

INTERACTION OF IRRADIANCE AND STOCKING DENSITY ON NUTRIENT
UPTAKE BY RED MACROALGAE. IMPLICATIONS FOR BIOREMEDIATION OF
FISH FARM EFFLUENTS

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2013

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*To my wife, Marcela, and my son, Vicente,
for their tremendous support during this
time of “back to school”*

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ABSTRACT

In land-based integrated aquaculture of marine finfish and macroalgae, rearing space is a large expense. Increasing algal stocking density can increase efficiency, but this may require greater irradiance because of self-shading. To determine the irradiance needed, experiments were conducted in one-litre flasks with enriched seawater under natural and artificial illumination. Under natural illumination, a Daily Photon Dose of $17 \text{ mol m}^{-2} \text{ d}^{-1}$ at 10 and 14 °C, *Palmaria palmata* cultured at 10 g L^{-1} grew 100 % faster and absorbed 20 % more nutrients than *Chondrus crispus*. However, Atlantic halibut farm effluent can reach up to 19 °C in summer, too high for *P. palmata*. Under artificial illumination, *C. crispus* performed better than under natural illumination. Light saturation curves indicated nutrient uptake by *C. crispus* at 10 g L^{-1} and 10 °C was highest at $23 \text{ mol m}^{-2} \text{ d}^{-1}$ irradiance, equivalent to $400 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 16 hours.

LIST OF ABBREVIATIONS USED

A	Absorbance
ATP	Adenosine-5'-triphosphate
AIC	Akaike's information criterion
α	<i>Alpha</i> . Initial slope of light saturation curve
APHA	American Public Health Association
NH ₃ -N	Ammonia-nitrogen
NH ₄ ⁺	Ammonium ion
NH ₄ ⁺ -N	Ammonium-nitrogen
HCO ₃ ⁻	Bicarbonate ion
BOD	Biological oxygen demand
C	Carbon
CO ₂	Carbon dioxide
cm	Centimetre
Chl <i>a</i>	Chlorophyll <i>a</i>
<i>ca.</i>	<i>Circa</i> . Approximately
DPD	Daily photon dose
DAL-AC	Dalhousie University - Agricultural Campus
DB	Dark bottle
DRR	Dark respiration rate
d	Day
°	Degree (plane angle)
°C	Degree Celsius
Ø	Diameter
DW	Dry weight
E	East
Eq.	Equation
<i>et al.</i>	<i>Et alii</i> . And others
Fig.	Figure
FB	Final bottle
DFO	Fisheries and Oceans Canada
ft-c	Foot-candle
FW	Fresh weight
GeO ₂	Germanium dioxide
g	Gram
Hz	Hertz
H	High
h	Hour
hh:mm	Hours and minutes
HPO ₄ ²⁻	Hydrogen phosphate ion
tanh	Hyperbolic tangent function
"	Inch
IB	Initial bottle

IMTA	Integrated multi-trophic aquaculture
ID	Internal diameter
Fe	Iron
I	Irradiance
K	Kelvin color temperature
Kg	Kilogram
Ly	Langley
L	Length
L:D	Light:Dark
Ltd.	Limited liability
L	Litre
Lm	Lumen
R _m	Maximum value of a response variable
MJ	Megajoule
M	Metre
μE	Microeinstein
μg	Microgram
μL	Microlitre
μmol	Micromol
μM	Micromolar
μW	Microwatt
mg	Milligram
mL	Millilitre
mm	Millimetre
min	Minute
'	Minute (plane angle)
M	Molar
nm	Nanometre
NRC	National Research Council Canada
Ln	Natural logarithm
NSERC	Natural Sciences and Engineering Research Council of Canada
NPR	Net photosynthetic rate
NO ₃ ⁻	Nitrate
NO ₂ ⁻	Nitrite
N	Nitrogen
N	North
N°	Number
N	Number of observations
PO ₄ ³⁻	Orthophosphate
PO ₄ ³⁻ -P	Orthophosphate- phosphorus
OD	Outside diameter
P	Page
%	Percent
pers. comm.	Personal communication
pers. observ.	Personal observation
P	Phosphorus

PPFR	Photosynthetic photon flux rate (flat sensor)
PPFFR	Photosynthetic photon flux fluence rate (spherical sensor)
PQ	Photosynthetic quotient
PAR	Photosynthetically active radiation
PS	Photosystem
PBP	Phycobiliprotein
PC	Phycocyanin
PE	Phycoerythrin
PVC	Polyvinyl chloride
PM	<i>Post meridiem.</i> After noon
psi	Pounds per square inch
psu	Practical salinity unit
PAM	Pulse amplitude modulated
r	Radius
<i>g</i>	Relative centrifugal force
R	Response variable
rpm	Revolutions per minute
RNA	Ribonucleic acid
s	Second
SGR	Specific growth rate
SE	Standard error of the means
t	Time in days
T	Time in hours
TAN	Total ammonia nitrogen
TPD	Total photon dose
UV	Ultraviolet radiation
NH ₃	Un-ionized ammonia
unpubl. data	Unpublished data
V	Volume
W	Watt
W	West
Wi	Wide

ACKNOWLEDGEMENTS

First of all, I would like to sincerely thank my “on campus” co-supervisor, Dr. Jim Duston for giving me the opportunity to take part of this project and his support beyond the academic. His enthusiasm and critical suggestions facilitated the success of this initiative. Many thanks to my “off-campus” co-supervisor, Dr. David J. Garbary, for his introduction in the phycology world. My acknowledgement goes also to my defense committee members, Dr. Thierry Chopin and Dr. David Percival for their suggestions to improve my manuscript.

This investigation could not have been possible without the funding given by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the algal material facilitated by Scotian Halibut Ltd., special thanks to its General Manager, Brian C. Blanchard.

I also appreciate the collaboration and good discussion with the NSERC project team, Dr. Jang K. Kim, Peter Corey and Scott Caines. The technical support of the undergraduate students who developed their fourth-year project in this interesting topic, Yichen Sun and Ryan Walker, and Zhuhui Ye (R.I.P.) who cooperated voluntarily in Experiment II. Special thanks to the Aquaculture Centre staff, Paul MacIsaac, Audrie-Jo McConkey, Mitchell Moore and Scott Jeffrey for their partnership during the time of my stay in Truro, and to Krista MacLeod, Greenhouse’s Manager for providing part of the greenhouse to conduct some of the experiments.

I cannot fail to thank the people who supported me before this research, Margaret Wood and Beth McAra from the Dalhousie Student Services. Dr. Derek Anderson, Dr. Santosh Lall, Dr. Toby Balch and Fernando Salazar who help me in the search for a master position.

Last, but not least thanks to my wife, Marcela, and my son, Vicente, who accompanied me in this adventure and that for long time only saw me the weekends that I did not have “to water the algae”. I extend my gratitude to my family in Chile, my mother, Lilian, and my siblings, Jorge and María Isabel, without their valuable support I could not have reached this goal.

CHAPTER 1 INTRODUCTION

In Atlantic Canada, the production of farmed salmonids has grown 10 % in the last five years, while in the same period in Pacific Canada it has fallen 4 % (Statistics Canada 2012). Atlantic salmon is the main salmonid species cultured (70 %) using net-pens as the culture system (Statistics Canada 2012). The expansion of the salmon farming industry in Canada has been constrained by concerns about its environmental, health and social impacts (Fraser et al. 2010; Grant 2010; Loucks et al. 2012). Nutrients released directly to the environment without any treatment are an increasing concern. Faeces and unconsumed feed result in the organic enrichment of the sediment and dissolved nutrients can result in localized eutrophication of the water column (Buschmann et al. 2009; Holmer 2010). An initiative to decrease the nutrient impact is to incorporate bivalves and macroalgae with the fish culture, a system known as Integrated Multi-Trophic Aquaculture (IMTA). Accordingly, the bivalves and macroalgae use the waste nutrients to grow (Chopin et al. 2008; Abreu et al. 2009). However, these open-water systems suffer the disadvantage of not being able to quantify the amelioration of the integrated culture because nutrients are diluted in the ocean and come from numerous sources, not just the farm assessed (Chopin et al. 2001). Environmental organizations, such as the World Wildlife Fund, the David Suzuki Foundation, The Conservation Fund, and the Atlantic Salmon Federation, among others, promote the establishment of only land-based closed-containment aquaculture systems (Pendleton et al. 2005; Summerfelt et al. 2013). The integrated culture of macroalgae and fish is also an ecological way to treat land-based aquaculture effluents (Abreu et al. 2011; Kang et al. 2011). In these systems,

a mass balance approach can be used to account for inputs and outputs. The advantages of using macroalgae over traditional effluent treatment systems are that they can remove the dissolved nutrients excreted by the fish to generate a value-added product (Buschmann et al. 1994). The fraction of nutrients excreted by the fish as dissolved compounds through the gills and urine corresponds to 80 % of nitrogen (N), 16 % of phosphorus (P) and 63 % of carbon (C), respectively (Ackefors and Enell 1994; Buschmann et al. 2009). The remainder, 20 % of N, 84 % of P and 37 % of C, respectively, corresponds to solid wastes. Traditional recirculation or effluent treatment systems only transform N into less toxic forms, through aerobic processing by autotrophic nitrifying bacteria (Lekang 2007). Phosphorus has been removed from aquaculture effluents using aluminum sulfate (alum residual), with 94 to 99 % efficiency in seawater and 68 % in freshwater but this has not been attempted at an industrial scale (Mortula and Gagnon 2007; Sharrer et al. 2009). However, the culture system must not only be environmentally friendly, its installation and operation costs must be commercially viable. Land-based integrated systems are expensive because they require a high investment in land, tank construction, pipe installation, and purchase of equipment for aeration, water circulation, and lighting. Their operation is also costly because of the electricity required to run the equipment mentioned above and its associated labour demand (Titlyanov and Titlyanova 2010; Hafting et al. 2012). For these reasons, it is important to optimize this culture system, by increasing nutrient removal and decreasing costs.

The use of macroalgae to treat effluent is not new, having been used independently or in conjunction with microalgae and bivalve molluscs to treat domestic waste (Ryther et al. 1972; Haines 1976), to treat effluent from bivalve molluscs (Ambler et al. 1988), fish (Vandermeulen and Gordin 1990) and gastropod molluscs (Shpigel and Neori 1996). However, it has long been recognized that these systems are case-specific; each system has to be adjusted to the local variables of illumination, temperature, nutrients, and species (Ryther et al. 1978). For this reason, it is not possible to generalize, and optimization of these systems necessitates a long-term research program.

The Faculty of Agriculture, Dalhousie University (formerly the Nova Scotia Agricultural College until 2012) and Scotian Halibut Ltd. were awarded a Strategic Project Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to investigate the integrated culture of Atlantic halibut [*Hippoglossus hippoglossus* (Linnaeus 1758)], and two red macroalgae, Irish moss [*Chondrus crispus* Stackhouse] and dulse [*Palmaria palmata* (Linnaeus) Weber and Mohr]. Atlantic halibut was selected because it is the principal product of the industrial partner. Red macroalgae are good candidates for integrated land-based culture because there are several species with known culture technology, and an established market. Furthermore, these algae require lower irradiance than other macroalgae (Raven and Hurd 2012). This 3-year NSERC project was the first step in developing an integrated system that focused on the optimization of macroalgal culture. The main factors studied during the first phase of the project were temperature, nutrient concentration and stocking density, while my study concentrated in the effect of irradiance at both low (2 g L^{-1}) and high (10 g L^{-1}) stocking density.

1.1 REVIEW: LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE EXPERIENCES WITH THE SPECIES SELECTED FOR THIS RESEARCH

The combination of Atlantic halibut, Irish moss and / or dulse has never been studied, except in the context of this NSERC project (Corey et al. 2012, 2013; Kim et al. 2013). Moreover, Atlantic halibut has not been used in any integrated culture study. Irish moss (hereafter *Chondrus crispus*) has been considered a good candidate for integrated culture since the earliest IMTA experiences because of its economic importance as a source of carrageenan (Ryther et al. 1972; Tenore et al. 1973). Originally, local wild *C. crispus* from Massachusetts, USA (41° 31' N, 70° 40' W) was integrated in tertiary domestic wastewater treatment. Wastewater was diluted in seawater and treated with microalgae. Microalgae were used to feed oysters. Macroalgae were utilized to successfully remove nutrients excreted by oysters and nutrients not removed by the microalgae. The whole system reduced the mean daily nutrient load into the environment from 4.0 to 1.6 kg d⁻¹ of ammonium-nitrogen (NH₄⁺-N) and from 0.7 to 0.5 kg d⁻¹ of orthophosphate-phosphorus (PO₄³⁻-P; Goldman et al. 1974¹). A similar study was conducted in Florida, USA (27° 32' N, 81° 15' W) with two strains of *C. crispus*, the same wild strain from Massachusetts and the clone T4. The clone T4 was a frond selected among 440 thalli in the beginning of the land-based aquaculture of *C. crispus* in Nova Scotia (NS), Canada. Clone T4 was selected for its high growth, lower susceptibility to epiphytes (bacteria and opportunistic micro- or macroalgae that grow upon another alga), and vegetative propagation by fragmentation. It got its name because it was thallus number four in tank T (Neish and Fox 1971). Although it was originally thought that T4 was a sterile clone, it was later determined to be a male gametophyte with

¹ Original loads in mg-atoms d⁻¹

viable sexual reproduction (Guiry 1981). *Chondrus crispus* has a triphasic isomorphic life history with male and female gametophytes, carposporophytes parasitic on the female gametophyte and free-living tetrasporophytes (Chen and McLachlan 1972). The results in the Florida experiment were unsuccessful because fronds suffered heavy epiphytism and clone T4 was quickly damaged by high temperatures, which ranged between 10 and 33 °C (Ryther et al. 1978). The failure of the Florida study was not a surprise since *C. crispus* was out of its natural latitudinal distribution. More details about the distribution and physiological characteristics of *C. crispus* appear in Chapter 2.

More recently, in a pilot scale system, Matos et al. (2006) used *C. crispus* in conjunction with *P. palmata* to treat effluent of 40 t of another flatfish, turbot [*Psetta maxima* (Linnaeus 1758)], and European seabass [*Dicentrarchus labrax* (Linnaeus 1758)]. In northern Portugal (41° 22' N, 08° 46' W) effluent from the fish was circulated into six 1500-L tanks filled with *C. crispus* stocked at 4.4 g L⁻¹. The resulting effluent was transferred to a second stage where *P. palmata* was stocked in six 300-L tanks at 5.0 g L⁻¹. This system was called “cascade” by the authors, because the tanks were in a series. In spring conditions with temperatures between 17 and 21 °C and salinity of 33 psu, *P. palmata* performed better than *C. crispus*. Accordingly, *P. palmata* gave 40 g dry weight (DW) m⁻² d⁻¹ and removed 41 % of the N excreted by the fish; this compared to 8 g DW m⁻² d⁻¹ and a removal of 14 % of the N by *C. crispus*. However, not all the nutrient removal corresponded to algal uptake. There was an external factor not cited by the authors; the mean concentration at the output of the first set of tanks was 10 % higher than at the input of the second set of tanks. Regrettably, *P. palmata* degenerated rapidly when the temperature reached 20 °C (Matos et al. 2006). In another experiment in central

Portugal (40° 47' N, 08° 40' W), also conducted with turbot effluent, *C. crispus* yield was independent of stocking density between 6.3 and 10 g L⁻¹ with a mean yield of 20 g DW m⁻² d⁻¹ (Matos et al. 2006). This experiment was conducted in 1500-L tanks in summer conditions with a mean temperature of 19 °C and a mean salinity of 20 psu (Matos et al. 2006). The authors also evaluated another red macroalga, *Gracilaria bursa-pastoris* (Gmelin) Silva, which performed better than *P. palmata* and *C. crispus* at this latitude. However, the authors concluded that using *G. bursa-pastoris* and *C. crispus* in a “cascade” system was optimal, because of the high nutrient uptake rate of *G. bursa-pastoris* and the high commercial value of *C. crispus*. They estimated that to remove 50 % of the N generated by 40 t turbot per year, it would be necessary to have 3,271 m² of algal surface-area. By comparison, in a lower temperature environment, a wild strain of *C. crispus* collected in the Irish Sea (54° 23' N, 05° 33' W) was cultured in effluent of sea trout [*Salmo trutta trutta* (Linnaeus 1758)], in Northern Ireland. In 20-L tanks, 4 to 10 cm frond lengths of *C. crispus* were cultured in spring conditions, 10 to 18 °C, pH 8.8 and salinity 30 to 33 psu (Holdt 2009). At a stocking density of 3 g L⁻¹, the mean yield was 2.9 g DW m⁻² d⁻¹, 36 % of that found by Matos et al. (2006) at a higher stocking density and a higher nutrient concentration (Holdt 2009²). In the sea trout effluent experiment, the nutrient removal was 37 % of the nitrate (NO₃⁻), 58 % of the nitrite (NO₂⁻) and 50 % of the orthophosphate (PO₄³⁻). Holdt (2009) suggested that stocking densities much greater than 3 g L⁻¹ were likely required for macroalgae to absorb all available nutrients. My research focus was to optimize irradiance needed to culture the selected red macroalgae at high stocking density (10 g L⁻¹) thus minimizing the light

² Data were reported originally as fresh weight (FW), a FW/DW ratio of 4.2 was considered to make them comparable with Matos et al. (2006)

limitation identified by Matos et al. (2006) when they increased the *Gracilariaria* stocking density from 6.3 to 8.3 g L⁻¹, and the nutrient uptake limitation identified by Holdt (2009) at low stocking density, 3 g L⁻¹.

These results with European strains need to be considered with some caution because several parameters differ from NS, especially those carried out in Portugal where strains were adapted to higher temperatures (annual range 13 to 21 °C) than in NS (annual range 0 to 18 °C). In addition, both species were not evaluated in the same conditions, *C. crispus* and *P. palmata* had different stocking densities and were grown in a sequential tank system; this means each species received different amounts of nutrients. However, in North America there are no other studies with the selected species in a land-based IMTA system. Currently there is only one study using *P. palmata* in the integrated cultivation of Atlantic salmon [*Salmo salar* Linnaeus 1758)], but in this case in sea net-pens in New Brunswick (NB, Chianale et al. 2012).

1.2 LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE IN NOVA SCOTIA

The initiative of Dalhousie University and Scotian Halibut Ltd. was supported by a Strategic Project Grant from NSERC between 2009 and 2012. As indicated earlier, Atlantic halibut was utilized as a source of nutrients to study the bioremediation of the effluent by red macroalgae. This carnivorous species is native to coastal waters off NS, and it is cultured on a small scale in the Maritime Provinces. Grow to market size occurs mainly in land-based facilities using either flow-through or recirculating systems (Brown 2010). Estimates of the excretion rate of dissolved N in Atlantic halibut range from 4 to

14 mg N kg fish⁻¹ h⁻¹, a wide range that inversely related to body size (Davenport et al. 1990; Fraser et al. 1998; Davies and Slaski 2003). Nitrogen is mainly excreted as ammonia (83 %), with the remaining 17 % as urea (Fraser et al. 1998). However, the form and excretion rate depend on the N concentration in the feed, feed rate, the size of the fish and the culture conditions (Handy and Poxton 1993). Phosphorus excretion has been less studied, and it is mainly excreted in the faeces as particulates. No values have been published on dissolved P excretion by Atlantic halibut. However, turbot excreted dissolved P at a rate of 0.3 to 0.7 mg P kg fish⁻¹ h⁻¹ (Aubin et al. 2006, 2009).

Macroalgae use N to synthesize amino acids to make protein, some of which are incorporated into photosynthetic pigments and enzymes utilized in the photosynthetic process. Phosphorus is used for the synthesis of nucleic acids, lipids and prosthetic groups and finally, in the formation of adenosine-5'-triphosphate (ATP, Falkowski and Raven 2007). In my study, I considered both N and P since both nutrients are important in the development of fish and macroalgae, and because of their potential environmental impact. In addition, to determine the absorption of the nutrients, it is important to analyze their accumulation in the algal tissue and their incorporation in the photosynthetic pigments.

At the Scotian Halibut Ltd. farm at Wood's Harbour, NS (43° 31.04' N, 65° 44.30' W), the highest nutrient concentration in the effluent was *ca.* 560 µM N and 25 µM P, up to 100-fold higher than in coastal seawater (Corey 2011). Currently there are no regulations in Canada for the disposal of marine aquaculture effluent, and for this reason, there is no target to meet. In July 2012 the Canadian "Wastewater Systems Effluent Regulations" were published, but these only apply to domestic sewage

(Department of Justice 2012). By comparison, Chile has regulations for the disposal in the coastal zone that are applicable to all industries, including aquaculture. With respect to nutrients, the standards are 50 mg L⁻¹ (3570 μM) of Total Kjeldahl nitrogen [sum of organic nitrogen, un-ionized ammonia (NH₃) and ammonium ion (NH₄⁺)], and 5 mg L⁻¹ (161 μM) of phosphorus (MINSEGPRES 2001). Due to the large water volumes used in aquaculture, these concentrations are rarely exceeded; however, nutrient loading may be harmful to the environment, especially if circulation in the receiving water body is low. For marine aquaculture effluents, the global trend is to follow guidelines for best management practices or codes of conduct rather than specific regulations (van Rijn 2013). This is because of the variability in dynamics of different coastal ecosystems and the characteristics of different farms, such as species and culture conditions. At Scotian Halibut farm, after 10 years of treatment with bacterial biofilters and one year of treatment with red macroalgae and / or bacterial biofilters, the halibut effluent has had no apparent deleterious effect on the receiving water body (White et al. 2011). Eutrophication indicator species, such as *Ulva* spp., were not found. On the contrary, the immediate aquatic ecosystem has benefited from the increased growth of the dominant intertidal brown macroalgae, rockweed [*Ascophyllum nodosum* (Linnaeus) Le Jolis].

