.33)	23.04 (±0.19)
270	30	23.93 (±0.43)	23.67 (±0.50)	24.19 (±0.24)	23.94 (±0.25)
30	270	23.77 (±0.58)	24.04 (±0.37)	23.93 (±0.40)	23.67 (±0.15)

Appendix V: Statistical Data, Experiments 2 and 3 (Chapter 3)

week ≡ time period of four-week culture experiment; Nit ≡ media nitrate/ammonium (treatments 1 to 5, see Table 3.1); Block ≡ tank

Table V.i Repeated Measures ANOVA (SAS) tables for specific growth rate (SGR).

Palmaria palmata

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F	P
week	3	95	142.8	<0.0001
nit	4	95	6.74	<0.0001
week*nit	12	95	2.15	0.0204

Tests of Effect Slices

Effect	Week	Num DF	Den DF	F	P
week*nit	1	4	95	8.5	<0.0001
week*nit	2	4	95	2.21	0.0737
week*nit	3	4	95	1.06	0.3804
week*nit	4	4	95	0.96	0.435

Chondrus crispus

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F	P
week	3	95	81.62	<0.0001
nit	4	95	1.02	0.4005
week*nit	12	95	0.5	0.9124

Tests of Effect Slices

Effect	Week	Num DF	Den DF	F	P
week*nit	1	4	95	0.68	0.6047
week*nit	2	4	95	0.12	0.9763
week*nit	3	4	95	1.04	0.3897
week*nit	4	4	95	0.75	0.5574

Table V.ii Regression formulas for specific growth rate.

Palmaria palmata

Treatment	Regression formula	r^2-value
1	$y = 6.206(x)^2 + 3.979(x) - 1.566$	0.762
2	$y = 4.687(x)^2 + 4.208(x) - 1.456$	0.781
3	$y = 6.074(x)^2 + 3.270(x) - 1.306$	0.588
4	$y = 7.535(x)^2 + 1.973(x) - 1.025$	0.771
5	$y = 5.417(x)^2 + 3.896(x) - 1.525$	0.651

Chondrus crispus

Treatment	Regression formula	r^2-value
1	$y = 5.026(x)^2 + 1.170(x) - 0.549$	0.575
2	$y = 4.962(x)^2 + 1.329(x) - 0.558$	0.704
3	$y = 4.533(x)^2 + 1.887(x) - 0.733$	0.651
4	$y = 4.891(x)^2 + 1.430(x) - 0.607$	0.645
5	$y = 4.856(x)^2 + 1.643(x) - 0.636$	0.712

Table V.iii.a ANOVA tables for nitrogen uptake by *Palmaria palmata*.

Analysis of Variance for week 3 nitrogen uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.7175	0.7175	0.1435	1.13	0.376
Nit	4	65.7206	65.7206	16.4301	129.45	0.000
Error	20	2.5384	2.5384	0.1269		
Total	29	68.9765				

Analysis of Variance for week 4 nitrogen uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	5.2087	5.2087	1.0417	1.71	0.179
Nit	4	16.0836	16.0836	4.0209	6.59	0.001
Error	20	12.2026	12.2026	0.6101		
Total	29	33.4949				

Table V.iii.b ANOVA tables for nitrogen uptake by *Chondrus crispus*.

Analysis of Variance for week 3 nitrogen uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.4222	0.4222	0.0844	0.38	0.858
Nit	4	6.6074	6.6074	1.6518	7.38	0.001
Error	20	4.4769	4.4769	0.2238		
Total	29	11.5065				

Analysis of Variance for week 4 nitrogen uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	2.1029	2.1029	0.4206	0.72	0.617
Nit	4	24.2606	24.2606	6.0652	10.36	0.000
Error	20	11.7046	11.7046	0.5852		
Total	29	38.0682				

Table V.iv.a ANOVA tables for tissue nitrogen (N) and carbon (C) in *Palmaria palmata*.