Ammonia excreted by the Atlantic halibut could be found in the effluent as the ammonium ion (NH₄⁺) or as the toxic un-ionized ammonia (NH₃), depending on its temperature, pH, and salinity (Bower and Bidwell 1978). Therefore, to reuse or recirculate this water to the halibut, it is necessary to reduce the NH₃ concentration to less than 3.6 μM NH₃ (0.07 mg NH₃ L⁻¹) to prevent a reduction in the growth rate (Brown 2010; Paust et al. 2011). The NH₃ is toxic to fish because it can enter through the gills

into the blood system and then penetrates into the brain causing neural damage, affecting the feeding behaviour and inducing stress (Weber et al. 2012). The toxic concentration of NH_3 , $3.6 \mu\text{M NH}_3$, is equivalent to $3 \mu\text{M}$ of ammonia nitrogen ($\text{NH}_3\text{-N}$) or $0.06 \text{ mg NH}_3\text{-N L}^{-1}$, which is the concentration of N present as ammonia (Handy and Poxton 1993). Corey (2011) reported a nitrogen concentration in the Atlantic halibut effluent up to $560 \mu\text{M N}$. Before any treatment, it is expected that all the N would be Total Ammonia Nitrogen (TAN, $\text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$). Considering that an IMTA system could have an efficiency of 50 % of N removal (Matos et al. 2006), the treated water still could be toxic to the fish when its temperature was higher than $6 \text{ }^\circ\text{C}$ (Fig. 1.1). This evaluation was conducted with the daily temperatures recorded at the Atlantic halibut farm between 2002 and 2012 (P. Corey pers. comm.), the pH recorded in 2011 (8.0, Corey 2011) and the percent ionized ammonium (Bower and Bidwell 1978). The period and toxicity depended if the calculation was conducted with the maximum daily temperature (2 to $18 \text{ }^\circ\text{C}$) or the minimum daily temperature (0 to $13 \text{ }^\circ\text{C}$). This is an exercise to demonstrate the importance of reporting the final nutrient concentration, not only the percent removed, and the need to increase the algal nutrient removal or maintain a traditional biofilter after the macroalgae.

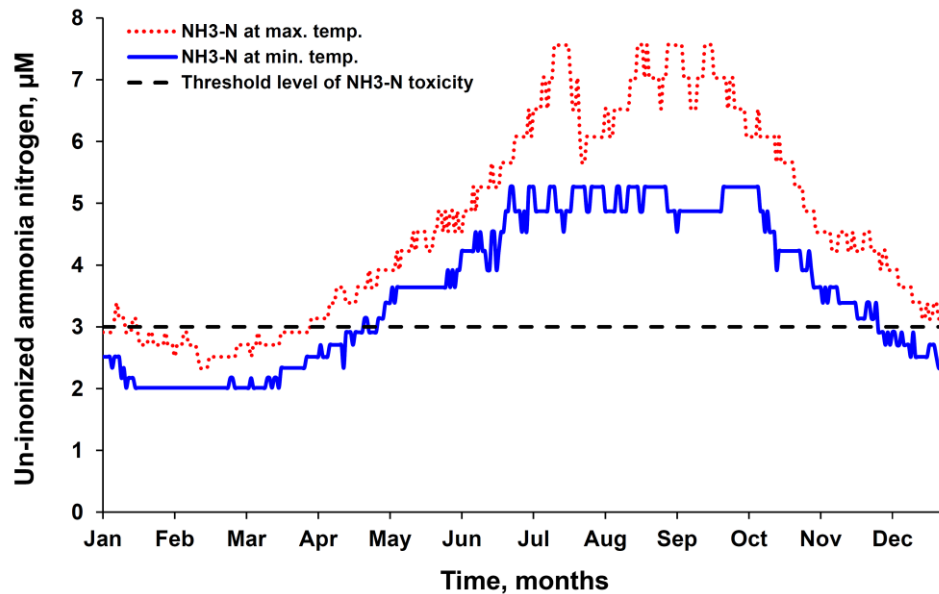


Figure 1.1. Un-ionized ammonia nitrogen (NH₃-N) in Atlantic halibut farm effluent after 50 % Total Nitrogen Ammonia (TAN) removal. Considering an initial value of 560 µM TAN, the highest (dotted line) and the lowest (solid line) daily temperature between 2002 and 2011, maximum pH recorded in the effluent, and Bower and Bidwell (1978) tables. The horizontal line represents the threshold level of toxicity (3 µM NH₃-N, Brown 2010).

The two species of red macroalgae selected to integrate the culture with Atlantic halibut, *C. crispus* and *P. palmata*, were once identified as “top candidates” for aquaculture in North America (Cheney 1999). The author considered *C. crispus* a valuable carrageenan source and *P. palmata* a desirable health food, as well as both being potential sources of eicosapentaenoic acid and pigments. In addition, these species met the requirements to be included in an IMTA system: both are native to NS, are fast-growing, have a known life cycle and their culture technology has been developed (Morgan et al. 1980a; Bidwell et al. 1985; McHugh 2003). Similar to other macroalgae, these species are susceptible to diseases and epiphytes (Craigie and Correa 1996; Gachon et al. 2010; Saunders and Clayden 2010).

The strain of *C. crispus* used in this project was from Basin Head, Prince Edward Island. This strain was selected because it is the only one in the Maritime Provinces in which the population is 100 % gametophytic and has asexual reproduction by fragmentation (Garbary et al. 2011). This strain can tolerate up to 20 °C and can live unattached in the water column (McCurdy 1980). In relocation studies, the Basin Head strain showed better growth than the traditional clone T4 which has been more intensively studied for cultivation (McCurdy 1980; Cheney et al. 1981; Chopin et al. 1999). The strain of *P. palmata* was derived from a commercially harvested wild population (Garbary et al. 2012) at Point Prim, NS (44° 41.5' N, 65° 47.2' W) and was first cultured by Corey et al. (2012, 2013) as part of this NSERC project.

Early in the aquaculture development of these species, during the 1970 – 1980's, many culture variables were studied and algal performance analyzed (see Chapter 2 for details). However, these studies were conducted with specific strains and in a wide range of growing conditions, as well as on different thallus portions, from millimetre segments to whole fronds. In addition, there have been many changes in instrumentation and units. For example, over 30 years ago, Ramus (1981) noted six different methods for measuring photosynthetic activity, and 12 units, which involved the evaluation of either carbon or oxygen. Currently, one of the techniques most utilized is chlorophyll *a* fluorescence of photosystem II (PS-II) by pulse amplitude modulated (PAM) fluorescence. It is a nonintrusive technique, which allows rapid determination of photosynthetic activity in the field or at the farm, as well as underwater (Schreiber et al. 1986; Figueroa et al. 2006). However, it has the disadvantage that respiration cannot be determined (Raven and Hurd 2012). In my research, the traditional light-dark bottle method was utilized to

measure photosynthetic activity by analyzing oxygen evolution (Thomas 1988). Light has been measured with a range of instruments that record different fractions of the electromagnetic spectrum, and express intensity by a variety of names and units (Arditti 2008; Björn 2008; Kirk 2011). To describe the effect of light on photosynthetic and nutrient uptake processes, in the present study the term “irradiance” was selected, according to Falkowski and Raven (2007). Irradiance was measured as the Photosynthetic Photon Flux Fluence Rate (PPFFR) in $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is the integral of photon flux of the Photosynthetic Active Radiation (PAR, 400 – 700 nm waveband) from all directions measured by a spherical sensor (Ramus 1985). The values of irradiance measured differ depending on the type of sensor utilized. A flat sensor, which measures the Photosynthetic Photon Flux Rate (PPFR), recorded 60 % of the value measured with a spherical sensor under the same conditions (pers. observ.). However, this difference will depend on the culture and illumination conditions. This point is important because both sensors express irradiance in the same units ($\mu\text{mol m}^{-2} \text{s}^{-1}$); therefore, in any study it is necessary to specify the model of sensor used. In Appendix A, there are examples the different type of light measurements and units used in the scientific literature and the conversion to the current units. The great variability in published results in terms of culture conditions and measurement methods means that they cannot be used directly to optimize the integrated culture with Atlantic halibut. Consequently, it is necessary to validate them with the available strains under farm-specific conditions.

To determine the effect of temperature, nutrient source and concentration, laboratory scale experiments were conducted, within the NSERC project, under controlled conditions: irradiance, as PPFFR, of $125 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod of 16:8 (ligh:dark, L:D) and a stocking density of 2 g L^{-1} (Corey et al. 2012; 2013). Both species were cultured in NO_3^- at a similar concentration to halibut effluent ($300 \mu\text{M N}$) and natural seawater ($30 \mu\text{M N}$). Three temperatures were evaluated, 6, 10 and $17 \text{ }^\circ\text{C}$; these covered the range of temperature during the growing season (May to November) of the south-west of NS, where the Atlantic halibut farm is located. *Chondrus crispus* grew best at $17 \text{ }^\circ\text{C}$, while *P. palmata* grew best at $6 \text{ }^\circ\text{C}$. According to Corey et al. (2012) the highest specific growth rate of *C. crispus* and *P. palmata* at $300 \mu\text{M N}$ was 5.5 and 5.8 % d^{-1} , respectively; whereas at $30 \mu\text{M N}$, growth rate was less than 3 % d^{-1} . Nitrogen removal in *C. crispus* of *ca.* $1 \text{ mg N g DW}^{-1} \text{ d}^{-1}$ was independent of temperature, while in *P. palmata* N removal decreased with temperature. For my research, the intermediate temperature ($10 \text{ }^\circ\text{C}$) was selected to not skew the response towards the temperature most favourable for each species. In addition, $10 \text{ }^\circ\text{C}$ was the only temperature within the range recommended to grow Atlantic halibut juvenile, 10 to $14 \text{ }^\circ\text{C}$ (Brown 2010). The nitrogen source was tested for *C. crispus* and *P. palmata* in similar conditions, PPFFR of $125 \mu\text{mol m}^{-2} \text{s}^{-1}$, 16:8 L:D, $10 \text{ }^\circ\text{C}$ and 2 g L^{-1} . Nitrate and NH_4^+ were combined from 0 to $300 \mu\text{M}$. Both species take up NH_4^+ better than NO_3^- , and total N uptake was highest at $300 \mu\text{M NH}_4^+$, $4.5 \text{ mg N g DW}^{-1} \text{ d}^{-1}$ for *P. palmata* and $3.4 \text{ mg N g DW}^{-1} \text{ d}^{-1}$ for *C. crispus* (Corey et al. 2013). Hence, NH_4^+ was selected as the N source in my research; moreover, it is the form of N excreted by fish.

A long-term pilot experiment of 11 months was conducted in two independent recirculation systems at the Atlantic halibut farm, with a third system operating with raw seawater (Corey 2011). *Palmaria palmata* was cultured in nine 1.25-m³ plastic cages (1.32 m² surface–area x 0.95 m deep) suspended inside each of the three concrete sump tanks (39 to 65 m³). The stocking density increased from 3 to 10 kg m⁻² as the study progressed. The natural light, via opaque roofing fibreglass panels, was supplemented with fluorescent tubes to ensure a minimum irradiance, as PPFFR, of 100 μmol m⁻² s⁻¹. One of the main conclusions from this pilot experiment was that, in winter conditions, to remove 50 % of the dissolved N generated by 100 t of Atlantic halibut, would require *ca.* 34,000 m² of algal rearing surface-area (P. Corey, pers. comm.). This rearing surface-area is 11.3 times larger than the area needed to culture the 100 t of Atlantic halibut, and would not be cost effective because of land cost, and the aeration and illumination operational expenses. To be economically feasible, the tank space devoted to macroalgae culture must be decreased. In addition, this surface-area was five-fold higher than the surface-area estimated by Matos et al. (2006) to remove the same percent of N generated by 100 t of turbot per year. The main difference in both estimations was the stocking density. Matos et al. (2006) suggested 6.3 g L⁻¹ for *G. bursa-pastoris* and 8.3 g L⁻¹ for *C. crispus*. Corey (2011) expressed the stocking density of *P. palmata* as surface-area, 4.4 kg m⁻². If the stocking density is expressed in terms of the volume of the cages, it is equivalent to 4.6 g L⁻¹. However, although the algae were contained in the cages, they had available all the volume water contained in the sump tank, so it is more realistic to express their stocking density by the sump volume. In this scenario, the stocking density would be 1.1 to 2.4 g L⁻¹, similar to the laboratory scale experiments. In both cases, the

stocking density was low compared with Matos et al. (2006), and the total algal rearing surface-area could be reduced by increasing the stocking density. Another smaller scale experiment in 50-L tanks was conducted as part of the NSERC project. The objective of this experiment was to determine the best stocking density to culture *C. crispus* and *P. palmata* in Atlantic halibut effluent (Kim et al. 2013). Five stocking densities from 1 to 50 g L⁻¹ were evaluated under saturated irradiance, PPFFR of 140 μmol m⁻² s⁻¹ and with a photoperiod of 16:8 L:D. This experiment indicated that 10 g L⁻¹ is a good stocking density to culture these red macroalgae in Atlantic halibut effluent (Kim et al. 2013). A high stocking density can result in self-shading; thus, a higher irradiance may be needed to sustain the photosynthetic rate. For this reason, the focus of my research was to determine the effect of irradiance on the performance of these red macroalgae cultured at this high stocking density (10 g L⁻¹), while taking into account previous experience with this type of integrated culture.

To determine the irradiance required to culture these red macroalgae at high stocking density, it is necessary to determine the light saturation point. This is achieved by producing light saturation curves to express the dependence of a response variable on the light irradiance (Ramus and Rosenberg 1980). In a general curve, the first phase corresponds to the light limited phase, where the response increases linearly with irradiance up to the saturation point (Fig. 1.2). In special cases, the light compensation point is also determined; this is the irradiance where the rate of photosynthesis exactly matches the rate of respiration. The change in the slope of the curve indicates the efficiency in the use of the light, with the highest slope indicating the highest efficiency. The second phase is a plateau where irradiance is no longer a limiting factor. Potential

limiting factors during the plateau phase could be some of the nutrients, such as carbon dioxide, nitrogen, phosphorus, and micronutrients. Assuming nutrients are not limiting, at some point, irradiance becomes excessive and photoinhibition occurs (Lobban and Harrison 1994). In nature, light saturation for growth occurs at a lower irradiance than that for photosynthesis, possibly because of nutrient limitation in seawater (Lüning 1990).

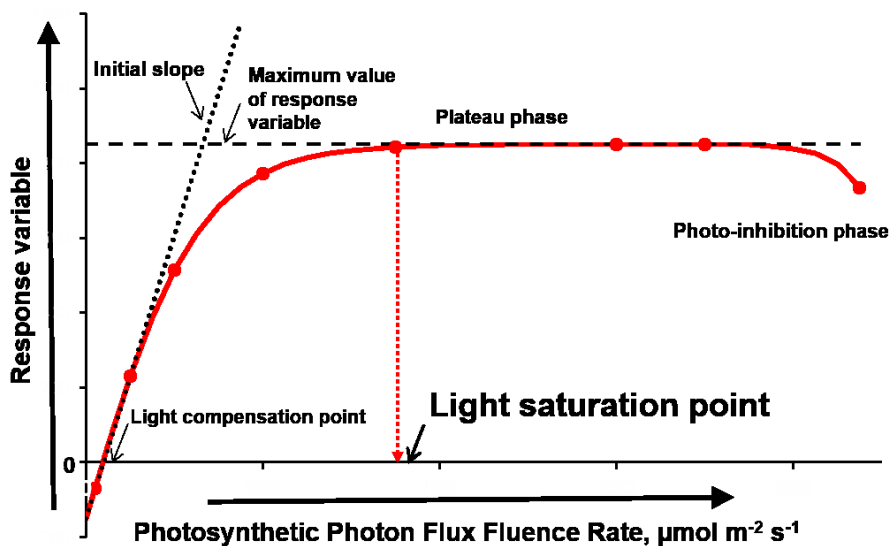


Figure 1.2. General light saturation curve. Irradiance as Photosynthetic Photon Flux Fluence Rate versus the different response variables, such as photosynthetic rate.

1.3 OBJECTIVES AND STRUCTURE OF THE THESIS

The primary goal of my research was to find the best irradiance to culture *C. crispus* and *P. palmata* at the stocking density of 10 g L⁻¹. To achieve this objective, three experiments were conducted on a laboratory scale at Dalhousie University's Agricultural Campus (DAL-AC) in 2011 and 2012. During the summer of 2011 both species were cultured simultaneously under natural light to determine the effect of irradiance at high stocking density (10 g L⁻¹) compared to the low stocking density

evaluated previously (2 g L⁻¹, Corey et al. 2012, 2013). This experiment was carried out twice, at water temperatures of 10 and 14 °C. This is the first experiment where both species have been evaluated at the same time and under the same culture conditions. In previous studies, they were evaluated consecutively, and they were not exposed to the same conditions (Corey et al. 2013; Kim et al. 2013). The null hypothesis was that the performance of both species would be the same, independent of both stocking density and irradiance.

The third experiment was conducted in the fall 2012 exclusively with *C. crispus*, at the same two stocking densities, 2 and 10 g L⁻¹, under six levels of artificial light and 10 °C. In this case, the objective was to determine the light saturation point by using light saturation curves, and to test the null hypothesis that the light saturation point for growth and photosynthesis are different even when nutrient levels are not limiting. In addition, I wanted to determine the light saturation points for nutrient uptake and the effect of irradiance on dark respiration and the nutrient and pigment content.

The thesis is divided into five chapters, Chapter 1 being the Introduction. Chapter 2 reviews the literature about physiology, ecology and culture of *C. crispus* and *P. palmata*, to provide the background needed for the optimization of their integration in the IMTA system. Chapter 3 describes experiments conducted under natural light and Chapter 4 describes the artificial light experiment. Finally, Chapter 5 synthesizes the earlier chapters from the perspective of applying the results to integrated aquaculture at a commercial scale.

CHAPTER 2 ECOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF IRISH MOSS AND DULSE

To optimize the capacity of Irish moss and dulse to remove the nutrients from the effluent of Atlantic halibut it is necessary to understand their performance in both the natural environment and culture. Their evolutionary background, their microhabitat preferences, and their geographic distribution determine the potential of these algae. The totality of these features will determine the physiological performance of individual algal strains and the ability to manipulate this for cultivation proposes. Understanding these variables is necessary to optimize nutrient uptake.

2.1 CLASSIFICATION OF STUDY ORGANISMS

Taxonomy is dynamic and interpretation often depends on the tools used to analyze the specimens. The currently accepted classification is indicated in Table 2.1.

Table 2.1. Taxonomic classification of Irish moss and dulse (Guiry and Guiry 2012).

Phylum	Rhodophyta	
Subphylum	Eurhodophytina	
Class	Florideophyceae	
Subclass	Nemaliophycidae	Rhodymeniophycidae
Order	Palmariales	Gigartinales
Family	Palmariaceae	Gigartinaceae
Genus	<i>Palmaria</i>	<i>Chondrus</i>
Species	<i>P. palmata</i> (Linnaeus) Weber and Mohr 1805	<i>C. crispus</i> Stackhouse 1797
Common name	Dulse	Irish moss

Dulse has a distinctive life cycle that was first described by van der Meer and Todd (1980). The life cycle is characterized by a great sexual dimorphism, with macroscopic male gametophytes (n) and microscopic female gametophytes (n). Tetrasporophytes (2 n) have a similar size and morphology to those of the male gametophytes. Tetrasporophytes develop directly on fertilized females without producing carposporophytes. These characteristics are shared with other members of the order Palmariales (van der Meer 1981; Bold and Wynne 1985). Dulse has a solid and flat thallus, like other members of the genus *Palmaria*. Its blade structure is pseudoparanchymatous and it develops from multiaxial apical systems. It can become cartilaginous with age and thalli can reach one metre in length (Bold and Wynne 1985; Guiry 2012a). The specific characteristics of dulse are its palmate branching with its finger-like extensions and its marginal proliferations at the base (Guiry 2012a).

Irish moss, like other members of class Florideophyceae has apical growth and multicellular thalli (Lee 2008). Like other members of the genus *Chondrus*, Irish moss has a compressed to flattened thallus, with subdichotomous branching and multiaxial apical systems. It is smaller than other species within the genus. For example, *C. crispus* grows to a maximum of 20 cm in length compared to 50 cm in *C. giganteus* Yendo (Yendo 1920; Guiry 2012b). It has highly variable branching, colour and thickness (Guiry 2012b). Some of the factors that affect *C. crispus* morphology are water motion, temperature and genetic structure (Gutierrez and Fernández 1992; Kübler and Dudgen 1996; Krueger-Hadfield et al. 2013).