Analysis of Variance for %C, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	3.663	3.663	0.733	0.54	0.741
Nit	4	0.733	0.733	0.183	0.14	0.967
Error	20	26.959	26.959	1.348		
Total	29	31.355				

Analysis of Variance for %N, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.26776	0.26776	0.05355	0.69	0.639
Nit	4	5.65445	5.65445	1.41361	18.13	0.000
Error	20	1.55911	1.55911	0.07796		
Total	29	7.48132				

Analysis of Variance for C:N ratio, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	1.8269	1.8269	0.3654	0.58	0.717
Nit	4	46.7001	46.7001	11.6750	18.42	0.000
Error	20	12.6781	12.6781	0.6339		
Total	29	61.2051				

Table V.iv.b ANOVA tables for tissue nitrogen (N) and carbon (C) in *Chondrus crispus*.

Analysis of Variance for %C, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.7790	0.7790	0.1558	1.20	0.344
Nit	4	2.2113	2.2113	0.5528	4.26	0.012
Error	20	2.5927	2.5927	0.1296		
Total	29	5.5830				

Analysis of Variance for %N, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.23383	0.23383	0.04677	0.58	0.716
Nit	4	0.49842	0.49842	0.12461	1.54	0.228
Error	20	1.61534	1.61534	0.08077		
Total	29	2.34759				

Analysis of Variance for C:N ratio, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.5583	0.5583	0.1117	0.75	0.597
Nit	4	0.6127	0.6127	0.1532	1.03	0.418
Error	20	2.9834	2.9834	0.1492		
Total	29	4.1543				

Table V.v.a ANOVA tables for nitrogen (N) and carbon (C) removal in *Palmaria palmata*.

Analysis of Variance for N-removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.018150	0.018150	0.003630	1.81	0.157
Nit	4	0.040631	0.040631	0.010158	5.05	0.006
Error	20	0.040201	0.040201	0.002010		
Total	29	0.098982				

Analysis of Variance for C-removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	3.7662	3.7662	0.7532	3.25	0.026
Nit	4	3.8113	3.8113	0.9528	4.11	0.014
Error	20	4.6376	4.6376	0.2319		
Total	29	12.2151				

Table V.v.b ANOVA tables for nitrogen (N) and carbon (C) removal in *Chondrus crispus*.

Analysis of Variance for N-removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.004126	0.004126	0.000825	0.58	0.714
Nit	4	0.020758	0.020758	0.005190	3.65	0.022
Error	20	0.028408	0.028408	0.001420		
Total	29	0.053293				

Analysis of Variance for C-removal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.12004	0.12004	0.02401	0.33	0.892
Nit	4	1.16114	1.16114	0.29029	3.93	0.016
Error	20	1.47605	1.47605	0.07380		
Total	29	2.75724				

Table V.vi.a ANOVA tables for phosphate uptake in *Palmaria palmata*.

Analysis of Variance for week 3 phosphate uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Nit	4	0.008960	0.008960	0.002240	0.58	0.679
Block	5	0.014533	0.014533	0.002907	0.76	0.592
Error	20	0.076871	0.076871	0.003844		
Total	29	0.100364				

Analysis of Variance for week 4 phosphate uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Nit	4	0.010108	0.010108	0.002527	0.37	0.824
Block	5	0.010191	0.010191	0.002038	0.30	0.906
Error	20	0.134793	0.134793	0.006740		
Total	29	0.155092				

Table V.vi.b ANOVA tables for phosphate uptake in *Chondrus crispus*.