2.2 HABITAT AND DISTRIBUTION

Palmaria palmata is indigenous to the North Atlantic Ocean. Its distribution also extends into the Arctic Ocean along both the American and European coasts to 80° N. Its southern limits are New Jersey, USA and northern Portugal, respectively, 40° N (Provan et al. 2005; Matos et al. 2006).

Palmaria palmata commonly inhabits the lower intertidal zone, but it can live to depths of 20 m in clear water (Guiry 2012a). It attaches to rocks or other hard substrates, such as mussels or submerged wood, but it is also an epiphyte of other algae such as *Laminaria* and *Fucus* (Garbary et al. 2012). Since it inhabits a wide variety of habitats and shows polymorphism, it is important to select a morph suitable for cultivation. In general, a high surface area:volume ratio favours nutrient uptake (Rosenberg and Ramus 1984). *Palmaria palmata* is characterized as a perennial with high vegetative growth, which facilitates cultivation (Bird and McLachlan 1992; Guidy 2012a). The wild strain used in this project was collected in the low intertidal zone of the Bay of Fundy (Point Prim, 44° 41.5' N, 65° 47.2' W). This coastline is exposed to a tidal range of up to eight metres and the water temperature does not usually exceed 15 °C (Chang et al. 2005; Corey et al. 2012; Garbary et al. 2012).

Chondrus crispus is also found in the North Atlantic Ocean, but its distribution extends to the North Pacific Ocean. In the northwest Atlantic, it is present from Newfoundland, Canada, 51° N to New Jersey, USA, 39° N, and in the northeast Atlantic from Norway, 65° N, to Morocco, 39° N (Pringle and Mathieson 1987; Provan and Maggs 2012). In contrast to *P. palmata*, *C. crispus* is not found in polar areas.

Chondrus crispus inhabits the intertidal and subtidal zones, from littoral pools to 18 m depth. Because this species is highly polymorphic it can grow well in a range of habitats, and it also tolerates a wide range of salinity, 10 to 58 psu (Bird et al. 1979). It lives almost exclusively attached to rocky substrata, but some strains, such as the one from Basin Head, can be found floating in sheltered waters or entangled with byssus threads of mussels (McCurdy 1980). It is a perennial species, with great power of regeneration (Guiry 2012b). The Basin Head strain used in this project is characterized by its tolerance to both high temperature and irradiance. In its natural habitat, due to the shallow depth (0.4 to 1.5 m) of this body of water, it experiences up to 25 °C and up to 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (McCurdy 1980; DFO 2009). However, the specific biological material used for my thesis work has been cultivated for at least four years in controlled conditions at the Marine Research Station of the National Research Council, NRC, Ketch Harbour, NS, so its characteristics may have changed. The conditions of its natural habitat have also changed and the population is endangered; there is less than 1 % of the biomass than in 1980 (Sharp et al. 2010). Consequently, it is difficult to compare the results published in the literature with the biological material used in this NSERC project.

2.4 AQUACULTURE

Early in the development of *C. crispus* and *P. palmata* cultivation, several factors affecting the growth rate, nutrient uptake and photosynthesis were studied (Neish and Fox 1971; Morgan et al. 1980a). These included nutrient concentration and sources, temperature, stocking density, and salinity. However, these studies were conducted with different strains; mainly clone T4 of *C. crispus* and a wild strain of *P. palmata* from NB.

In addition, the experiments were conducted in a wide range of growth conditions, as well as on different parts of thalli. For this reason, these results cannot be used directly to optimize the current integrated culture with Atlantic halibut, and it is necessary to validate them with our own strains. Nevertheless, it is valuable to review culture conditions and light response curve parameters for both species.

2.4.1 *Chondrus crispus*

Growth studies. The focus of my research was the effect of irradiance on the performance of *C. crispus*. However, other factors modify its effect; therefore, it is important to consider these interactions. Temperature is one of these factors, and tolerance to high irradiance generally increases with temperature up to 20 °C (Mathieson and Norall 1975; Enright and Craigie 1981). A wide temperature range, between 10 and 20 °C, has been identified as “optimum” for growth of *C. crispus*. This range depends upon temperature acclimation (season), temperature adaptation (strain), irradiance used, nutrient level and culture conditions. Acclimation time response to environmental changes may be hours or days, in contrast to adaptation where the time response is in thousands or millions of years (Raven and Geider 2003). The lower limit for optimal growth, 10 °C, was reported in fronds from the North Sea (54° 11' N, 07° 53' E) when 2-cm segments were cultivated in 60-L aquaria at 0 to 25 °C. However, no significant difference was found in the growth rate of fronds cultured at either 10 or 15 °C (Fortes and Lüning 1980). The upper limit for optimal growth, 20 °C, was reported in wild fronds from Cape Sambro, NS (44° 28' N, 63° 33' W) cultured in 100-L tanks at 5 g L⁻¹ and ambient temperature, pH and photoperiod between February and December (Neish et

al. 1977). The same optimal temperature (20 °C) was found when the clone T4 of *C. crispus*, was cultured in 400-L tanks at 1 g L⁻¹ under natural light and temperature between 3 and 25 °C. However, its growth rate was only 1.2 times higher than at 15 °C (Simpson et al. 1978a). Since 1982 the clone T4 was replaced by another clone of the Basin Head strain in experiments by the NRC because the latter grew better (Craigie 1990). Prior to this NSERC project, the optimal temperature for growth of the Basin Head strain was unknown. The last revisions of culture protocol of *C. crispus* were based on studies with the clone T4 (Craigie and Shacklock 1995). The Basin Head strain grew better at 17 °C than at 6 or 10 °C, when 4- to 7-cm segments were cultured in laboratory scale at 2 g L⁻¹, an irradiance of Photosynthetic Photon Flux Fluence Rate (PPFFR) of 125 μmol m⁻² s⁻¹, at both 30 and 300 μM nitrate (Corey et al. 2012). However, because temperatures higher than 17 °C were not assessed, we cannot determine if the optimum temperature for cultivation of the Basin Head strain is the same as for the clone T4.

Information on the photosynthetic response of *C. crispus* under natural light is scarce. Moreover, saturation points vary depending on the experimental conditions and strains. Growth of a strain from the North Sea (*ca.* 58° 14' N, 11° 26' E) was saturated at a Daily Photon Dose (DPD) between 2.5 and 5 mol m⁻² d⁻¹, when it was tested between 15 and 16 °C (Strömngren and Nielsen 1986³). Daily Photon Dose is the integration of the PPFFR during the time that the algae are exposed to the irradiance (Sagert and Schubert 2000). Under artificial light, the compensation point for *C. crispus* also from the North Sea (56° 07' N, 10° 20' E) at 7 °C was 0.44 μmol m⁻² s⁻¹, as Photosynthetic Photon Flux Rate (PPFR, Markager and Sand-Jensen 1992). The saturation point for growth of NS strains, varied between 50 and 145 μmol m⁻² s⁻¹ at 15 °C and 14° to 17 °C, respectively

³ Original irradiance 10 and 20 W m⁻² over 14 h

(Bird et al. 1979; Enright and Craigie 1981⁴). As a DPD, the saturation point ranged from 3 to 8 mol m⁻² d⁻¹. Fronds from the English Channel (48° 52' N, 03° 08' W) did not show a saturation point for growth in the range of irradiance evaluated as PPFR, DPD up to 11 mol m⁻² d⁻¹ (Braud and Delépine 1981⁵). These fronds were cultured at a low stocking density (2 g L⁻¹) and temperatures between 8 and 20 °C. However, photoinhibition for growth of clone T4 from NS only occurred when a DPD mean of 29 mol m⁻² d⁻¹ was reached (Enright and Craigie 1981⁶). In this case, fronds were cultured at temperatures between 14 and 17 °C with a photoperiod of 16:8 L:D, equivalent to the summer solstice at 45° N. There is a wide range of irradiance at which *C. crispus* can be cultured. My research evaluated a range of irradiance that included the lowest light saturation point for growth (50 μmol m⁻² s⁻¹) and the highest value where photoinhibition was found (DPD of 29 mol m⁻² d⁻¹, equivalent to a PPFR of 500 μmol m⁻² s⁻¹ over 16 h).

Photosynthetic studies. The light saturation point for photosynthesis of *C. crispus* has been reported only under artificial light. A wide range of saturation points has been reported from 116 to 450 μmol m⁻² s⁻¹ (Mathieson and Norall 1975⁷; Cabello-Pasini et al. 2000). However, this range overlaps the irradiance level reported by photoinhibition, 324 μmol m⁻² s⁻¹ (Brinkhuis and Jones 1974⁸). As stated earlier, macroalgal responses to irradiance vary with external environmental factors, particularly temperature acclimation. The lowest light saturation point (116 μmol m⁻² s⁻¹) was in fronds from New Hampshire, USA (ca. 42° 59' N, 70° 37' W) collected in fall-winter. Fronds from the same area

⁴ Original irradiance 4 MJ m⁻² d⁻¹ of PAR over 16 h

⁵ Original irradiance 198 μE m⁻² s⁻¹ over 16 h

⁶ Original irradiance 14.4 MJ m⁻² d⁻¹ PAR

⁷ Original irradiance units μE m⁻² s⁻¹

⁸ Original irradiance 2400 ft-c

collected in spring-summer had a higher saturation point, $263 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Mathieson and Norall 1975⁹). The saturation point was more affected by seasonal temperature acclimation than the depth and reproductive phase (Mathieson and Norall 1975). The highest saturation point, PPFR up to $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ was recorded from fronds from Maine, USA (*ca.* $43^{\circ} 48' \text{ N}$, $69^{\circ} 54' \text{ W}$) cultured in Ensenada, Mexico ($31^{\circ} 52' \text{ N}$, $116^{\circ} 40' \text{ W}$) at 17°C and measured as electron transport rate (Cabello-Pasini et al. 2000; Zertuche-González et al. 2001). Temperature also affects the photoinhibition point. Accordingly, fronds collected in winter from Long Island Sound, USA (*ca.* $40^{\circ} 57' \text{ N}$, $73^{\circ} 07' \text{ W}$) were photoinhibited at $324 \mu\text{mol m}^{-2} \text{s}^{-1}$; whereas fronds collected in summer were not photoinhibited at the same irradiance (Brinkhuis and Jones 1974¹⁰). Another factor that affects the photosynthetic activity is salinity. Changes in the salinity alter intracellular water potential, intracellular ion concentrations, and cellular turgor pressures and volumes (Kirst 1989; Reed 1990). Hyposaline environments inhibited the photosynthetic activity in more species of red macroalgae than hypersaline environments (Krist 1989). For example, *C. crispus* performs well between 10 to 58 psu, but better at 30 psu (Craigie 1990). This is consistent with its habitat preferences, semi-exposed shores and estuaries (Chopin 1986). In the present study, the salinity used was 30 psu, the same as conditions at the Atlantic halibut farm.

⁹ Original irradiance units $\mu\text{E m}^{-2} \text{s}^{-1}$

¹⁰ Original irradiance 2400 ft-c

2.4.2 *Palmaria palmata*

Growth studies. The methods for vegetative culture of *P. palmata* have not advanced over the last 20 years, according to the literature. Fronds from the Bay of Fundy, NB (*ca.* 44° 42' N, 66° 44' W), at a stocking density of 6.5 g L⁻¹ had the highest growth at temperatures between 6 and 15 °C, when a range of 3 to 30 °C was evaluated (Morgan et al. 1980a). Similarly, a wide range of light saturation points for growth also has been reported for *P. palmata* from the North Sea, DPD of 3 to 8 mol m⁻² d⁻¹ (Kübler and Raven 1995¹¹; Sagert and Schubert 2000¹²). By comparison, for fronds from NB cultured at 6 g L⁻¹ at temperatures between 6 and 15 °C, the range of the light saturation point was narrower, DPD of 5 to 7 mol m⁻² d⁻¹ (Morgan and Simpson 1981a,b¹³).

Photosynthetic studies. The light compensation point for photosynthesis in *P. palmata* fronds collected in Duncans Cove, NS (*ca.* 44° 30' N, 63° 30' W) was 6.6 µmol m⁻² s⁻¹, at 10 to 15 °C (Robbins 1978¹⁴). This temperature range was defined as “optimum” for photosynthesis by Robbins (1978). The saturation point for photosynthesis varied between 200 and 400 µmol m⁻² s⁻¹. This range was found in both sun- and shade-acclimated wild fronds from the Bay of Biscay (*ca.* 43° 34' N, 06° 11' W, Martínez and Rico 2008). The lowest value agrees with the light saturation point determined in wild fronds from NS (212 µmol m⁻² s⁻¹, Robbins 1978¹⁵). Photosynthesis in *P. palmata* is also affected by salinity, pH and total carbon dioxide (CO₂) concentration; optimum conditions were 32 psu, pH between 6.5 to 7.5 and CO₂ between 6.0 to 7.0 mM (Robbins

¹¹ Original irradiance 75 µmol photon m⁻² s⁻¹ over 12 h

¹² Original irradiance 113 mol photon m⁻² d⁻¹⁴ over 16 h

¹³ Original irradiance 53 and 68 ly d⁻¹ during 16 h

¹⁴ Original irradiance units µE m⁻² s⁻¹

¹⁵ Original irradiance units µE m⁻² s⁻¹

1978). Temperature acclimation affects photosynthetic activity, and winter fronds had a higher photosynthetic activity than summer fronds at 5 to 15 °C whereas summer fronds had the highest photosynthetic activity at 20 and 25 °C. In contrast, temperature acclimation did not affect the respiration rate. Consequently, the photosynthesis to respiration ratio decreased as temperature increased between 5 and 20 °C (Robbins 1978).

2.5 FACTORS AFFECTING OPTIMIZATION OF NUTRIENT UPTAKE BY RED MACROALGAE

2.5.1 Nutrient uptake

Nutrient uptake is the removal of nutrient from the environment and its transport into cells (Berges 1997). Algal nutrient uptake depends on chemical, physical, and biological factors. The main chemical factors are available nutrient concentrations and their chemical form. The physical factors are primarily light, temperature, water motion (diffusive boundary layer) and desiccation. Biological factors include surface-area:volume ratio, filament formation, type of tissue, age, nutritional history, and inter-thallus variability (Lobban and Harrison 1994; Harrison and Hurd 2001). In an integrated culture system, the nutrient concentration depends on the feeding regimen of the fish and the nutrient concentration of the fish feed (Handy and Poxton 1993). The chemical form of the nutrients depends on the pH of the culture water and on the species of fish; some species excrete more urea than ammonia (Bower and Bidwell 1978; Handy and Poxton 1993). In the integrated culture system with Atlantic halibut, the pH varied from 7.0 to 8.0 (Corey 2011). In this pH range, nutrients are mainly as ammonium ion (NH_4^+),

hydrogen phosphate ion (HPO_4^{2-}) and bicarbonate ion (HCO_3^- ; Kester and Pytkowicz 1967; Ip et al. 2001; Emerson and Hedges 2008).

Light, specifically its irradiance, was selected as the experimental factor because of its importance to nutrient uptake. Red macroalgae can passively uptake un-ionized ammonia (NH_3) and carbon dioxide (CO_2 , Reed 1990; Raven and Hurd 2012). However, as indicated above, due to the pH of the effluent, these forms are not very abundant. To uptake the other nutrient forms, red macroalgae need to expend energy, which comes from photosynthetic processes (Raven and Hurd 2012). Therefore, photosynthetic activity affects nutrient uptake, and the use of these nutrients affects the photosynthetic activity in a feedback system. Irradiance provides the energy for algae to fix carbon (C) and to combine it with phosphorus (P) and nitrogen (N) absorbed. These elements are used to make a wide variety of organic compounds, such as, photosynthetic pigments, photosynthetic enzymes and adenosine-5'-triphosphate (ATP, Falkowski and Raven 2007).

2.5.2 Effect of light on algal physiology

Light acts on plants in three ways: time of exposure or photoperiod, light quality or spectral energy distribution measured in wavelength, and irradiance or the number of photons reaching the algal photosynthetic pigments (Falkowski and Raven 2007). In the present study, a constant photoperiod was used, equivalent to that at the summer solstice at a NS latitude (45°N , 16:8 h L:D). There is a little difference in the photoperiod if the hours of illumination are considered between the Civil Twilight Time, 16.93 h, or between the sun rise and sun set, 15.67 h (NRC 2011). The latter was selected because

the irradiance was not detected in the dawn or dusk periods. The effect of the light quality was not considered in my experiments. To avoid the interference of the light quality, lamps selected were the closest to sun light with a wavelength range between 350 to 750 nm. However, the spectrum was not evaluated in any experiments, and irradiance was the principal experimental factor analyzed and quantified (more information about the artificial light used can be found in Chapter 4).

The effect of irradiance on algae is species-specific (Enríquez et al. 1994). Moreover, different populations within the same species exhibit different responses depending on photoacclimation (Häder and Figueroa 1997). However, a common trait is that photosynthesis, nutrient uptake and growth increase with irradiance until nutrients become limiting, or irradiance becomes excessive (Lüning 1981). The algal responses to light saturation points are determined by light saturation curves, which are mathematical models to express the dependence of a response variable on the irradiance (Ramus and Rosenberg 1980).

Red macroalgae have physiological characteristics that reduce the energy cost of photosynthesis, which allow them to adapt to low irradiance. Accordingly, even at a low irradiance, such as $12 \mu\text{mol m}^{-2} \text{s}^{-1}$, they have a positive photosynthetic rate, which allows them to grow (Eggert and Wiencke 2000). Red macroalgae, including *C. crispus* and *P. palmata*, the species selected for this project, have phycobiliproteins (PBP) as photosynthetic antennae or accessory pigments, which allow them to capture low irradiance levels. Due to the high energy cost necessary to generate these phycobiliproteins (Sargert et al. 1997), red macroalgae reduce their energy expenditure by favouring the uptake of NH_4^+ and CO_2 as opposed to nitrate (NO_3^-) and HCO_3^- .

(Kübler and Raven 1994; Raven and Hurd 2012). Shade-acclimated red macroalgae also display a physical change, their thalli are thin to reduce the amount of non-photosynthetic tissue and to increase the light absorption per unit of photosynthetic tissue weight (Häder and Figueroa 1997).

As irradiance increases, either because of the sun rising or improved light transmission through the water, the ratio of PBP to chlorophyll *a* (Chl *a*) declines due to a decrease in PBP and an increase in Chl *a* concentration (López-Figueroa 1992). These changes correspond to photoacclimation, actions to mitigate photoinhibition (Häder and Figueroa 1997). Macroalgae use the energy captured to generate photosynthetic enzymes and photoprotective structures; they also reduce photosynthetic antenna size to prevent photoinhibition (Raven and Hurd 2012). Photoacclimation can also occur over a longer time scale, causing morphological changes, such as increasing frond thickness or complexity (Häder and Figueroa 1997). Photoinhibition occurs in two stages: dynamic photoinhibition (or photoprotection) and chronic photoinhibition (or photoinactivation). The first includes a process of decreasing the excitation of photosystem II (PS-II) in the reaction centres (Raven and Hurd 2012). The second process occurs when the function of PS-II is lost, especially by destruction of the reaction centre protein D₁. D₁ can be repaired in dim light, but if the damage rate exceeds repair, photodamage results (Häder and Figueroa 1997). Repair of D₁ stimulates synthesis of antioxidants, which are by-products that may be extracted for pharmaceutical uses (Raven and Hurd 2012). The recovery time depend on the species and light-acclimation. A high stocking density could prevent photoinhibition because it allows the fronds to have more time to recover under dim light, due to self-shading (Cabello-Pasini et al. 2000). Extra nutrients are

necessary for a rapid recovery from photoinhibition. Phosphorus is necessary to produce the enzymes and ribonucleic acid (RNA) to synthesize D_1 , and iron (Fe) is needed to synthesize photosystem I (PS-I, Raven and Hurd 2012). Red macroalgae exposed to higher irradiance can use NO_3^- as source of N. Although NO_3^- assimilation requires more energy than NH_4^+ , because NO_3^- must be transformed to NH_4^+ , NO_3^- has the advantage that its accumulation is not toxic to red macroalgae (Raven and Hurd 2012). However, in addition to energy, algae require more Fe to synthesize the nitrate reductase enzyme (Morel et al. 2003).

In conclusion, the response of the macroalgae to irradiance is not uniform, and it is regulated by a number of factors. This helps to explain the differences in results from similar studies over the last 40 years with the same species. Macro- and micro-nutrient concentrations are very important, and these differ in experiments with effluents, natural or enriched seawater. Therefore, it is important to specify not only the conditions in which experiments were carried out but also the conditions preceding. In my research enriched seawater, specially formulated for red macroalgae (von Stosch 1964), and supplemented with CO_2 was utilized to optimize growth and to evaluate the effect of irradiance.

2.5.3 Effect of stocking density

Nutrient uptake is ultimately limited by the contact surface of the macroalgae with the aquatic environment (Wallentinus 1984; Baird and Middleton 2004). To increase the nutrient uptake it is necessary to increase the contact surface, a simple approach being to increase the algal biomass. However, the contact surface will depend on the morphology

on the thallus, which varies among species and depends on culture conditions (Gutierrez and Fernández 1992; Kübler and Dudgeon 1996). In natural populations, maximum productivity is when the biomass is about 10 kg m^{-2} (Lüning and Pang 2003). In culture, this same value was determined as the optimal stocking density for *C. crispus* cultivated in tanks in NS (Bidwell et al. 1985). In the coastal zone, light absorption by macroalgae is determined by the algal and substratum surface-area; however, in tanks, unattached macroalgae can use the whole water column. Therefore, biomass on a given surface-area can be increased. To ensure all fronds are exposed to light, it is necessary to keep them moving. The most suitable system has been the tumble culture approach developed by Bidwell et al. (1985). This system generates water circulation cells within the tank by placing parallel perforated tubes in the tank floor through which air and seawater are injected independently. Previous systems only injected air (Huguenin 1976; Lapointe et al. 1976; Waaland 1976). The correct air pressure, bubble size, duration and interval of aeration will depend on the physical characteristics of the species and tank design (Msuya and Neori 2008). Circulation of fronds increases nutrient uptake and photosynthetic activity because it gives them time to recover in dim light, minimizes the diffusive boundary layer and homogenizes temperature, nutrients and gases (Neish and Knuton 1978; Greene and Gerard 1990; Pang and Lüning 2004). The water circulation in tumble culture also favours air-water gas exchange. However, excessive agitation can damage the fronds and result in loss of CO_2 and NH_4^+ to the atmosphere (Braud and Amat 1996). In addition, excessive aeration increases the operating cost of producing compressed air without benefits. During the Wood's Harbour experiments, the highest incremental cost of the inclusion of macroalgae to the Atlantic halibut culture was the electrical power

needed to produce compressed air. This is a serious limiting factor to the economic viability of land-based IMTA (Corey 2011).

In tumble culture, a high stocking density can result in self-shading, thus a higher irradiance may be needed to sustain the photosynthetic rate. At the other extreme, excessive irradiance produces photoinhibition (Cabello-Pasini et al. 2000). Hence, to optimize an integrated culture system incorporating red macroalgae, it is necessary to identify the correct irradiance for a defined stocking density. This was the central focus of my research.

Increasing the stocking density increases productivity, but also decreases the risk of one of the main problems in algal culture, the growth of epiphytes (Neish and Knutson 1978). Epiphytes are bacteria and opportunistic micro- or macroalgae that generally exhibit fast growth. This inhibits the growth of the target species by competing for nutrients and reducing the exposed surface of the algae for absorption of light and nutrients (Lüning and Pang 2003). Another way to avoid epiphytes is to establish axenic cultures with antibiotics and disinfectants (Liu and Kloareg 1992). Germanium dioxide (GeO_2), a silicon metabolism inhibitor, it is commonly used to avoid the growth of diatoms. It is also toxic to other algae, the dose depends on the species to control: to diatoms, $0.2 \text{ mg GeO}_2 \text{ L}^{-1}$; to Phaeophyta, 1.1 mg L^{-1} ; and to Chlorophyta, especially *Ulva* spp., 2.2 mg L^{-1} (Markham and Hagmeier 1982; Shea and Chopin 2007; Tarakhovskaya et al. 2012). Unfortunately, this approach is impractical in IMTA systems; the algal rearing tanks are microcosms, similar to a tidal pool (pers. observ.). There are different herbivore invertebrate species, which can help to keep clean the fronds (Fletcher 1995). Another treatment is to increase or diminish nutrient

concentration, depending on the epiphyte that one wants to control (Bidwell et al. 1985). However, this last method is also not applicable to a recirculation system, because the goal is to clean the actual rearing water, not manipulate it. Selection of algal strains resistant to local epiphytes would be a long-term solution (Santelices and Ugarte 1990). In my research, to reduce the problem with epiphytes, only the cleanest fronds from the Atlantic halibut farm were selected, and for the experiments, only the first few centimeters below the apex were used.

In conclusion, increasing stocking density increases nutrient uptake per unit tank space, if the irradiance is adequate. Considering that red macroalgae are adapted to absorb low irradiance levels because their high concentration of phycobiliproteins, I hypothesized the irradiance needed to maintain the photosynthetic activity and nutrient uptake would not be extremely high.

CHAPTER 3 EFFECT OF NATURAL IRRADIANCE ON PERFORMANCE OF RED MACROALGAE CULTIVATED AT TWO STOCKING DENSITIES

3.1 ABSTRACT

In land-based algal culture, the rearing space is a high investment expense. The goal is to maximize productivity per rearing surface-area. Increasing stocking density is a way to increase productivity, but this may require greater irradiance because of self-shading. Performance of red macroalgae was assessed at laboratory scale (one-litre flasks) in two experiments at 10 and 14 °C, respectively. Three levels of irradiance: 100, 50 and 20 % of incident sunlight, two stocking densities: 2 and 10 g L⁻¹ and two species, *Chondrus crispus* and *Palmaria palmata*, were used. Nutrient uptake by *P. palmata* was 25 % higher than that by *C. crispus* at the mean of the six treatment conditions of both experiments. *Palmaria palmata* also showed higher tissue carbon retention than *C. crispus* at both stocking densities; this retention was directly related to irradiance. Nitrogen retention was inversely related to irradiance due the consumption of the 500 µM ammonium contained in the flasks. Moreover, it was lower in *P. palmata* than *C. crispus*, resulting in a high molar C/N ratio (22 and 18 at 10 and 14 °C, respectively). Photosynthetic rate was higher in *P. palmata* than in *C. crispus*. In the range studied, *P. palmata* performance better than *C. crispus* and its use is recommended at 10 kg m⁻³, under a Daily Photon Dose of 17 mol m⁻² d⁻¹. However, in the Atlantic halibut farm, the effluent temperature can reach 19 °C, temperature at which *P. palmata* degraded. *Chondrus crispus* can better withstand these high temperatures.

3.2 INTRODUCTION

Land-based integrated culture of macroalgae and fish is expensive because it requires a high investment in land, tank construction, pipe installation, and purchase of equipment for aeration, water circulation, and lighting. Its operation is also costly because of the electricity required to run the equipment, the necessary labour and the nutrients monitoring (Troell et al. 2003; Titlyanov and Titlyanova 2010). An approach to offset these costs is to maximize the algal stocking density. More algal biomass increases the contact surface for nutrient uptake, gives algal fronds time to recover in dim light, and decreases the risk of one of the main problems in culture of macroalgae, the growth of epiphytes (Neish and Knutson 1978; Hanelt et al. 1992; Pang and Lüning 2004). However, high stocking density may result in self-shading. Thus, a higher irradiance may be needed to sustain the photosynthetic rate (Bidwell et al. 1985). Conversely, excessive irradiance may produce photoinhibition, the declination of photosynthetic activity (Cabello-Pasini et al. 2000). Consequently, determination of the best irradiance at high stocking density is necessary to maximize productivity (increase of biomass per time unit) and nutrient uptake.

The optimal stocking density to culture the red macroalgae, *Chondrus crispus* (Irish moss) and *Palmaria palmata* (dulse) under artificial light in Atlantic halibut effluent was suggested to be 2 kg m^{-2} (Kim et al. 2013). This stocking density, equivalent to 10 g L^{-1} , was the best compromise between growth and nutrient uptake. Kim et al. (2013) evaluated five stocking densities from 1 to 50 g L^{-1} , at an irradiance (Photosynthetic Photon Flux Fluence Rate, PPFRR) of $140 \mu\text{mol m}^{-2} \text{ s}^{-1}$, that was assumed to be saturated at the lower stocking density studied, and with a 16:8 L:D

photoperiod. Artificial lighting has hardware investment and operational costs, so a more cost effective approach is to use natural light.

In addition to irradiance, photoperiod is an important factor in macroalgal cultivation. Photoperiod determines the time that the algae are exposed to light, which allows them to photosynthesize and grow. In some species, it also controls reproductive phenology (Lüning 1993). Although continuous light (24 h) resulted in higher growth rate of *C. crispus* compared to shorter photoperiods, the use of artificial lighting during the night increases operations costs (Shacklock et al. 1975; Fortes and Lüning 1980). The photoperiod recommended to culture both species *C. crispus* and *P. palmata* in land-based systems is 16:8 L:D (Bird et al. 1979; Morgan and Simpson 1981a). This is the maximum natural daylight between sunrise and sunset in Nova Scotia (NS) latitude 45 ° N (NRC 2011).

Working with natural irradiance increases uncertainty because the weather, specifically the cloud cover, is unpredictable. Consequently, experiments under these conditions are difficult to replicate. For this reason, I considered it is important to assess the performance of both species simultaneously. Three irradiance levels were selected based on the mean daily local irradiance curve of Halifax, NS (Craigie and Shacklock 1995). Considering summer (June) conditions as 100 % irradiance, fall (October) conditions represent 50 % and winter (December) conditions 20 % irradiance. To analyze the performance of these species at different temperature conditions, two temperatures, 10 and 14 °C, were selected based on the temperature range recommended to culture Atlantic halibut juvenile, 10 to 14 °C (Brown 2010).

Laboratory scale experiments allow for better control of culture conditions to determine the effect of a specific factor. However, in batch culture systems inorganic carbon (CO₂) becomes limiting since it is quickly consumed by macroalgae during photosynthesis, resulting in an increase in pH of the medium (Simpson et al. 1978a). The optimal pH for photosynthesis in *C. crispus* and *P. palmata* is ca. 7.0 and it is significantly reduced at pH 9.0 (Robbins 1978; Simpson et al. 1978a; Craigie 1990). However, pH 8.5 is recommended as optimal for culture, since excessive addition of CO₂ does not generate benefits, since it tends to escape to the atmosphere (Braud and Amat 1996).

The aim of this research was to determine the best irradiance to culture *C. crispus* and *P. palmata* at high stocking density (10 g L⁻¹), using 2 g L⁻¹ as control, when they are simultaneously exposed to natural light in a controlled laboratory experiment.

3.3 MATERIALS AND METHODS

Algal material: The Basin Head strain of *Chondrus crispus* and the Point Prim strain of *Palmaria palmata* were obtained in April 2011 from Scotian Halibut Ltd., Wood's Harbour, NS (43° 31.04' N, 65° 44.30' W). Both species were maintained from June 2009 in Atlantic halibut effluent with the nutrient concentration ranging from 6 to 564 µM N, at natural temperature from 1 to 18 °C, and natural illumination via opaque roofing fibregrass panels supplemented with fluorescent tubes to have at least a PPFFR of 100 µmol m⁻² s⁻¹ (Corey 2011). Previously, *C. crispus* had been cultured for at least four years at the Marine Research Station, NRC, Ketch Harbour, NS (44° 28' N, 63° 33' W). *Palmaria palmata* was collected from the wild at Point Prim, Digby County, NS (44° 42'

N, 65° 47' W, Corey et al. 2012). Segments, 3.5 cm long, were trimmed from the apical parts of thalli.

Place and dates: Two experiments were conducted in Greenhouse N° 1 at Dalhousie University's Agricultural Campus (DAL-AC), Truro, NS (45° 22.42' N, 63° 15.89' W). The first experiment was conducted between May 31 and June 25, 2011, and photosynthetic rates were measured on June 28 and 29, 2011. The second experiment was carried out between July 18 and August 12, 2011 and photosynthetic rates were measured on August 17 and 18, 2011.

Experimental design: Forty-eight one-litre Florence flasks (4060-1L, Pyrex, MA, USA) were arranged in a split plot factorial design with four blocks (four rectangular fibreglass troughs, 2.8 m L x 0.4 m W x 0.2 m H). Three whole plots (three irradiance levels: high, 100 %; medium, 50 % and low, 20 % of the sunlight inside of the greenhouse) were randomized within each block. A sub-plot with 2 x 2 factorial of stocking density (2 and 10 g L⁻¹ fresh weight [FW]) and species (*C. crispus* and *P. palmata*) were randomized within each irradiance level (Figs. 3.1, 3.2; Appendix B).

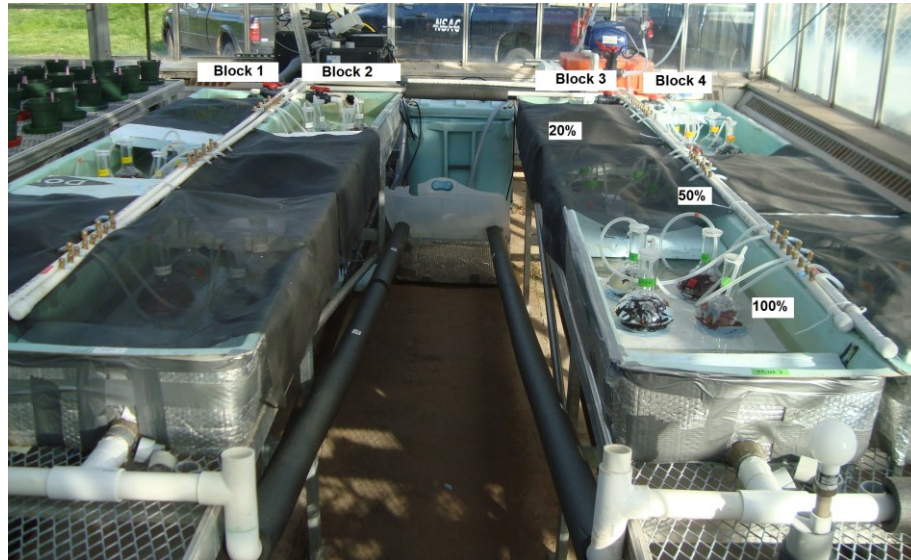


Figure 3.1. Experimental facility in Greenhouse N° 1 at Cox Institute, DAL-AC, Truro. Four troughs (blocks), three irradiance levels (100, 50 and 20 % of incident sunlight; achieved with zero, one and three layers of fiberglass cloth screen), two stocking densities (2 and 10 g L⁻¹ FW) and two species (*Chondrus crispus* and *Palmaria palmata*)



Figure 3.2. One-litre Florence flasks stocked with *Chondrus crispus* at the two stocking density treatments (2 and 10 g L⁻¹ FW)

Set up and operation: Ambient irradiance and photoperiod inside the greenhouse were the only illumination. The 50 and 20 % levels of irradiance were achieved using one and three layers of fibreglass cloth screen, respectively (FCS7350 Micro mesh, Saint-Gobain, NY, USA). The full Photosynthetic Active Radiation (PAR, 400 – 700 nm waveband) was logged every 15 min at the level of the culture flasks using a spherical quantum sensor (LI-193SA, LI-COR, NE, USA). This sensor measures the irradiance as Photosynthetic Photon Flux Fluence Rate (PPFFR, $\mu\text{mol m}^{-2} \text{s}^{-1}$). Since PPFFR varied constantly due the cloud cover, to improve the reproducibility of the results, Daily Photon Dose (DPD, $\text{mol m}^{-2} \text{d}^{-1}$) and Total Photon Dose (TFD, $\text{mol m}^{-2} \text{26 d}^{-1}$) were calculated, integrating the PPFFR during daylight hours (Sagert and Schubert 2000).

To maintain a constant temperature of either 10 or 14 °C, the flasks were placed in a water bath in each trough using freshwater recirculated through two chillers (Arctica DA-500B, Aquarium Products Inc., CA, USA). Water temperature was logged every 10 min (Minilog 8-bit, Vemco, NS, Canada).

To achieve the tumble culture, air was bubbled into each flask via an acrylic tube (4.76 mm OD, 16007, Dynamic Aqua-Supply Ltd., BC, Canada) connected to the air manifold by silicone tubing (4.78 mm ID, 515-013, Dow Corning, MI, USA), and regulated individually with a brass valve (VBR1, Dynamic Aqua-Supply Ltd., BC, Canada). Aeration was generated by a diaphragm air pump (SL94 Sweetwater, Aquatic Eco-System, Inc., FL, USA). To control pH, four 1/2" PVC air manifolds were set up, one for each species-stocking density condition. The carbon dioxide (CO₂) supply to each manifold was controlled by a globe valve (1/2", GVA005VT, Chemline Plastics Ltd., ON, Canada). The valves were connected to a CO₂ cylinder with clear PVC tubes

(6.35 mm ID, Clearforce - RH28504, EATON, ON, Canada). pH was monitored manually every two hours during daylight, in four flasks of a random sub-plot, with 100 % irradiance using a hand-held meter (Electrode 13-620-AP50 and Accumet AP61, Fisher Scientific, Singapore). If pH exceeded 8.5 in any treatment combination, CO₂ was bubbled in through the air supplies of this species-stocking density condition manifold (See details in Fig. 3.1).

Culture medium and samples: Filtered (0.5 µm) and UV-sterilized seawater from the Marine Research Station, NRC was enriched with a modification of von Stosch's enrichment medium which has an concentration of nitrogen and phosphorus equivalent to the maximum concentration found in Atlantic halibut effluents (von Stosch 1964; Corey 2011). Unenriched seawater had a mean of 14 µM of ammonium, 0.4 µM of nitrate and 0.2 µM of orthophosphate. Nutrient enriched seawater contained 500 µM of ammonium chloride as the nitrogen source and 30 µM of sodium phosphate as the phosphorus source. Vitamins were added, according to the specifications for this medium (Andersen et al. 2005, p. 518). Each flask contained one litre of culture medium (Fig. 3.2) and *ca.* 25 and 125 initial segments at 2 and 10 g L⁻¹, respectively. The culture medium was replaced twice per week (batch culture system). In addition to regular pH monitoring, at each exchange of the culture medium, temperature and pH were recorded to determine the conditions inside each flask. Weekly, the algae were dried with paper towel to remove excess of water, and weighed, after which, whole segments were removed to return each flask to the original stocking density. Specific growth rate (SGR) was expressed in percent per day (Eq. 3.1; Evans 1972).

Eq. 3.1
$$\text{SGR} = [\ln (\text{FW}_2 / \text{FW}_1) / (t_2 - t_1)] \times 100$$

where: \ln is the natural logarithm, FW_2 and FW_1 are the final and initial fresh weights and t_2 and t_1 are the final and initial day of the sampling period.

To analyze C and N, tissue samples were dried at 60 °C at the start and end of each experiment. These samples also were used to calculate the fresh weight /dry weight ratio (FW/DW ratio). To determine ammonium and orthophosphate uptake, seawater was sampled at each medium exchange and frozen at -20 °C (APHA et al. 2005). During the culture media exchange, fronds were examined, photographed to register the colour change and epiphytes were removed using a scalpel.

Net photosynthetic rate (NPR): At the end of each experiment, a sample of fronds from each treatment was used to determine the NPR using the light-dark bottle method (Thomas 1988). Dissolved oxygen was measured with a polarographic electrode (Accumet Self-Stirring Biological Oxygen Demand [BOD] Probe, Fisher Scientific, USA) connected to a benchtop dissolved oxygen meter (AB40, Fisher Scientific, Singapore). The segments were stocked in triplicate in 300-mL BOD bottles at the same stocking density as the Florence flasks (2 or 10 g L⁻¹ FW). The BOD bottles were held in the same troughs that the experimental Florence flasks to maintain the same experimental temperature and exposed to irradiance for about one to two hours until oxygen bubbles were observed in the light bottles. Photosynthetic rate was determined sequentially for each species and light treatment over the course of two days. The light treatment level (20, 50 or 100 % irradiance) corresponded to the percent of the natural solar radiation

inside the greenhouse, at the measurement time. During each measurement, PPFRR was recorded every minute with the spherical sensor indicated above (LI-193SA). To determine the irradiance during the NPR measurement, the mean PPFRR was calculated. Net photosynthetic rate ($\text{mg C g}^{-1} \text{FW h}^{-1}$) was determined with Eq. 3.2 (Thomas 1988).

$$\text{Eq. 3.2} \quad \text{NPR} = [(\text{FB} - \text{IB}) \times 375.9] / (\text{T} \times \text{PQ}) / (\text{FW} \times 3333)$$

In this equation, FB and IB are the final and initial oxygen levels in the light bottle in mg L^{-1} , 375.9 is the factor to standardize volume (1 m^3), T is incubation time in h, PQ is the photosynthetic quotient (1.2 was assumed), FW is the fresh weight of the fronds in g and 3333 is the factor to standardize to a litre basis.

Tissue analysis: Dried tissues were ground to a powder for 1.5 min at a vibrational frequency of 19.5 s^{-1} in a ball mill (MM301, Retsch, Germany), and then a 200 mg DW aliquot was analyzed in an elemental analyzer (CNS-100, Leco, MI, USA) using sulfamethazine as a standard. Results were expressed in percent of total N and C in dry tissue, and molar C/N ratio was calculated for each sample (Atkinson and Smith 1983). Due to the small amount of dry tissue available in the low light treatment, samples from the four blocks were pooled, Block 1 was pooled with Block 2, and Block 3 with Block 4.

Water nutrients: Ammonium and orthophosphate in the water samples were determined on an auto-analyzer (Technicon Auto Analyzer II, Technicon Industrial Systems, NY, USA) by the Greenhouse Gases and Nutrient Analysis Laboratory at the Department of Environmental Sciences, DAL-AC. Ammonium was determined using the salicylate-

hypochlorite method (Bower and Holm-Hansen 1980), and orthophosphate was measured using the molybdenum blue method (Murphy and Ripley 1962).

Culture conditions. Both experiments were carried out during summer conditions. Experiment I included the summer solstice, therefore, its photoperiod showed less variation than Experiment II, when the daylight declined through time (Table 3.1). Despite the shorter daylength during Experiment II, it resulted in a higher Daily Photon Dose (DPD) and Total Photon Dose (TPD) because cloud cover was less than during Experiment I (Table 3.1). The irradiance exposure of the fronds at the mid and low light treatment had an overlap due to the inevitable changes in cloud cover between each culture medium exchange (Appendix D, Table D.1; nutrient uptake) and between each weighing (Appendix D, Table D.2; specific growth rate). Daily changes in the angle of incident sunlight, also affected the irradiance entering the greenhouse during Experiment II, when the sunlight entered through the lateral window walls in the late afternoon (Fig. 3.3).