Analysis of Variance for time 4 phosphate uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.016980	0.016980	0.003396	1.81	0.156
Nit	4	0.029229	0.029229	0.007307	3.90	0.017
Error	20	0.037461	0.037461	0.001873		
Total	29	0.083670				

Analysis of Variance for time 7 phosphate uptake, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Block	5	0.006880	0.006880	0.001376	0.81	0.556
Nit	4	0.022599	0.022599	0.005650	3.33	0.030
Error	20	0.033953	0.033953	0.001698		
Total	29	0.063431				

Appendix VI: Phosphate Uptake, Experiments 2 and 3 (Chapter 3)

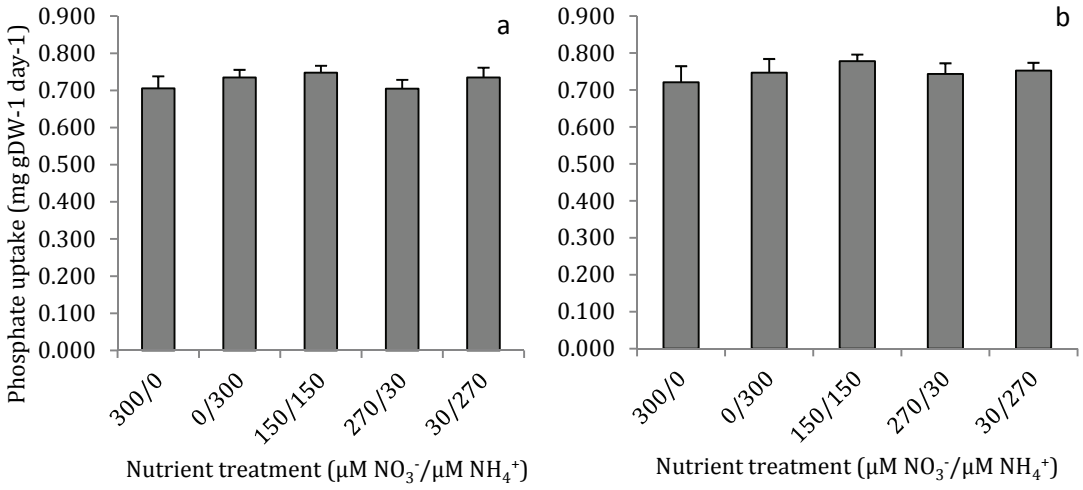

Figure VI.i Phosphate uptake by *Palmaria palmata* at various combinations of nitrogen media during week 3 (a) and week 4 (b) of a four-week culture experiment (mean ±SE). Uptake was calculated as a function of change in media phosphate concentration over four days.

Table VI.i Phosphate uptake by *Chondrus crispus* at various combinations of nitrogen media during a four-week culture experiment (mean ±SD). Uptake was calculated as a function of change in media phosphate concentration over four days. Statistical results for one-way ANOVA of each time period.

Time	Phosphate uptake (µM PO_4^{3+}, NO_3^-/NH_4^+ treatment)							F-value
(days)	300/0	0/300	150/150	270/30	30/270	F-value	P-value	for block
4	0.466 (±0.02)	0.452 (±0.03)	0.457 (±0.03)	0.473 (±0.02)	0.476 (±0.05)	0.48	0.75	0.70
7	0.507 (±0.04)	0.470 (±0.02)	0.504 (±0.05)	0.531 (±0.06)	0.533 (±0.08)	1.17	0.35	0.99
11	0.520 (±0.03)	0.477 (±0.03)	0.515 (±0.03)	0.498 (±0.03)	0.495 (±0.03)	1.32	0.30	0.53
14	0.520 (±0.02)	0.434 (±0.03)	0.500 (±0.04)	0.501 (±0.04)	0.516 (±0.06)	3.90	0.02	1.81
18	0.513 (±0.02)	0.473 (±0.05)	0.488 (±0.05)	0.474 (±0.04)	0.484 (±0.03)	1.89	0.15	6.73
21	0.548 (±0.05)	0.472 (±0.09)	0.505 (±0.08)	0.526 (±0.06)	0.480 (±0.03)	1.62	0.21	2.56
25	0.542 (±0.05)	0.474 (±0.02)	0.492 (±0.01)	0.517 (±0.05)	0.468 (±0.03)	3.33	0.03	0.81
28	0.511 (±0.07)	0.437 (±0.02)	0.487 (±0.08)	0.459 (±0.04)	0.474 (±0.06)	1.61	0.21	2.44

Appendix VII: Ammonium Control, Experiments 2 and 3 (Chapter 3)

Table VII.i Results of t-test for initial and final concentrations of ammonium in control flasks (seaweed absent) at treatment combinations 2, 3, 4, and 5 (see Table 3.1), Experiment 2 involving *P. palmata*.