The water bath system worked well, each target temperature of the freshwater in the troughs was constant during both experiments (Table 3.1). The seawater temperature inside the flasks was not significantly influenced by incident sunlight at the different treatments (Appendix D, Table D.3). By contrast, pH was significantly affected by light treatment in both experiments ($p < 0.05$; Appendix D, Table D.3). In both experiments, the mean pH was maintained below the threshold of 8.5 in the 100 % light treatment. However, at lower irradiances, the pH was lower (7.7) because of the lower need for CO₂ due to the low photosynthetic activity. This effect was clearest in Experiment I

(Appendix D, Table D.3), specifically in *P. palmata* at high stocking density (Appendix D, Table D.4). This occurred because at the highest stocking density, *P. palmata* required greater addition of CO₂ to maintain pH, and this was followed by *C. crispus*, at the same stocking density. At the low stocking density, both species showed the same trend. Accordingly, *C. crispus* needed very little supplementary CO₂ to maintain pH, and the pH was similar at all light treatments.

Table 3.1. Culture conditions inside the greenhouse in Experiment I and Experiment II. The photoperiod is according to NRC (2011). Total Photon Dose and Daily Photon Dose are computed from the Photosynthetic Photon Flux Fluence Rate recorded every 15 min with a spherical quantum sensor (LI-193SA). Three light treatments: 100, 50, and 20 % of the incident sunlight. Water temperature was recorded every 10 min with a datalogger (Minilog 8-bit). All means \pm SE.

	<i>Experiment I</i>	<i>Experiment II</i>
Period, dates	May 31 - Jun. 25	Jul. 18 – Aug. 12
Photoperiod, (L:D), h	15.6:8.4 \pm 0.02	14.8:9.2 \pm 0.06
Total Photon Dose, mol m ⁻² 26 d ⁻¹	343	414
100 % Daily Photon Dose, mol m ⁻² d ⁻¹	13.1 \pm 1.3	16.6 \pm 1.6
50 % Daily Photon Dose, mol m ⁻² d ⁻¹	6.5 \pm 0.6	8.3 \pm 0.8
20 % Daily Photon Dose, mol m ⁻² d ⁻¹	2.6 \pm 0.3	3.3 \pm 0.3
Water bath system temperature, °C	10.0 \pm 0.01	14.0 \pm 0.01

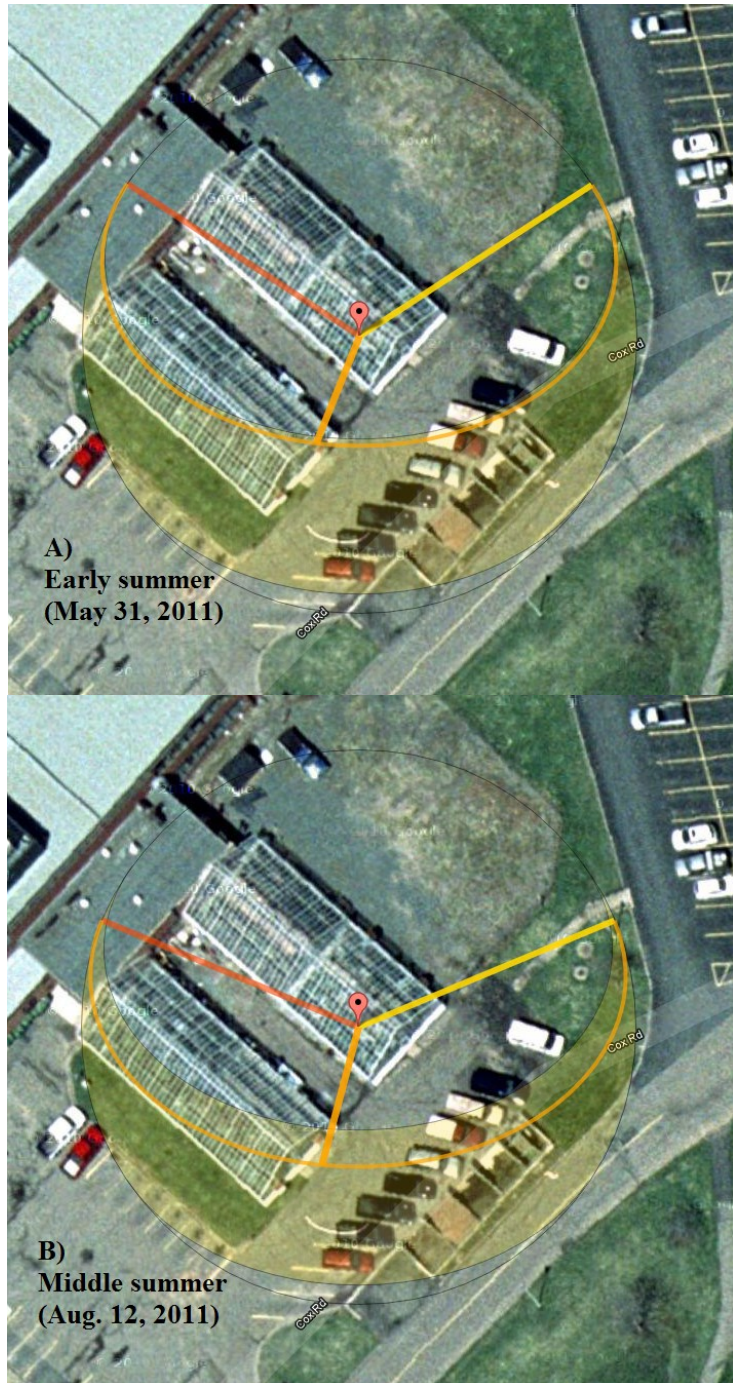


Figure 3.3. Photographs showing the maximum difference of the angle of incident sunlight during Experiment I and Experiment II conducted in early-summer (May 31 to June 25, 2011) and mid-summer (July 18 to August 12, 2011), respectively. The experiments were conducted in the corner of the greenhouse indicated with with the red icon. The yellow line represents the sunrise, the orange curve the Sun's trajectory, and the red line the sunset. The light yellow area is the variation of sun trajectories during the year; the closer is the yellow line to the centre, the closer is the angle of incident sunlight to the azimuth. <http://www.suncalc.net>.

In Experiment I, irradiance during the Net Photosynthetic Rate (NPR) measurements was more stable than in Experiment II. Mean PPFFR from 123 to 313 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and from 41 to 1,435 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Therefore, the PPFFR, which photosynthesis was measured at each light treatment for both species, exhibited no significant differences (Appendix D, Table D.5). In contrast, in Experiment II at the same light treatment, both species were exposed to a significantly different PPFFR (Appendix D, Table D.6). However, in both experiments, the PPFFR was significantly different at the three light treatments for both species.

Statistical analysis: Data were analyzed using a mixed effects model where block was the random factor. The fixed factors were the whole plot (three light levels; 20, 50, and 100 % incident sunlight), and the factorial sub-plot: two species (*C. crispus* and *P. palmata*) x two stocking densities (2 and 10 g L^{-1} FW). This model allows determination of the differences in the means of the response variables. For nutrient uptake and specific growth rate, a repeated measures analysis was conducted to include the effect of the different sampling periods. Different types of covariance structure were tested, and those with a lower Akaike's Information Criterion (AIC) that met the normality requirement were selected to run the Mixed procedure (Elliot and Woodward 2010). The Anderson-Darling normality test (Anderson and Darling 1952) was conducted in Minitab 16 (Minitab Inc., State College, PA, USA), using the residuals of the Mixed procedure of SAS statistical software (SAS 9.2, Institute Inc., Cary, NC, USA). Uptakes for both nutrients from Experiment I were square transformed to meet the normality criterion. In Experiment II ammonium uptake was also square transformed, while orthophosphate

uptake was cubic transformed. For tissue nutrients and molar C/N ratio, a single time was analyzed. Least-squares means analysis ($p=0.05$) was used as a *post hoc* test to determine pairwise relationships among all treatment combinations.

3.4 RESULTS

Nutrient uptake efficiency. In Experiment I, only ammonium (NH_4^+) uptake efficiency was significantly affected by the interaction of all the four experimental factors, irradiance, stocking density, species and sampling period (Table 3.2). By contrast, nutrient uptake efficiency was not affected by the three-way interaction between irradiance, stocking density and sampling period ($p = 0.133$, Table 3.2). This can be explained by the overlap in the irradiance among the different light treatments and sampling periods indicated in the culture conditions section (Appendix D, Table D.1). For the same reason, NH_4^+ uptake efficiency also was not significantly affected by the interaction between stocking density, species and sampling period ($p = 0.153$, Table 3.3). Ammonium uptake efficiency was significantly affected by stocking density alone or in combination with the other experimental factors. However, as indicated above, in combination with sampling period and a third factor (irradiance or species), NH_4^+ was not significantly affected. The effect of self-shading at high stocking density was not evident in *P. palmata* because at both stocking densities fronds consumed, between each media exchange almost 100 % of the NH_4^+ available in the flasks (Fig. 3.4.A). Orthophosphate (PO_4^{3-}) uptake efficiency was significantly affected by any interaction between three of the four experimental factors. PO_4^{3-} uptake efficiency, as with NH_4^+ uptake efficiency, was not significantly affected by the interaction between sampling period, light treatment

and species because of the overlap in the irradiance between the different light treatments (Table 3.3). However, it was possible to detect that *P. palmata* had an overall higher uptake efficiency than *C. crispus* for both nutrients, and the uptake was higher at 10 g L⁻¹ than at 2 g L⁻¹.

Chondrus crispus at 2 g L⁻¹ was the treatment most sensitive to irradiance, and NH₄⁺ uptake efficiency was significantly different at the three light treatments at all four measurement intervals (Fig. 3.4.A.a). In contrast, at 10 g L⁻¹, NH₄⁺ uptake was no significantly different at the 50 and 100 % light treatments. At the lowest light treatment, NH₄⁺ uptake efficiency increased through the sampling periods up to being independent of irradiance at the fourth sampling period (Fig. 3.4.A.b). *Palmaria palmata* NH₄⁺ uptake at 2 g L⁻¹ was similar at 100 and 50 % light treatment ($p > 0.05$), and at the 20 % light treatment was lower, but there was high variation (Fig. 3.4.A.c). At 10 g L⁻¹, *P. palmata* uptake of NH₄⁺, in most of the periods, was independent of irradiance consuming almost 100 % of the NH₄⁺ available in the flasks (Fig. 3.4.A.d).

Orthophosphate (PO₄³⁻) uptake efficiency by *C. crispus*, at 2 g L⁻¹ consistently increased in a stepwise manner with irradiance (Fig. 3.4.B.a). At 10 g L⁻¹, PO₄³⁻ uptake was equally high (78 % of the PO₄³⁻ available in the flasks) in 50 and 100 % light treatment, both significantly higher than at the 20 % light treatment (Fig. 3.4.B.b). At both stocking densities of *P. palmata*, there was no significant difference in PO₄³⁻ uptake between the 100 and 50 % light treatment, in both cases fronds consumed 80 % of the PO₄³⁻ available in the flasks (Figs. 3.4.A.c and d). However, at 20 % light treatment, PO₄³⁻ uptake was significantly higher at 10 than at 2 g L⁻¹.

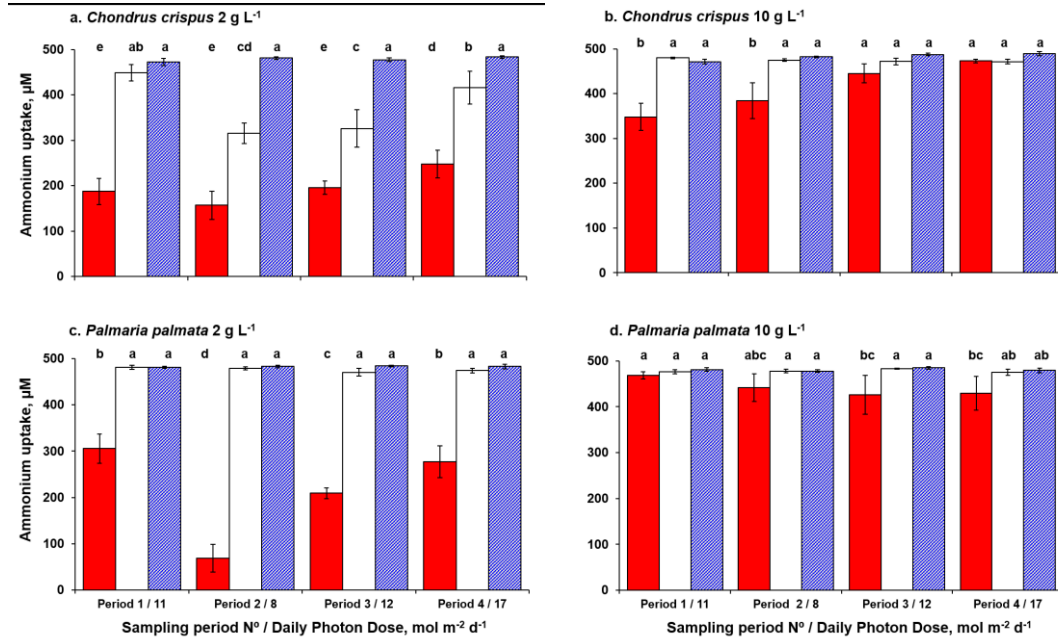
Table 3.2. Significance of experimental factors alone and in combination for Experiment I and Experiment II. N uptake = Ammonium uptake, μM . P uptake = Orthophosphate uptake, μM . SGR = Specific Growth Rate, $\% \text{ d}^{-1}$. Values highlighted indicate no significance (α at 0.05).

<i>Factors</i>	<i>Experiment I</i>			<i>Experiment II</i>		
	<i>N uptake</i>	<i>P uptake</i>	<i>SGR</i>	<i>N uptake</i>	<i>P uptake</i>	<i>SGR</i>
Irradiance (20, 50, and 100 %)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Stocking density (2 and 10 g L ⁻¹)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Species (<i>C. crispus</i> and <i>P. palmata</i>)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sampling period (1, 2, 3 and 4)	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
Irradiance x density	<0.001	0.043	<0.001	<0.001	0.117	<0.001
Irradiance x species	0.004	0.008	<0.001	<0.001	0.432	0.031
Irradiance x sampling period	0.013	0.381	0.649	<0.001	<0.001	0.192
Density x species	0.011	0.010	0.003	0.029	<0.001	0.636
Density x sampling period	0.018	0.033	<0.001	0.015	0.489	0.669
Species x sampling period	0.001	0.144	0.010	<0.001	0.007	<0.001
Irradiance x density x species	0.001	<0.001	0.209	<0.001	<0.001	0.596
Irradiance x density x sampling period	0.133	0.004	0.491	<0.001	<0.001	0.416
Irradiance x species x sampling period	<0.001	0.006	0.087	<0.001	<0.001	0.087
Density x species x sampling period	0.153	0.010	0.036	0.003	<0.001	0.010
Irradiance x density x species x sampling period	0.008	0.370	0.856	<0.001	0.037	0.034

In Experiment II, conducted later in the summer at 14 °C, uptake of both nutrients was influenced significantly by the interaction of the four experimental factors, irradiance, stocking density, species and sampling period (Table 3.3). Ammonium uptake efficiency also was significantly affected by the experimental factors alone or in any combination. However, PO_4^{3-} uptake was independent of the interaction between irradiance and stocking density, irradiance and species, and stocking density and sampling period (Table 3.3). This was because the irradiance was not significantly different at the four sampling periods and there was an overlap in the irradiance between the light treatments (Appendix D, Table D.1). Similar to Experiment I, NH_4^+ available in the flasks was almost 100 % consumed at each media exchange at the 50 and 100 % light treatments. In the case of PO_4^{3-} , the uptake was also not significantly different at 50 and 100 % light treatments, *ca.* 85 % of the PO_4^{3-} available. In Experiment II, nutrient uptake efficiency by *P. palmata* was higher than *C. crispus*, and at 10 g L⁻¹ the nutrient uptake efficiency was higher than at 2 g L⁻¹, mainly at the lower light treatment.

At 2 g L⁻¹, NH_4^+ uptake efficiency in Experiment II by *C. crispus* was significantly affected by irradiance (Fig. 3.5.B.a). At 10 g L⁻¹, NH_4^+ uptake by *C. crispus* at 100 and 50 % light treatment was similar ($p > 0.05$, Fig. 3.5.A.b). Ammonium uptake by *P. palmata* also was not significantly different at 100 and 50 % light treatment, all nutrient were absorbed (Fig. 3.5.A.c). At 20 % light treatment, in contrast to *C. crispus*, NH_4^+ uptake by *P. palmata* was more constant. At 10 g L⁻¹, NH_4^+ uptake by *P. palmata* was independent of irradiance because the high nutrient consumption (Fig. 3.5.A.d).

A. Ammonium



B. Orthophosphate

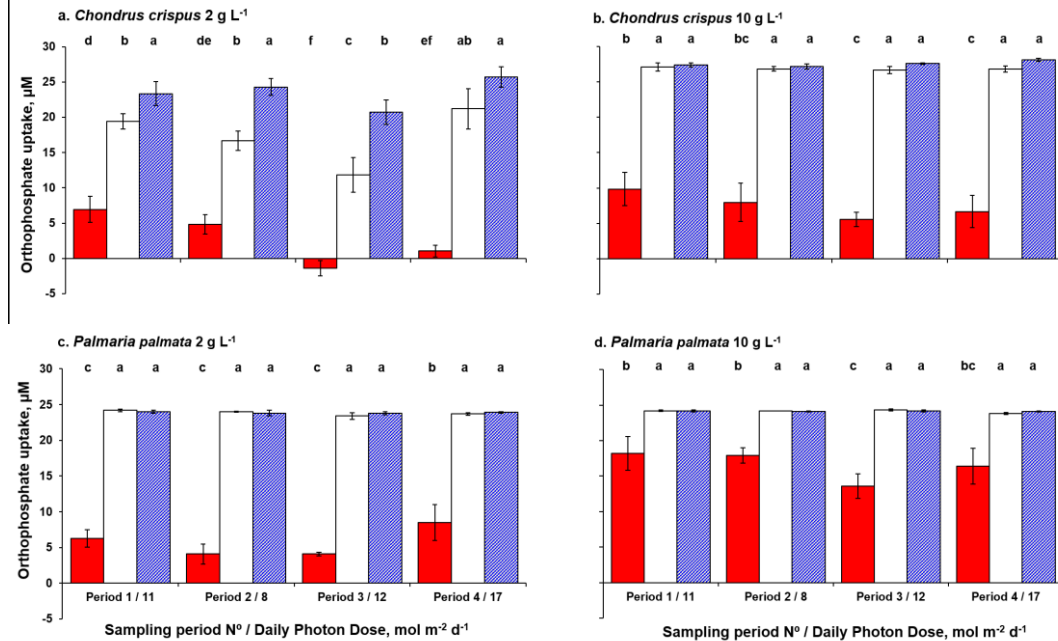


Figure 3.4. Nutrient uptake efficiency in Experiment I, May 31 – June 25, 2011. A) Ammonium. B) Orthophosphate. *Chondrus crispus* (a and b) and *Palmaria palmata* (c and d) were cultivated at 10 °C at two stocking densities (2 g L⁻¹ FW, a and c and 10 g L⁻¹ FW, b and d) under three levels of natural light (20, 50 and 100 % incident sunlight). Nutrient uptake was measured at each culture medium exchange, twice a week. Mean Daily Photon Dose (DPD) at 100 % irradiance is reported at each sampling period because natural light varied in time (Period N° / DPD). Irradiance levels: solid bar (20 %); clear bar (50 %) and patterned (100 %). Error bars = SE. Bars sharing the same letter are not significantly different (Least-square means test, p>0.05).