Treatment	Initial concn	Final concn *	Concn change	P-value **
25 Feb. - 1 Mar. '10				
2	160.67	171.50	10.83	0.758
3	97.50	213.07	115.57	0.000
4	19.68	18.76	-0.92	0.757
5	252.40	292.80	40.40	0.532
4 - 8 Mar. '10				
2	245.90	268.80	22.90	0.567
3	197.10	191.10	-6.00	0.827
4	22.52	22.27	-0.25	0.729
5	284.40	271.50	-12.90	0.740
11 - 15 Mar. '10				
2	276.95	250.30	-26.66	0.449
3	138.47	132.69	-5.78	0.746
4	20.00	18.95	-1.05	0.542
5	185.20	236.91	51.71	0.012
18 - 22 Mar. '10				
2	253.29	283.25	29.96	0.115
3	166.62	196.61	29.99	0.095
4	23.41	21.86	-1.55	0.276
5	350.36	342.84	-7.51	0.685

* Control held four days without seaweed biomass, under fluorescent light and continuous aeration.

** Two-sample t-test, Minitab15.

Notes:

n=3 for all initial concentrations

n=4 for final samples 25 Feb-1 Mar; n=6 for all other final samples

all samples from 25 Feb-1 Mar and 4-8 Mar were analyzed in duplicate

Table VII.ii Results of t-test for initial and final concentrations of ammonium in control flasks (seaweed absent) at treatment combinations 2, 3, 4, and 5 (see Table 3.1), Experiment 3 involving *C. crispus*.

Treatment	Initial concn	Final concn *	Concn change	P-value **
10 - 14 Mar. '11				
2	267.82	403.69	135.88	0.111
3	165.10	276.63	111.53	0.003
4	23.40	34.43	11.03	0.002
5	307.96	430.55	122.58	0.137
14 - 17 Mar. '11				
2	372.61	393.22	20.61	0.590
3	204.78	289.88	85.10	0.002
4	25.40	33.10	7.70	0.041
5	381.00	400.96	19.96	0.544

* Control held four days without seaweed biomass, under fluorescent light and continuous aeration.

** Two-sample t-test, Minitab15.

Notes:

n=2 for all initial concentrations

n=6 for final samples

Appendix VIII: Tissue Nitrogen and Carbon of *Palmaria palmata* (Chapter 4)

Table VIII.i Tissue nitrogen, carbon, and carbon: nitrogen ratio (on dry weight basis) of *Palmaria palmata* integrated with Atlantic halibut recirculating aquaculture (Effluent 1; mean±SD; n = 3).