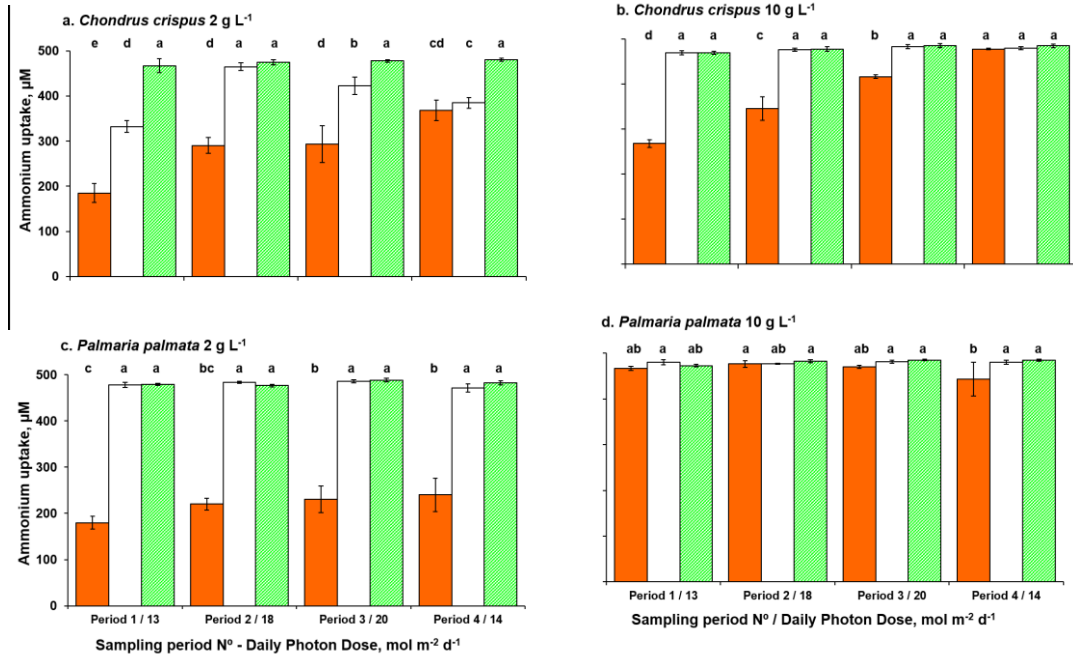
Orthophosphate uptake efficiency by *C. crispus* at 2 g L⁻¹ was variable in the different sampling periods. In some periods uptake was clearly influenced by the light treatment, while in other periods the PO₄³⁻ uptake efficiency at 50 % light treatment was similar to the 20 and 100% light treatments (Fig. 3.5.B.a). At 10 g L⁻¹, the difference between 100 and 50 % light treatments was not significant throughout the experiment, both resulting in consumption of about 85 % of the available PO₄³⁻ (Fig. 3.5.B.b). In *C. crispus*, PO₄³⁻ uptake efficiency at the 20 % light treatment was similar at both stocking densities. At 2 g L⁻¹, PO₄³⁻ uptake efficiency by *P. palmata* showed the same trend as *C. crispus* at 10 g L⁻¹ (Fig. 3.5.B.c), with no significant difference between the 100 and 50 % light treatments, but mean PO₄³⁻ uptake efficiency was lower at the 20 % light treatment. At 10 g L⁻¹, PO₄³⁻ uptake by *P. palmata* was significantly lower at the lowest light treatment compared to 50 and 100 % light treatment where the fronds consumed 86% of the available PO₄³⁻ (Fig. 3.5.B.d). In *P. palmata*, PO₄³⁻ uptake efficiency at the 20 % light treatment was lower at 2 g L⁻¹ than at 10 g L⁻¹ at all the sampling periods.

Specific growth rate (SGR). In Experiment I, SGR was not significantly influenced by the interaction of the four experimental factors, irradiance, stocking density, species and sampling period (Table 3.3). The highest level interaction was a three-way interaction between stocking density, species and sampling period (p = 0.036, Table 3.3). Irradiance affected significantly SGR when it was analyzed alone and in combination with stocking density or species, but at highest interaction levels, the irradiance effect was hidden (Table 3.3). This was due to the high nutrient consumption at the 50 and 100 % light treatments indicated above. Specific growth rate also was not significantly influenced by

the interaction among irradiance and sampling period because the overlap in the irradiance among all the light treatments at the different sampling period indicated in the culture condition section (Appendix D, Table D.2). Specific growth rate followed the same trend as nutrient uptake, with a higher growth rate in *P. palmata* than in *C. crispus*. However, contrary to nutrient uptake, growth rate was higher at 2 than at 10 g L⁻¹ (Fig. 3.6.A).

In Experiment II, SGR was significantly influenced by the interaction of the four experimental factors, irradiance, stocking density, species and sampling period (Table 3.3). However, SGR was not significantly affected by the other high interaction levels, with exception of the interaction among the stocking density, species and sampling period ($p = 0.01$, Table 3.3). In Experiment II, irradiance also affected significantly SGR only when it was analyzed alone or in combination with stocking density or species. This, due to the high nutrient consumption at the 50 and 100 % light treatments and the overlap in the irradiance among the light treatments indicated above. In Experiment II, at the 50 and 100 % light treatments, SGR followed the same trend that in Experiment I, a higher growth rate in *P. palmata* than in *C. crispus* and at 2 g L⁻¹ than at 10 g L⁻¹ (Fig. 3.6.B). However, at the lower light treatment, the difference between species was not evident.

A. Ammonium



B. Orthophosphate

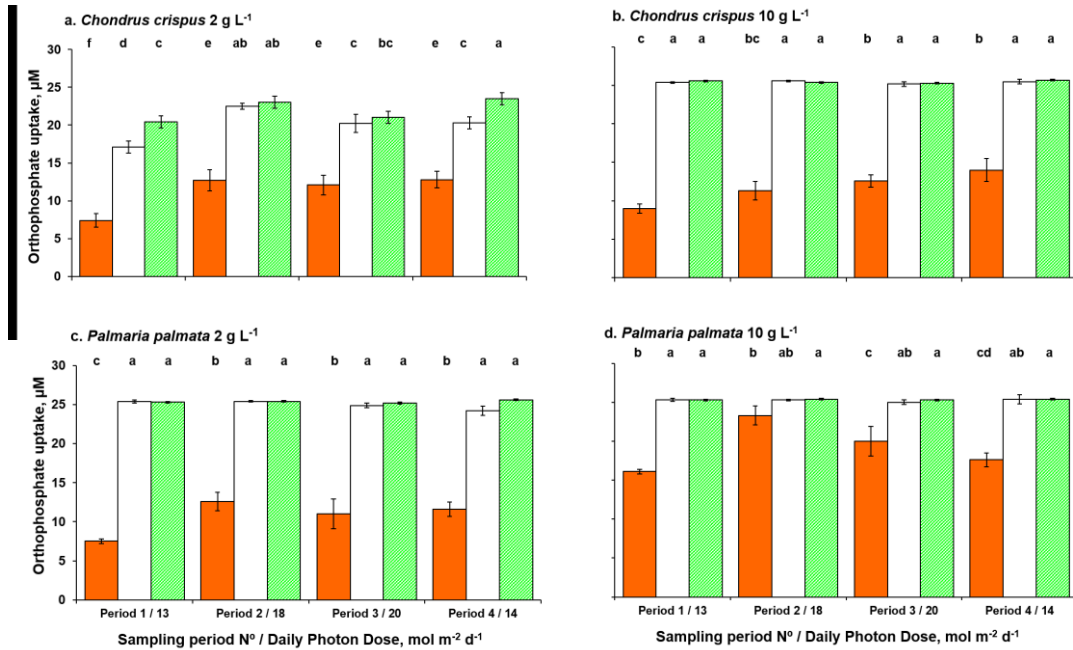
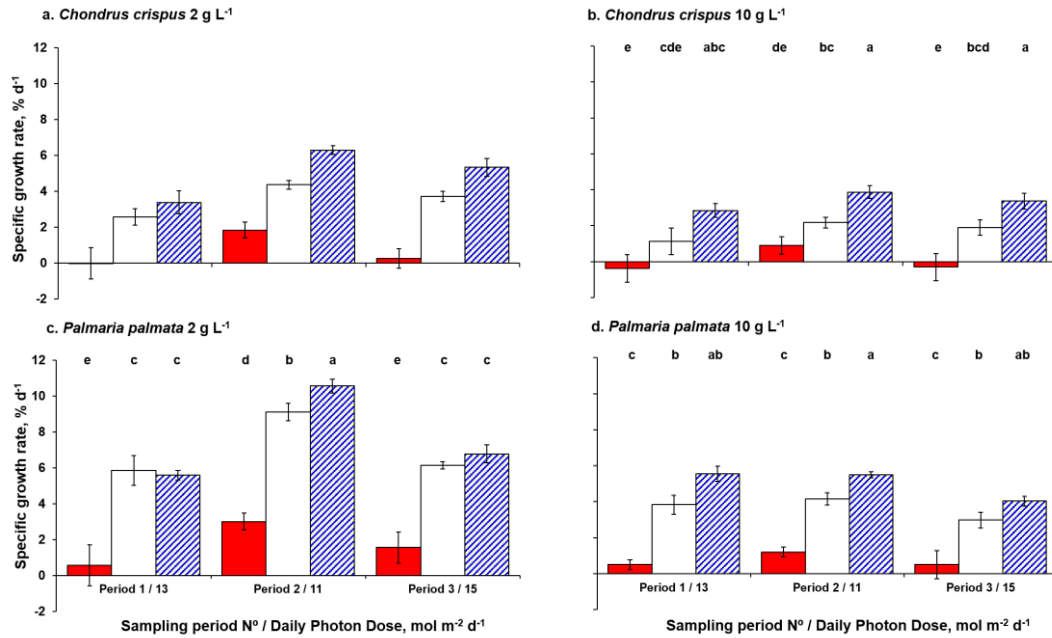


Figure 3.5. Nutrient uptake efficiency in Experiment II, July 18 – August 12, 2011. A) Ammonium. B) Orthophosphate. *Chondrus crispus* (a and b) and *Palmaria palmata* (c and d) were cultivated at 14 °C at two stocking densities (2 g L⁻¹ FW, a and c and 10 g L⁻¹ FW, b and d) under three levels of natural light (20, 50 and 100 % incident sunlight). Nutrient uptake was measured at each culture medium exchange, twice a week. Mean Daily Photon Dose (DPD) at 100 % irradiance is reported at each sampling period because natural light varied in time (Period N^o / DPD). Irradiance levels: solid bar (20 %); clear bar (50 %) and patterned (100 %). Error bars = SE. Bars sharing the same letter are not significantly different (Least-square means test, p>0.05).

The highest mean SGR of the three sampling periods was the same in both experiments (7.6 % d⁻¹), which corresponded to *P. palmata* cultured at 2 g L⁻¹ at 100 % light treatment. At the same culture conditions, the highest mean SGR for *C. crispus* was 5.0 % d⁻¹ at 10 °C (Experiment I) and 6.9 % d⁻¹ at 14 °C (Experiment II; Fig. 3.6). In Experiment I, the highest SGR was during the second sampling period, when the Daily Photon Dose (DPD) was 11 mol m⁻² d⁻¹. For *C. crispus* at 2 g L⁻¹ was 6.3 % d⁻¹, in the other two periods, SGR was not significantly different at the 100 and 50 % light treatments, but at 20 %, growth was poor or even negative (Fig. 3.6.A.a). At 10 g L⁻¹, the highest SGR of *C. crispus* decreased to 3.9 % d⁻¹ (Fig. 3.6.A.b). In the case of *P. palmata*, the highest SGR at 2 g L⁻¹ was 10.6 % d⁻¹ (Fig. 3.6.A.c). In the other periods, there was no significant difference between the 100 and 50 % light treatments. At 10 g L⁻¹, the same pattern was observed, with a higher SGR of 5.6 % d⁻¹, but this was at a different sampling period (Fig. 3.6.A.d). In Experiment II, SGR of *C. crispus* cultured at 2 g L⁻¹ was affected significantly by each increase in irradiance within each sampling period, and over the whole experiment (Fig. 3.6.B.a). The highest SGR was 7.1 % d⁻¹ at 100 % light treatment in the third sampling period, DPD of 17 mol m⁻² d⁻¹. At 10 g L⁻¹, the highest SGR of *C. crispus* was 3.6 % d⁻¹, but there was no significant difference in growth at the 100 and 50 % light treatments (Fig. 3.6.B.b). The higher SGR of *P. palmata* was at the higher DPD (19 mol m⁻² d⁻¹), 8.7 % d⁻¹ at 2 g L⁻¹ and 5.6 % d⁻¹ at 10 g L⁻¹. Within each sampling period, irradiance had a significant effect on SGR of *P. palmata*, with higher irradiance resulting in higher growth rate (Fig. 3.6.B.c and d). However, the overall mean SGR of the three sampling periods showed that at 10 g L⁻¹ there was no significant difference between growth at the 50 and 100 % light treatments.

A. Experiment I



B. Experiment II

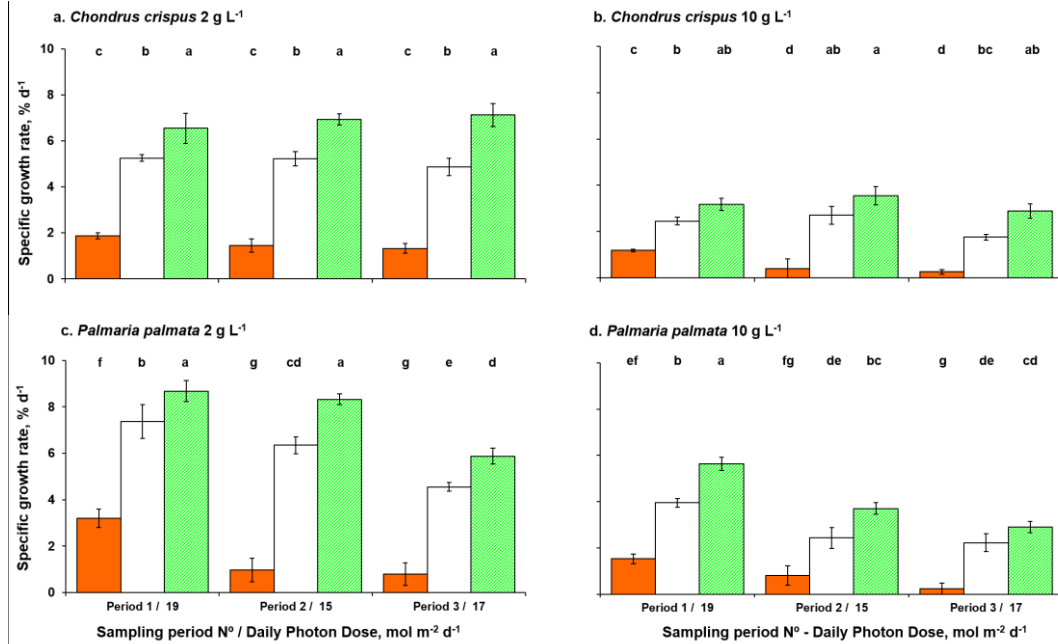


Figure 3.6. Specific Growth Rate. A. Experiment I (10 °C). B. Experiment II (14 °C). *Chondrus crispus* (a and b) and *Palmaria palmata* (c and d) were cultivated at two stocking densities (2 g L⁻¹ FW, a and c, and 10 g L⁻¹ FW, b and d) under three levels of natural light (20, 50, 100 % incident sunlight). Algae were weighed every week (sampling period). Mean Daily Photon Dose (DPD) at 100 % irradiance for sampling period differed because natural light varied (Period N° / DPD). Irradiance levels: solid bar (20 %); clear bar (50 %) and patterned (100 %). Error bars = SE. Bars sharing the same letter are not significantly different (Least-square means test, p>0.05).

Tissue nutrient content. In Experiment I, carbon (C) content, nitrogen (N) content and molar C/N ratio were not significantly affected by the interaction of the three experimental factors, irradiance, stocking density and species (Table 3.4). Carbon content also was not significantly affected by the interaction stocking density and species because the high effect of irradiance on *P. palmata* C content. At the lowest light treatment, C content in *P. palmata* was similar to *C. crispus* (Fig. 3.7.A). In *P. palmata* at the lower light treatment, the C content remained constant throughout the trial at 28%. However, in the 100 % light treatment C content increased to *ca.* 35 % C at both stocking densities (Fig. 3.7.A). By contrast, the C content of *C. crispus* (30 % C) remained constant independent of treatment (Fig. 3.7.A). This explains why the significance in the interaction irradiance with species was higher than in the interaction irradiance with stocking density ($p = 0.024$; Table 3.4). Nitrogen content also was not significantly affected by the interaction between irradiance and stocking density, only in *P. palmata* at 2 g L^{-1} was there a significant effect of irradiance on N content (Table 3.4, Fig. 3.7.B).

Nitrogen content was inversely related to irradiance in each factorial treatment (stocking density – species, Fig. 3.7.B). Nitrogen content decreased from 4.5 to 2.8 % N in *C. crispus* and from 4.7 to 1.9 % N in *P. palmata*. The most pronounced decrease was in *P. palmata* at 10 g L^{-1} , and less marked in *C. crispus* at 2 g L^{-1} . The molar C/N ratio was significantly affected by each of the experimental factors and by all of their combinations (Table 3.4). The initial molar C/N ratio was 7.8 and 7.0 for *C. crispus* and *P. palmata*, respectively. Molar C/N ratio increased with irradiance in both species (Fig. 3.7c). The highest molar C/N ratio was 22 in *P. palmata* at 10 g L^{-1} and 100 % light treatment and 13 in *C. crispus* at the same treatment (Fig. 3.7c).

Table 3.3. Significance of experimental factors alone and in combination for tissue analysis in Experiment I (10 °C) and Experiment II (14 °C). C = Tissue carbon content, %. N = Tissue nitrogen content, %. C/N ratio = Tissue molar carbon/nitrogen ratio. Values highlighted indicate no significance (α at 0.05).

<i>Factors</i>	<i>Experiment I</i>			<i>Experiment II</i>		
	<i>C</i>	<i>N</i>	<i>C/N ratio</i>	<i>C</i>	<i>N</i>	<i>C/N ratio</i>
Irradiance (20, 50, and 100 %)	0.023	0.002	0.002	0.137	0.003	0.004
Stocking density (2 and 10 g L ⁻¹)	0.011	<0.001	<0.001	0.113	<0.001	<0.001
Species (<i>C. crispus</i> and <i>P. palmata</i>)	<0.001	0.019	<0.001	<0.001	<0.001	0.068
Irradiance x density	0.024	0.080	<0.001	0.236	<0.001	<0.001
Irradiance x species	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
Density x species	0.063	0.033	0.001	0.009	<0.001	<0.001
Irradiance x density x species	0.814	0.394	0.060	0.105	0.043	0.001

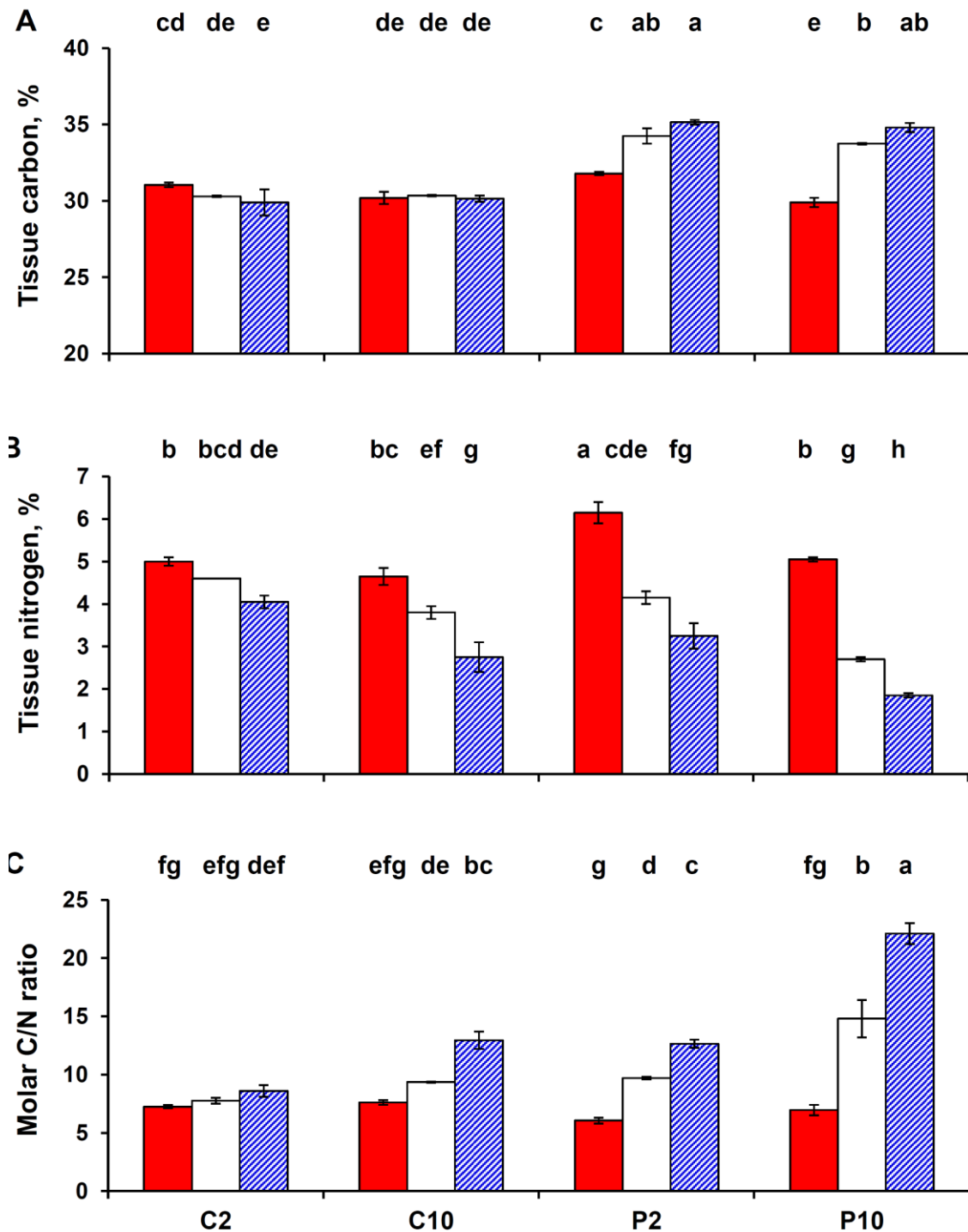


Figure 3.7. Experiment I, May 31 – June 25 2011. Tissue nutrient content of two red macroalgae cultured for 26 days at 10 °C at two stocking densities under three levels of natural light. A) Carbon; B) Nitrogen; C) Molar C/N ratio. C2 = *Chondrus crispus* at 2 g L⁻¹; C10 = *C. crispus* at 10 g L⁻¹; P2 = *Palmaria palmata* at 2 g L⁻¹; P10 = *P. palmata* at 10 g L⁻¹. Irradiance levels: solid bar (20 %); clear bar (50 %) and patterned bar (100 %). Error bars = SE. Bars sharing the same letter are not significantly different (Least-square means test, p>0.05).