Date	%N	%C	C:N Ratio
4-Nov-09	3.40 ±0.20	39.76 ±0.38	11.71 ±0.74
10-Nov-09	3.74 ±0.40	40.14 ±1.00	10.83 ±1.44
17-Nov-09	3.38 ±0.29	40.15 ±0.63	11.95 ±1.14
24-Nov-09	3.47 ±0.04	38.87 ±0.77	11.21 ±0.34
8-Dec-09	3.64 ±0.14	39.22 ±0.46	10.79 ±0.40
15-Dec-09	3.79 ±0.21	39.05 ±0.61	10.33 ±0.75
5-Jan-10	4.33 ±0.16	41.89 ±0.29	9.68 ±0.32
19-Jan-10	4.32 ±0.06	41.63 ±0.16	9.63 ±0.10
10-Feb-10	4.21 ±0.05	41.34 ±0.89	9.83 ±0.26
23-Feb-10	3.98 ±0.19	40.81 ±0.21	10.26 ±0.47
9-Mar-10	4.25 ±0.64	42.36 ±1.16	10.09 ±1.15
22-Mar-10	4.25 ±0.06	44.52 ±3.47	10.47 ±0.96
6-Apr-10	4.70 ±0.32	41.94 ±1.40	8.95 ±0.54
26-Apr-10	4.51 ±0.19	40.78 ±0.12	9.05 ±0.36
4-May-10	4.58 ±0.19	38.34 ±1.25	8.37 ±0.29
31-May-10	4.14 ±0.13	38.44 ±1.04	9.28 ±0.51
15-Jun-10	4.27 ±0.18	38.68 ±0.64	9.07 ±0.50
28-Jun-10	4.21 ±0.04	39.74±0.20	9.44 ±0.14
13-Jul-10	4.12 ±0.22	39.48 ±1.36	9.60 ±0.50
27-Jul-10	4.20 ±0.17	38.94 ±0.56	9.27 ±0.44
10-Aug-10	4.33 ±0.16	39.98 ±0.31	9.24 ±0.31
24-Aug-10	4.00 ±0.29	39.91 ±0.32	10.00 ±0.75
7-Sep-10	4.34 ±0.42	40.41 ±0.21	9.37 ±0.88
23-Sep-10	4.52 ±0.09	39.38 ±0.64	8.71 ±0.14
12-Oct-10	4.63 ±0.22	41.03 ±0.42	8.88 ±0.43
26-Oct-10	4.72 ±0.39	39.57 ±0.32	8.42 ±0.64
Min	3.38	38.34	8.37
Max	4.72	44.52	11.95
Mean	4.16	40.24	9.79

Table VIII.ii Tissue nitrogen, carbon, and carbon: nitrogen ratio (on dry weight basis) of *Palmaria palmata* integrated with Atlantic halibut recirculating aquaculture (Effluent 2; mean±SD; n = 3).

Date	%N	%C	C:N Ratio
4-Nov-09	3.53 ±0.35	40.52 ±0.78	11.57 ±1.30
11-Nov-09	3.42 ±0.20	40.42 ±0.76	11.84 ±0.91
19-Nov-09	3.45 ±0.07	40.18 ±0.32	11.65 ±0.21
25-Nov-09	3.52 ±0.30	40.51 ±0.41	11.57 ±1.11
8-Dec-09	3.85 ±0.11	40.52 ±0.20	10.52 ±0.25
16-Dec-09	4.03 ±0.37	39.22 ±0.57	9.79 ±1.00
6-Jan-10	4.21 ±0.09	42.46 ±0.25	10.08 ±0.23
20-Jan-10	4.23 ±0.19	42.20 ±0.81	9.98 ±0.54
11-Feb-10	4.33 ±0.18	40.16 ±0.28	9.29 ±0.37
24-Feb-10	4.56 ±0.43	40.68 ±0.98	8.98 ±1.02
10-Mar-10	4.69 ±0.19	42.03 ±0.25	8.97 ±0.34
19-Mar-10	4.20 ±0.17	36.88 ±1.47	8.80 ±0.71
23-Mar-10	4.65 ±0.23	40.45 ±1.37	8.70 ±0.34
7-Apr-10	4.78 ±0.19	40.28 ±1.22	8.44 ±0.59
26-Apr-10	4.82 ±0.13	39.78 ±1.55	8.25 ±0.53
4-May-10	4.37 ±0.09	38.88 ±0.32	8.90 ±0.11
1-Jun-10	4.29 ±0.21	38.44 ±0.50	8.98 ±0.34
16-Jun-10	4.69 ±0.08	37.48 ±1.27	8.00 ±0.39
6-Jul-10	4.85 ±0.14	38.81 ±0.59	8.01 ±0.34
14-Jul-10	4.68 ±0.07	37.81 ±0.06	8.07 ±0.13
28-Jul-10	4.60 ±0.10	38.81 ±0.15	8.44 ±0.21
11-Aug-10	4.71 ±0.12	39.21 ±0.50	8.33 ±0.22
8-Sep-10	4.41 ±0.22	39.94 ±0.46	9.07 ±0.35
23-Sep-10	4.92 ±0.11	40.31 ±0.61	8.20 ±0.12
12-Oct-10	4.84 ±0.18	40.13 ±0.21	8.31 ±0.33
26-Oct-10	5.02 ±0.21	40.57 ±0.38	8.09 ±0.35
Min	3.42	36.88	8.00
Max	5.02	42.46	11.84
Mean	4.37	39.87	9.26