In Experiment II, the three-way interaction between species, stocking density and species had a significant effect on N content but not C content (Table 3.4). Moreover, C content was not affected by irradiance, stocking density or their combination (Table 3.4). Only in *P. palmata* cultured at 10 g L⁻¹ was there a significant effect of irradiance on C content, the initial C content (33 % C) increased to 36 % C at the highest light treatment (Fig. 3.8.A). The C tissue content of *C. crispus* remained unchanged in all treatments at 29 % C. Nitrogen content was significantly affected by each experimental factors and by all of their combinations (Table 3.4). The initial N content of *C. crispus* (4.0 % N) only was reduced at the highest light treatment at 10 g L⁻¹ (2.5 % N). The initial N content of *P. palmata* (4.1 % N) was increased at the lowest light treatment at both stocking densities but was reduced at highest irradiances, mainly at 10 g L⁻¹ (2.3 % N; Fig. 3.8.B).

The molar C/N ratio in Experiment II was significantly affected by the three-way interaction between irradiance, stocking density and species (Table 3.4). This was due to the molar C/N ratio of *C. crispus* remaining relatively constant, while in *P. palmata* the C/N ratio decreased significantly at low irradiance and increased significantly at high irradiance, then the mean ratio of both species was similar (Fig. 3.8.C). The initial molar C/N ratio of *C. crispus* (8.6) only increased at 100 % light treatment at 10 g L⁻¹. By contrast, in *P. palmata* the molar C/N ratio was proportional to irradiance. However, at 2 g L⁻¹ all the values were lower than the initial ratio (9.3). At 10 g L⁻¹, the ratio also decreased at the 20 % light treatment but increased to 18 at the 100 % light treatment (Fig. 3.8.C). In Experiment II, *P. palmata* had lower C/N ratios than in Experiment I, but still it presented the highest and lowest molar C/N ratios (Fig. 3.8.C). In *C. crispus* molar C/N ratio was higher in Experiment II than in Experiment I.

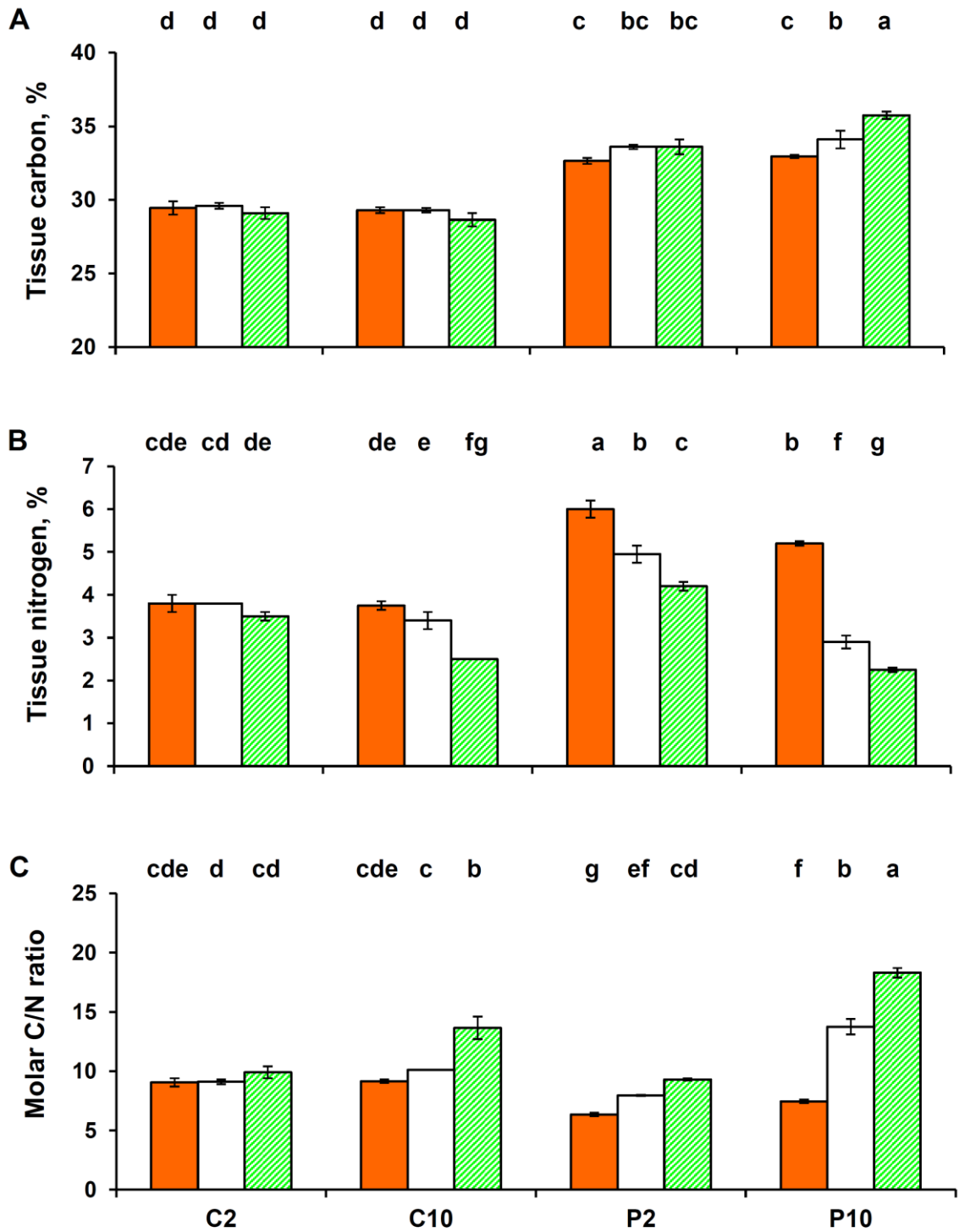
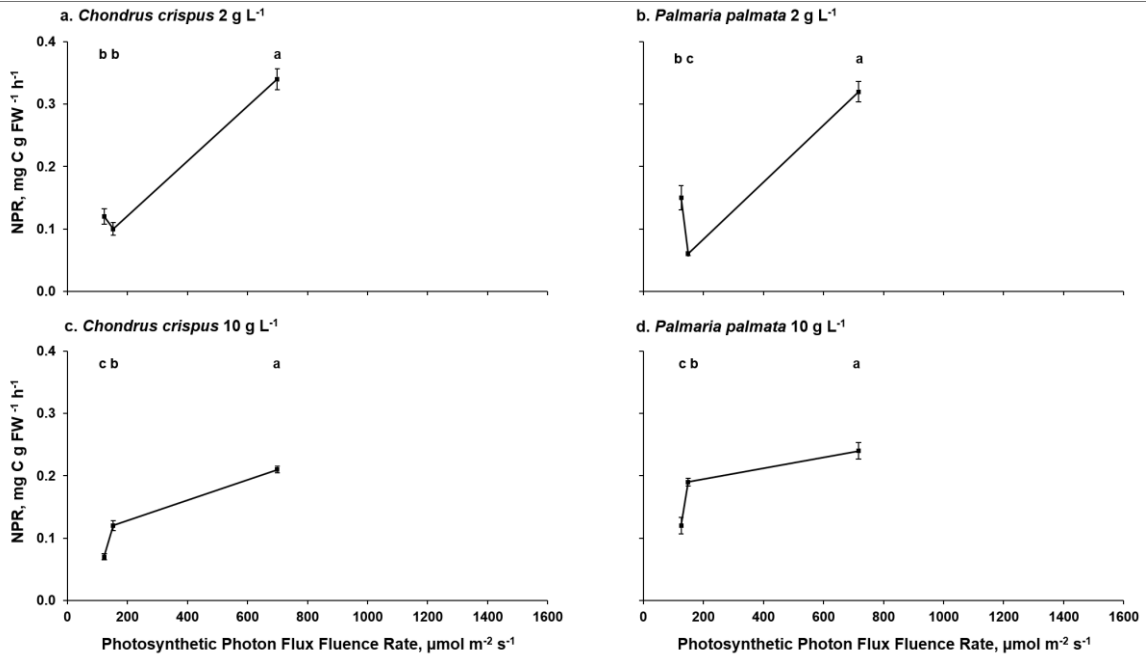


Figure 3.8. Experiment II, July 18 – August 12 2011. Tissue nutrient content of two red macroalgae cultured for 26 days at 14 °C at two stocking densities under three levels of natural light. A) Carbon; B) Nitrogen; C) Molar C/N ratio. C2 = *Chondrus crispus* at 2 g L⁻¹; C10 = *C. crispus* at 10 g L⁻¹; P2 = *Palmaria palmata* at 2 g L⁻¹; P10 = *P. palmata* at 10 g L⁻¹. Irradiance levels: solid bar (20 %); clear bar (50 %) and patterned bar (100 %). Error bars = SE. Bars sharing the same letter are not significantly different (Least-square means test. $p > 0.05$).

Net photosynthetic rate (NPR). In Experiment I, NPR was greater at the highest irradiance level for each species under both stocking densities (Fig. 3.9.A). At the highest irradiance, the highest NPR was in *C. crispus* at 2 g L⁻¹, 0.34 mg C g FW⁻¹ h⁻¹, at a mean Photosynthetic Photon Flux Fluence Rate (PPFFR) of 699 μmol m⁻² s⁻¹ (Fig. 3.9.A.a). By contrast, the lowest NPR was in the same species at the same PPFFR but at 10 g L⁻¹, 0.21 mg C g FW⁻¹ h⁻¹ (Fig. 3.9.A.c). *Palmaria palmata* NPR had a higher NPR, slightly less than *C. crispus* at 2 g L⁻¹ stocking density, but slightly greater than *C. crispus* at 10 g L⁻¹, at a mean PPFFR of 717 μmol m⁻² s⁻¹ (Figs. 3.9.A.b and d). At low stocking density, 2 g L⁻¹, both species showed a lower NPR at 50 % light treatment (PPFFR of ca. 150 μmol m⁻² s⁻¹) than at 20 % light treatment (PPFFR of ca. 125 μmol m⁻² s⁻¹; Figs. 3.9.A.a and b). However, this difference was significant only in *P. palmata*.

In Experiment II, the NPR of *C. crispus* was similar at 50 and 100 % light treatments at both stocking densities (Figs. 3.9.a and c). *Palmaria palmata* showed the same trend only at 2 g L⁻¹ (Fig. 3.9.b). The highest NPR was in *P. palmata* at 2 g L⁻¹ (0.33 mg C g FW⁻¹ h⁻¹) at a PPFFR of 1,435 μmol m⁻² s⁻¹. The lowest NPR at 100 % light treatment (PPFFR of 817 μmol m⁻² s⁻¹) was in *C. crispus* (0.21 mg C g FW⁻¹ h⁻¹) at 10 g L⁻¹ (Fig. 3.9.B.c). *Chondrus crispus* at 10 g L⁻¹ and *P. palmata* at 2 g L⁻¹ maintained the same NPR in both experiments despite the change in the irradiance. In Experiment II, *C. crispus* at 2 g L⁻¹ reduced its NPR with respect to Experiment I, while *P. palmata* at 10 g L⁻¹ increased its NPR.

A. Experiment I



B. Experiment II

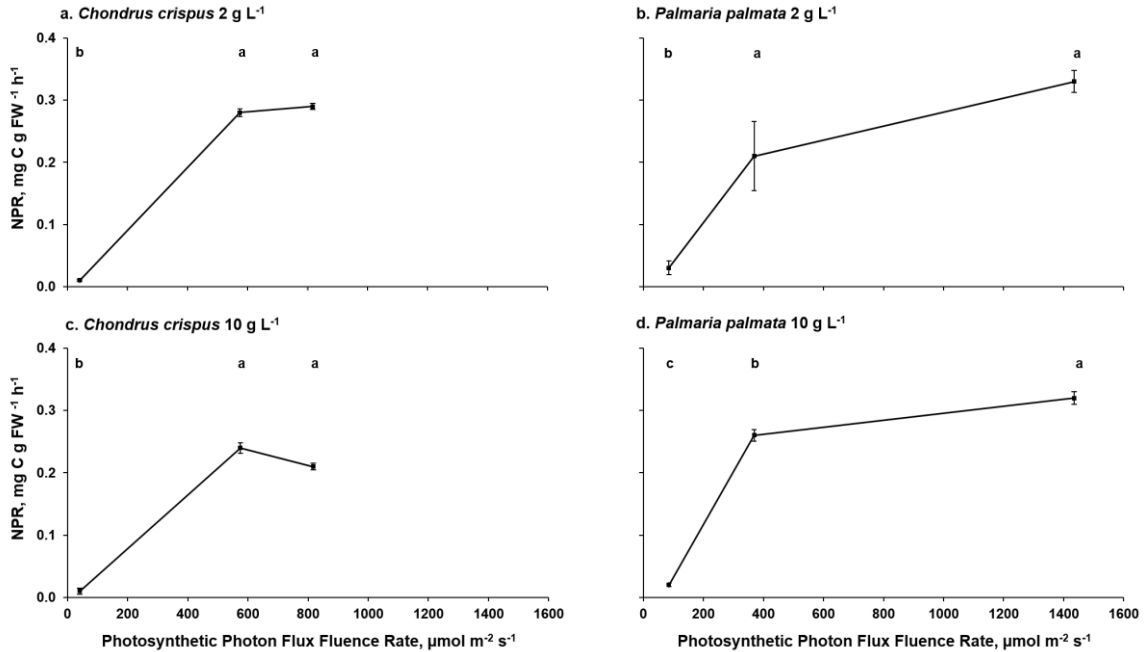


Figure 3.9. Net Photosynthetic Rate (NPR). A. Experiment I (10 °C). B. Experiment II (14 °C). *Chondrus crispus* (a and c) and *Palmaria palmata* (b and d) under natural light after their cultivation during 26 days at two stocking densities (2 g L⁻¹, a and b, and 10 g L⁻¹, c and d) under three levels of natural light (20, 50 and 100 % incident light). Error bars = SE.

Macroalga colour and epiphytes. Frond colour was affected by irradiance. At low irradiance, fronds were bright red, and this was associated with limited growth. In contrast, fronds exposed to high irradiance were greenish (*C. crispus*) or pale pink (*P. palmata*).

During Experiment I, starting in the second week, *C. crispus* at 2 g L⁻¹ exhibited bleaching of the apical and/or basal part of fronds, mainly at the 20 and 50 % light treatments (Fig. 3.10.A). Epiphytes also appeared in the second week at the 50 and 100 % light treatments, and in week three they were more abundant. Epiphytes were mainly *Ulva* spp. (Fig. 3.10.B). At 10 g L⁻¹, bleaching also began in week two at 20 and 50 % light treatment, but even in week one, a few fronds presented bleaching at 100 % light treatment. Epiphytes began appearing in week two at 50 % light treatment and in week three at 100 % light treatment. There were no epiphytes at 20 % light treatment at either stocking density. *Palmaria palmata*, at 2 g L⁻¹, developed small white holes that turned green, indicating a secondary infection of endophytic green algae (Fig. 3.10.C). This occurred throughout the experiment at 20 % light treatment, but at 50 and 100 % light treatment, it occurred only during weeks one and two. Small epiphytes were present only at week three, and only at 100 % light treatment. At 10 g L⁻¹ 'green spots' were present at 20 and 50 % light treatment during weeks one and two, but at 100 % light treatment, it was only during the first week.

During Experiment II, *C. crispus* at 2 g L⁻¹ exhibited no bleaching in any treatment, and epiphytes were *Ulva* spp. and diatoms. These epiphytes appeared in week two at 100 % light treatment, and in week three at 50 % light treatment. At 20 % light treatment, only diatoms were present. At 10 g L⁻¹, some fronds exhibited bleaching only

in week one, and were removed when the stocking density was reduced to the initial level. Epiphytes were absent on *C. crispus* at 10 g L⁻¹. In Experiment II, *P. palmata* at 2 g L⁻¹ also showed 'green spots', but only during week one, at 50 and 100 % light treatments. However, at 20 % light treatment, 'green spots' were present in weeks two and three. Several fronds were infected with a carpet-like epiphyte at 50 and 100 % light treatments, which was difficult to remove (Fig. 3.10.D). At 10 g L⁻¹, *P. palmata* exhibited 'green spots' during week one and two at 20 and 50 % light treatments, but at 100 % light treatment, 'green spots' appeared throughout the experiment. Only a few carpet-like epiphytes were present at 100 % light treatment.



Figure 3.10. A) Bleaching in *Chondrus crispus*; B) Epiphytes (*Ulva* spp.) on *C. crispus* C) 'Green spots' on *Palmaria palmata*; D) 'Carpet-like' epiphytes on *P. palmata*.

3.5 DISCUSSION

Daily Photon Dose (DPD) between 6.5 and 8.3 mol m⁻² d⁻¹ were sufficient for *C. crispus* and *P. palmata* to remove all the nutrients present in the culture flasks in 3 or 4 days, which initial concentration (500 μM NH₄⁺, 30 μM PO₄³⁻) were similar to concentrations in the Atlantic halibut effluent. However, in effluent with a constant nutrients supply, could be a difference between light treatments or species. The above irradiance levels corresponded to the mid light treatment during each of my experiments. Nevertheless, the summer of 2011 was cloudier than previous years (Environmental Canada 2011). Usually, in NS, the highest DPD is in June (43 mol m⁻² d⁻¹), followed by July (42 mol m⁻² d⁻¹) and August (36 mol m⁻² d⁻¹; Craigie and Shacklock 1995). Because of cloudiness and a screen on the greenhouse roof, the irradiance at the level of the flasks was only 30 and 44 % of the theoretical summer value during Experiment I and II, respectively. The high illumination (DPD of 13 and 16 mol m⁻² d⁻¹, respectively) corresponded to winter conditions. For this reason, maximizing nutrient uptake efficiency in full summer light conditions will require that irradiance should be reduced to 60 % but with constant monitoring of the DPD, because of weather changes. Irradiance did not change the overall trend of *P. palmata* out-performing *C. crispus*. However, this was not clear in some response variables because both species consumed almost 100 % of the ammonium available at mid and high light treatments. At the highest light treatment, the ammonium uptake efficiency was similar in both species. At the lowest light treatment, C storage was not significantly different in Experiment I, nor was the specific growth rate in Experiment II. However, the combined effect of irradiance and stocking density showed more cases in which the difference between

species was not significant. The exception to this trend was in Experiment II, where at the low light treatment and low stocking density, *C. crispus* had better ammonium uptake than *P. palmata*.

The similar nutrient uptake efficiency between species at the high and mid light treatments can be explained in two ways. First, high variability of the Photosynthetic Photon Flux Fluence Rate (PPFFR) was caused by variable cloud cover. This resulted in the mean PPFFR of the high light treatment being similar to the mean of the mid light treatment. In addition, at these light conditions, both species consumed almost all the nutrients available in the flasks. This was a limitation of the batch culture system, where culture medium was exchanged twice per week. Although this procedure has been extensively used in previous experiments (Kübler and Dudgeon 1996; Pereira et al. 2006; Corey et al. 2012, 2013), this was the first time that it was used at a high stocking density (10 g L^{-1}). Nevertheless, the total consumption of the nutrient does not invalidate the results. We can expect an improvement in the uptake efficiency when the macroalgae are cultured in fish effluent, where nutrients are not a limiting factor. Effluents are a constant source of nutrients for the macroalgae, at level up to $560 \text{ }\mu\text{M N}$ and $25 \text{ }\mu\text{M P}$ (Corey 2011). However, despite the constant nutrient supply, the studies with Atlantic halibut effluent showed a lower specific growth rate, no more than 1 \% d^{-1} (Corey 2011; Kim et al. 2013), compared to up to 9 \% d^{-1} in the present study. There are at least three possible explanations for this large difference in growth rate among studies associated with irradiance, specimen size and micronutrients concentration. The experiments in Atlantic halibut effluent were conducted at 8 to $14 \text{ mol m}^{-2} \text{ d}^{-1}$ as the Daily Photon Dose, whereas in my study the maximum specific growth rate was obtained at $16 \text{ mol m}^{-2} \text{ d}^{-1}$. An

increase in the irradiance would have helped to improve the growth. At a DPD of 15 mol m⁻² d⁻¹, *C. crispus* cultured in the effluent, increased its specific growth rate to 2.2 % d⁻¹ (S. Caines, pers. com. May 2012). In the effluent experiments, apical segments up to 6-cm long were used, while at laboratory scale I used apical segments up to 3.5 cm long. The effect of specimen size on growth rate was verified in laboratory conditions when segments with different initial sizes, 1 to 7 cm, were cultured in the same conditions. One-centimetre segments grew 130 % more than 7-cm segments (Fig. 3.11, Manríquez-Hernández and Walker unpubl. data). Another factor could be the low concentration of iron in the seawater. Iron limitation may limit the conversion of nitrate to ammonium at the necessary rate (Hurd et al. 1995; Maldonado and Price 1996). Thus, it is possible that the macroalgae, despite having high nutrient availability (mainly nitrogen as nitrate), could not use the entire available nitrate because they could not assimilate it. In the laboratory studies, enriched seawater was used, which contained up to 40-fold the iron concentration in the North-West Atlantic Ocean (Achterberg et al. 2001). Iron is normally supplemented in biomass land-based production, for example for *C. crispus* (Neish and Fox 1971). Thus in integrated culture micronutrients supplementation may be critical for maximizing algal growth (Demetropoulos and Langdon 2004; Waaland 2004).