Table VIII.iii Tissue nitrogen, carbon, and carbon: nitrogen ratio (on dry weight basis) of *Palmaria palmata* integrated with Atlantic halibut recirculating aquaculture (Ambient; mean±SD; n = 3).

Date	%N	%C	C:N Ratio
3-Nov-09	3.51 ±0.08	39.92 ±0.79	11.38 ±0.24
11-Nov-09	3.24 ±0.11	39.83 ±0.59	12.31 ±0.59
19-Nov-09	3.32 ±0.34	39.80 ±0.34	12.07 ±1.35
25-Nov-09	3.44 ±0.04	39.59 ±0.18	11.50 ±0.15
8-Dec-09	3.97 ±0.47	40.52 ±2.90	10.25 ±0.63
16-Dec-09	3.83 ±0.17	40.15 ±0.59	10.50 ±0.62
6-Jan-10	4.11 ±0.22	42.56 ±0.01	10.38 ±0.54
20-Jan-10	4.55 ±0.39	42.30 ±0.81	9.35 ±0.92
12-Feb-10	4.29 ±0.09	40.59 ±0.43	9.47 ±0.29
25-Feb-10	4.32 ±0.16	40.42 ±1.19	9.36 ±0.55
11-Mar-10	4.68 ±0.40	40.46 ±0.45	8.70 ±0.74
23-Mar-10	4.50 ±0.15	39.18 ±0.75	8.71 ±0.12
7-Apr-10	4.36 ±0.24	39.18 ±0.25	9.01 ±0.56
26-Apr-10	3.25 ±0.08	35.11 ±1.07	10.80 ±0.50
4-May-10	3.62 ±0.30	37.94 ±0.62	10.54 ±0.99
1-Jun-10	3.59 ±0.41	38.78 ±0.35	10.90 ±1.18
16-Jun-10	3.50 ±0.51	37.68 ±1.27	10.88 ±1.30
14-Jul-10	3.24 ±0.17	39.64 ±0.75	12.25 ±0.73
28-Jul-10	3.28 ±0.10	39.38 ±0.59	12.00 ±0.19
11-Aug-10	3.33 ±0.15	39.34 ±0.53	11.84 ±0.59
8-Sep-10	3.03 ±0.04	39.64 ±0.40	13.06 ±0.29
23-Sep-10	3.12 ±0.21	39.98 ±0.72	12.84 ±1.05
12-Oct-10	3.00 ±0.20	39.73 ±0.38	13.28 ±1.01
26-Oct-10	3.06 ±0.24	39.73 ±0.38	13.03 ±0.91
Min	3.00	35.11	8.70
Max	4.68	42.56	13.28
Mean	3.67	39.64	11.02

Appendix IX: Tissue Analysis of Macroalgal Samples from Natural Locations

Table IX.i Tissue analysis of seaweed collected from natural habitats.
Values for percent nitrogen and carbon are on dry weight basis.

Species	Sample id	Date	DW:FW	%N	%C	C:N ratio
P. palmata [1]	Point Prim, Digby, NS	27 May '10	0.191	3.76	37.60	10.00
P. palmata	Point Prim, Digby, NS	27 May '10	0.214	3.35	39.10	11.67
P. palmata	Point Prim, Digby, NS	27 May '10	0.185	3.81	38.00	9.97
	Mean		0.196	3.64	38.23	10.55
P. palmata	Charlesville, NS	23 Sep. '10		2.13	30.60	14.37
P. palmata	Charlesville, NS	23 Sep. '10		1.62	34.00	20.99
P. palmata	Charlesville, NS	23 Sep. '10		1.99	26.60	13.37
	Mean			1.91	30.40	16.24
C. crispus [2]	Charlesville, NS	3 Apr. '11	0.195	4.36	30.50	7.00
C. crispus	Charlesville, NS	3 Apr. '11	0.191	4.38	30.60	6.99
C. crispus	Charlesville, NS	3 Apr. '11	0.191	4.83	31.70	6.56
	Mean		0.192	4.52	30.93	6.85
C. crispus	SHL effluent stream [3]	8 Apr. '11	0.249	6.96	30.70	4.41
C. crispus	SHL effluent stream	8 Apr. '11	0.246	6.25	32.60	5.22
C. crispus	SHL effluent stream	8 Apr. '11	0.218	6.56	28.00	4.27
	Mean		0.238	6.59	30.43	4.63

[1] *Palmaria palmata*; [2] *Chondrus crispus*

[3] Scotian Halibut Ltd., Wood's Harbour, NS, land-based Atlantic halibut farm

Appendix X: Timeline of Activities, Experiments 1, 2, and 3.

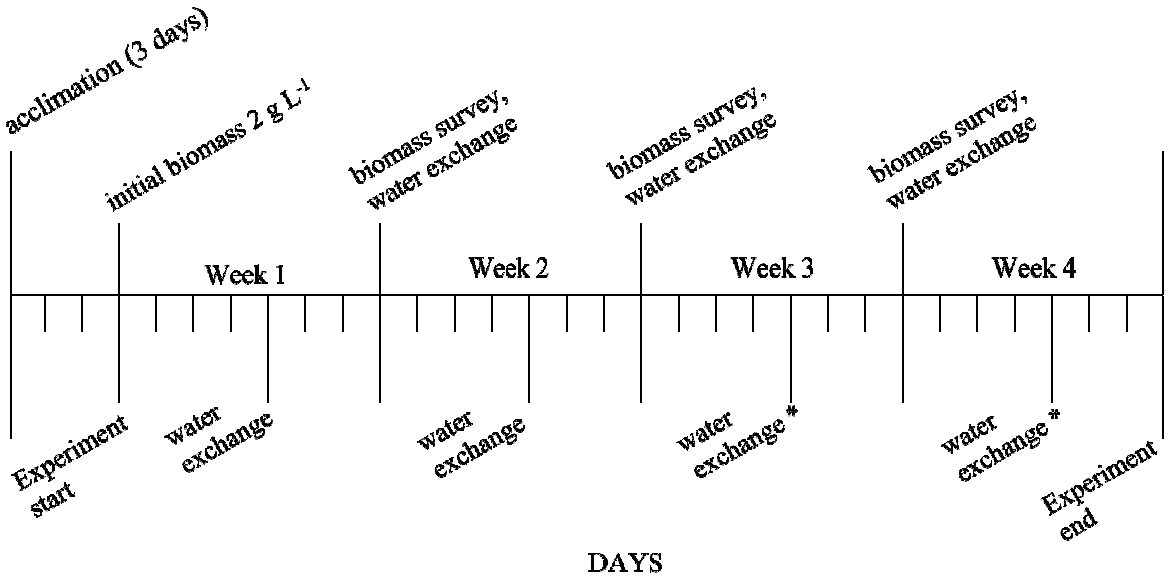

Figure X.i: Timeline of sampling activities for laboratory-based seaweed cultivation experiments. Water samples were collected at all water exchanges, Experiment start, and Experiment end. Tissue samples were collected at all biomass surveys, Experiment start, and Experiment end. (* For experiments 2 and 3, water samples were collected at water exchange, 12-hours post-exchange, and 24-hours post-exchange.)

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