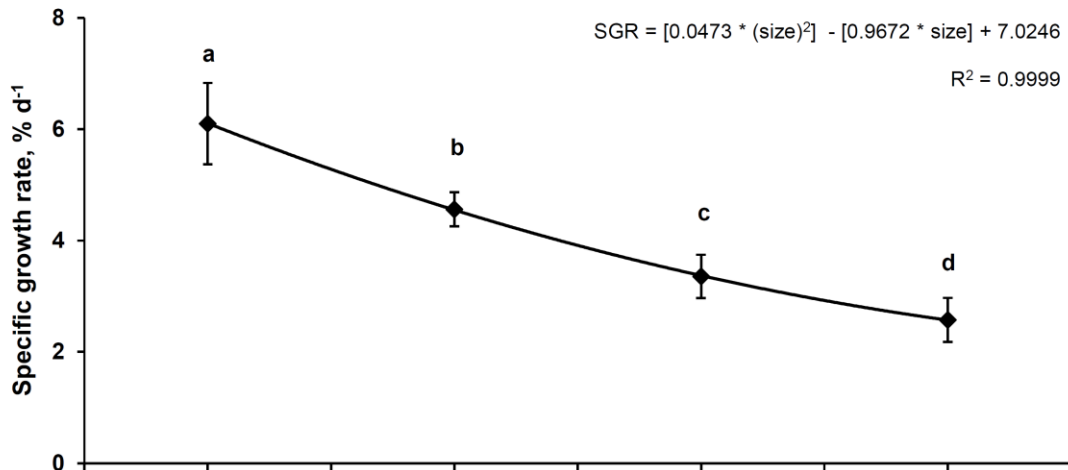


Figure 3. 11. Specific growth rate of *Chondrus crispus* cultivated at 17 °C under a Photosynthetic Photon Flux Fluence Rate of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16:8 L:D photoperiod. Four initial segment size, 1, 3, 5 and 7 cm were evaluated in a block design with four replicates. All are means. Error bar = SE, n = 16 (Four replicates x four weeks).

The effect of stocking density, without interaction with irradiance, showed the same trend as the overall analysis, *P. palmata* had a higher performance than *C. crispus* at both stocking densities. Both species grew better at 2 g L^{-1} than at 10 g L^{-1} , regardless of culture temperature. Under the former conditions, fronds had comparatively more nutrients available, consumed less CO_2 and had more time exposed to light. These are the conditions that commercial biomass producers are looking for (Craigie and Shacklock 1995). However, this low stocking density also has disadvantages, as it favours epiphyte growth and, depending on irradiance, there could be photoinhibition, which limits photosynthesis and growth (Cabello-Pasini et al. 2000). Stocking density also influences the tolerance to ammonium concentration. At 0.1 g L^{-1} , *P. palmata* grew better at 50 or 100 $\mu\text{M NH}_4^+$ than at the 500 $\mu\text{M NH}_4^+$ used in my experiments (Sanderson 2006). At higher ammonium concentrations growth rate decreased further, while 3,520 $\mu\text{M NH}_4^+$ was toxic (Sanderson 2006). Despite the fact that, in my experiments, in the mid-illumination treatment, both species consumed the same amount of nutrients as at high

illumination, they showed a higher growth at high illumination. This means macroalgae can uptake more nutrients than needed for the immediate needs for growth and maintenance, and store them for subsequent use when irradiance conditions are optimal for growth; this process is known as luxury nutrient uptake (Lüning 1990).

The fact that *P. palmata* accumulated C indicates that, despite having a greater SGR than *C. crispus*, it did not use it to grow, but accumulated it as fixed C. This is also reflected in the percent tissue N, which decreased from its initial level under the same conditions of irradiance and stocking density, while C was accumulating. This indicates that fronds could not continue to grow due to nitrogen limitation. That was clearly reflected in the high values of the molar C/N ratio, mainly in *P. palmata* at high light treatment, in both experiments. However, at 2 g L⁻¹ and low irradiance, both nutrients accumulated, indicating that irradiance was limiting for growth. For *C. crispus*, this effect of irradiance on nutrient uptake was not so marked. Only at high stocking density and the high light treatment was there possibly an effect, suggesting that *C. crispus* quickly used the absorbed nutrients. In nature, the molar C/N ratios in macroalgae vary from 6 to 60 with a mean of 22 (Atkinson and Smith 1983). There is no specific value for molar C/N ratio to determine if a macroalga is growing under nitrogen limiting conditions. Currently, values from 10 to 20 have been used as nitrogen limiting criteria in studies with red macroalgae (Al-Hafedh et al. 2012; Corey et al. 2012). However, one should consider that the highest value was originally determined for brown algae (Dean and Hurd 2007). Brown algae tend to have a higher molar C/N ratio than red macroalgae because of the nitrogenous pigments in the latter, for example the phycobiliproteins (Raven and Hurd 2012). In addition, the molar C/N ratio varies within species and

reproductive stages, and therefore depends on the metabolism of both nutrients, and the place and mechanism of storage (Atkinson and Smith 1983; Hanisak 1990; Dean and Hurd 2007). To define the critical values of nutrients for growth for a species (or strain), it is necessary to analyze the growth rate as function of the molar C/N ratio under irradiance and nitrogen limitation (Lapointe and Duke 1984). For this reason, I prefer to compare the changes in nutrient tissue levels to the initial value, rather than refer to a specific value for the molar C/N ratio.

Despite my experiments being conducted at a relatively low irradiance (DPD, 13 and 19 mol m⁻² d⁻¹), the irradiance to which the segments were exposed was in the range of light saturation points for growth described for both species. These light saturation ranges, as DPD, are 3.2 to 8.0 mol m⁻² d⁻¹ for *P. palmata* and 2.7 to 13.0 mol m⁻² d⁻¹ for *C. crispus* (Strömberg and Nielsen 1986; Greene and Gerard 1990; Kübler and Raven 1995; Sagert and Schubert 2000). The irradiance to which the fronds were exposed during the photosynthetic rate measurement was greater than the light saturation points for photosynthesis reported for these species, ca. 200 μmol m⁻² s⁻¹ (Mathieson and Norall 1975; Robbins 1978; Martínez and Rico 2008). During Experiment I, the mid light treatment was close to this threshold; however, at low stocking density both species showed a very low Net Photosynthetic Rate (NPR). The NPR was even lower than at the low light treatment, although there was not much difference in the irradiance levels. This measurement was carried out in the afternoon (ca. 4 PM), so the fronds were photoinhibited by the high mid-day illumination (Sagert et al. 1997). Due to this low NPR value, it cannot be determined whether or not photoinhibition was occurring at 700 μmol m⁻² s⁻¹. Previous studies of the photosynthetic activity of *C. crispus* and *P. palmata*

used a great variety of methods and expressed their results in different units, making it impossible to compare the value of this response variable. In Experiment II at 14 °C, *C. crispus* was photoinhibited. It presented a lower, or no, significant difference in the NPR between mid and high light treatment, despite the relative low irradiance, PPFFR of 817 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In contrast, despite the fact that *P. palmata* was exposed at a higher irradiance, PPFFR of 1,435 $\mu\text{mol m}^{-2} \text{s}^{-1}$, it was not photoinhibited. At both stocking densities, the highest NPR of *P. palmata* was at the highest irradiance. Thus, the photoinhibition of *C. crispus* explains its lower growth than *P. palmata*.

In conclusion, *P. palmata* at 10 g L⁻¹ is the best candidate to include in an integrated aquaculture system with Atlantic halibut. To maintain ammonium as the main nitrogen source the bacterial filter should be placed after the algae rearing tanks. The temperature must be maintained at no more than 14 °C, the highest temperature recommended to grow juvenile Atlantic halibut (Brown 2010). Regrettably, the Woods Harbour farm does not meet these requirements, the main nitrogen source is nitrate and temperature has a seasonal fluctuation up to 18 - 19 °C in summer conditions. For this reason, *P. palmata* started to degenerate in summer, due to sexual maturation. In the short term, *C. crispus* could be used. However, it is necessary to develop a *P. palmata* hatchery because its highest nutrient removal efficiency and potential market. Natural irradiance should be reduced to a DPD of 17 mol m⁻² d⁻¹. Based on the Nova Scotia annual irradiance curve, it would be necessary to supplement this with artificial light only during winter (Craigie and Shacklock 1995). Regardless, this model needs to be tested at a pilot scale with effluent to determine if daily fluctuations in nutrient concentrations have a greater effect than expected from my batch culture experiments. If the culture of

Atlantic halibut at Wood's Harbour does not change to a closed-containment system, we can expect that the red macroalgae will be exposed to highest temperatures that were recorded in this study. This means that the red macroalgae will increase their light saturation point and then need more irradiance than the determined in this study. Under these conditions, the response of *C. crispus* could change since it is better adapted to highest temperatures and irradiation than *P. palmata*. Another important change at farm scale is the size of the fronds, in my experiments 3-cm tips were used, where *P. palmata* had a higher surface-area:volume ratio. Fronds of the same length had different width, in general *P. palmata* were wider than *C. crispus*. If whole thalli are used, this relation could increase markedly in *C. crispus* because of its branched morphology.

CHAPTER 4 LIGHT SATURATON CURVES AND NUTRIENT ASSIMILATION OF *Chondrus crispus* UNDER ARTIFICIAL LIGHT

4.1 ABSTRACT

Irradiance is an important factor in macroalgal growth. The determination of the light saturation point for growth and photosynthesis is critical for the optimization of cultivation in land-based integrated culture systems, especially when stocking density was incremented. The light saturation curves for growth, photosynthetic activity, and nutrient uptake was determined for *Chondrus crispus* cultivated in one-litre flasks at 10 °C, at two stocking densities (2 and 10 g L⁻¹ fresh weight) under six light treatments (Photosynthetic Photon Flux Fluence Rate, 50 to 500 μmol m⁻² s⁻¹). At high stocking density (10 g L⁻¹), the light saturation point for growth was at a PPFFR of 335 μmol m⁻² s⁻¹, and for net photosynthetic rate was at a PPFFR of 186 μmol m⁻² s⁻¹. At high stocking density, *C. crispus* consumed in three days almost all nutrient available in the flasks, therefore the saturation points were lower than for the low stocking density or control (2 g L⁻¹). There was a marked effect of irradiance on the accumulation of tissue nutrient and pigment contents, mainly at high stocking density. At PPFFR < 200 μmol m⁻² s⁻¹, the highest nitrogen and phycoerythrin percentage were recorded. At low stocking density, it was possible to determine a target for optimal culture conditions (5 % N). If the content is lower, there is a possibility to optimize the culture condition for *C. crispus*. In Atlantic halibut effluent with a continuous nutrient supply, a PPFFR of 400 μmol m⁻² s⁻¹ is recommended. At higher irradiances, the stocking density could be increased over 10 g L⁻¹, reducing the rearing surface-area, and the installation costs.

4.2 INTRODUCTION

Irradiance is a key factor in the ecology and culture of macroalgae; it can explain 65 % of photosynthetic activity, 64 % of growth, and 42 % of nutrient uptake. Nutrient concentration is the other key factor (Lapointe and Duke 1984). These processes increase in proportion to irradiance, until it become excessive (photoinhibition) or nutrients become limiting (Häder and Figueroa 1997). In general, the response of each plant to irradiance depends on photoacclimation more than its phylogenetic origin (Enríquez et al. 1994). Hence, there is no consensus on the optimal irradiance for cultivation of macroalgae. For example, for *Chondrus crispus* the range of light saturation points reported is from 46 to > 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Strömngren and Nielsen 1986; Cabello-Pasini et al. 2000). Red macroalgae have accessory nitrogenous pigments (phycobiliproteins, PBP) which allow them to absorb lower irradiances than other algal phyla (Rhee and Briggs 1977). Plants acclimated to low irradiances have high pigment concentrations to maximize the absorption of the scarce photons available (Rosenberg and Ramus 1982). In contrast, plants acclimated to high irradiances have low concentrations of PBP, to avoid photoinhibition (Häder and Figueroa 1997). For this reason, the ratio PBP to chlorophyll *a* decreases as irradiance increases (López-Figueroa 1992). The acclimation to specific irradiances implies a diminution in the photosynthetic activity when plants are exposed to a change in their light environment. Plants acclimated to low irradiance are quickly photoinhibited in high irradiance, whereas high irradiance acclimated plants have insufficient pigment to photosynthesize at low irradiance (Lapointe and Duke 1984).

In nature, macroalgae live in environments with continuously changing irradiances. Irradiance changes with respect to time of day, water depth (tides), cloud cover, and turbidity. Day length also changes seasonally due to the ecliptic orbit of the Earth around the Sun (Kirk 2011). In controlled land-based facilities incorporating a tumble culture system, variation in irradiance also results from changes in stocking density. In tumble culture individual plants are exposed only momentarily to full illumination, which normally only comes from the tank surface, and they have time to recover their photosynthetic systems (Neish and Knutson 1978; Häder and Figueroa 1997). Increasing the stocking density reduces the growth rate of individual thalli, but increases the yield per area unit; therefore, nutrient uptake efficiency increases (Neish and Knutson 1978; Chopin et al. 2001). Increasing nutrient uptake efficiency is essential for optimizing the integrated culture of macroalgae and fish (Pereira et al. 2006). Increasing the algal stocking density also has other economic advantages: it reduces tank space requirements and other installation and operational costs (Chopin et al. 2001), increases formation of mycosporine-like amino acids which have a market as antioxidant and UV screening agents (Figueroa et al. 2008), reduces epiphytism (Bidwell et al. 1985) and prevents photoinhibition (Cabello-Pasini et al. 2000).

The goal of this NSERC project was to optimize the integration of *C. crispus* and *P. palmata* with Atlantic halibut in NS. In previous laboratory experiments with natural light (Chapter 3 of this thesis), *P. palmata* performed better than *C. crispus* in the range of temperature studied, 10 and 14 °C. However, in the third year of its cultivation, *P. palmata* deteriorated because of the high summer temperatures of up to 19 °C at the Atlantic Halibut farm. Therefore, it might be necessary to propagate *P. palmata* from

spores, which would make its incorporation in the IMTA system more costly and risky. Hence, in the current culture conditions, *C. crispus* could be incorporated at a commercial scale. The Basin Head strain of *C. crispus* used in this project reproduces through fragmentation, thus avoiding having to start cultures from spores and grows well throughout the year (Corey et al. 2012). Therefore, *C. crispus* was selected to determine the saturation curve under artificial light. Use of artificial light incurs extra installation and operational costs than natural light, but it allows irradiance to be accurately controlled to produce macroalgae with a consistent quality as required by the food and pharmaceutical industry (Hafting et al. 2012; Ibañez and Cifuentes 2012).

Chondrus crispus grew better under artificial light than natural light when it was evaluated in a preliminary trial using the same culture conditions as in the greenhouse experiments described in Chapter 3. A similar result was observed in *P. palmata* (Morgan and Simpson 1981b). The difference in growth rate of *C. crispus* between artificial and natural light was inversely related to Daily Photon Dose (DPD), 300 % higher under artificial than under natural light at $3 \text{ mol m}^{-2} \text{ d}^{-1}$ and only 8 % at $17 \text{ mol m}^{-2} \text{ d}^{-1}$. The DPDs with artificial light, in my preliminary trial, were achieved with a photoperiod of 16:8 L:D under a Photosynthetic Photon Flux Fluence Rate (PPFFR) of 50 and $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively. However, as in the greenhouse experiments, the nutrients in the culture medium were consumed quickly by the algae. For this reason, it was not possible to conclude if the reduction in the difference in the growth rate was an effect of the irradiance or due to nutrients being limiting. To resolve this question, a new experiment, which is described in this chapter, was conducted at a higher nutrient concentration ($1,300 \mu\text{M NH}_4^+$). Other studies within this NSERC project were also

conducted under artificial light. In laboratory scale experiments at 2 g L⁻¹ in one-litre flasks under a PPFRR of 125 μmol m⁻² s⁻¹ (DPD, 7 mol m⁻² d⁻¹; 16:8 L:D) *C. crispus* absorbed ammonium (NH₄⁺) faster than nitrate (NO₃⁻), up to 300 μM NH₄⁺ in 24 h vs. up to 111 μM NO₃⁻ in 24 h (Corey et al. 2013). However, this stocking density is low compared to a commercial-scale farm, which rears *C. crispus* at 5.6 to 11.1 g L⁻¹ in large tanks (2000 m² x 0.9 m depth, Craigie and Shacklock 1995). An additional trial in Atlantic halibut effluent was conducted in 50-L tanks to determinate the best stocking density to culture *C. crispus* at a PPFRR of 140 μmol m⁻² s⁻¹ (DPD, 8 mol m⁻² d⁻¹; 16:8 L:D). A stocking density of 10 g L⁻¹ was identified as the best compromise between nutrient uptake and productivity (Kim et al. 2013). A recent study indicated a higher irradiance is needed to increase biomass of *C. crispus* (Cornish et al. 2013). They used 155 to 165 μmol m⁻² s⁻¹ but with a shorter photoperiod (14:10 L:D), hence the DPD was similar to Kim et al. (2013), 8 mol m⁻² d⁻¹. An irradiance of 75 to 80 μmol m⁻² s⁻¹ (DPD, 4 to 5 mol m⁻² d⁻¹; 16:8 L:D) was indicated as sub-saturated (Cornish et al. 2013). By comparison, the 50 μmol m⁻² s⁻¹ (DPD, 3 mol m⁻² d⁻¹; 16:8 L:D) was considered the light saturation point for growth for a strain from NS (Bird et al. 1979).

The aim of my research was to determine the light saturation point for nutrient uptake efficiency, growth, and photosynthetic rate, and the effect on the dark respiration rate, and tissue nutrients and pigments. This information will allow the determination of the irradiance needed for the cultivation of *C. crispus* in Atlantic halibut effluent with nutrient levels 100-fold higher than seawater (Corey 2011). The approach was to compare the response of *C. crispus* culture in one-litre flasks at low stocking density (2 g L⁻¹ FW) and high stocking density (10 g L⁻¹ FW). The irradiance levels tested were

within the range indicated for light saturation of this species (PPFFD of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$; DPD of 3 to 29 $\text{mol m}^{-2} \text{d}^{-1}$ over 16 h light).

4.3 MATERIALS AND METHODS

Biological material: *Chondrus crispus*, Basin Head strain, was obtained from Scotian Halibut Ltd., Wood's Harbour, NS (43° 31.04' N - 65° 44.30' W). This stock has been maintained since June 2009 in Atlantic halibut effluent at ambient seawater temperatures (1 to 18 °C), with nutrient concentrations ranging from 6 to 564 $\mu\text{M N}$ and daytime illumination over a PPFFR of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Corey 2011). Previously, this material was cultured for several years at the Marine Research Station, NRC, Ketch Harbour, NS. Segments, 3.0 to 3.5 cm long, were trimmed from the apical part of the thalli.

Place and dates: The determination of the light saturation curves of *C. crispus* was conducted in the Aquaculture Centre at Dalhousie University's Agricultural Campus (DAL-AC), Truro, NS (45° 22.42' N - 63° 15.89' W). An exploratory assay was conducted to determine the initial nutrient concentration to ensure nitrogen was not limiting (March 29 to April 2, 2012). The experiment ran from April 22 to May 17, 2012 and photosynthetic and respiration rates were measured on May 18 and 19, 2012.

Experimental design: Forty-eight one-litre Florence flasks (4060-1L, Pyrex, MA, USA) were arranged in six fiberglass circular tanks (1.5 m diameter, Fig. 4.1). Eight flasks were randomly placed in each tank, four flasks at low stocking density comprised the control (2 g L^{-1} fresh weight [FW]) and the other four flasks had the high stocking density

(10 g L⁻¹ FW, Appendix E). To remove any organic residue, flasks were cleaned and rinsed with 10 % hydrochloric acid, then were filled with UV-sterilized seawater enriched with a modification of von Stosch's enrichment medium (von Stosch 1964). Ammonium was used as nitrogen source, instead of the original nitrate. Vitamins were added, according to the specifications for this medium (Anderson et al. 2005, p. 518). The culture medium was replaced every third or fourth day (batch culture system). During the experiments the mean nutrient content of unenriched seawater was 14 μM NH₄⁺, 0.4 μM NO₃⁻, 0.2 μM PO₄³⁻ and 30 psu salinity. Previous experiments (Chapter 3) determined that the original 500 μM of nitrogen proposed by von Stosch (1964) was limiting to *C. crispus* in this batch culture system. An assay with four nutrient levels (500 to 2,900 μM ammonium, NH₄⁺) determined that 1,300 μM NH₄⁺ is suitable for *C. crispus* in this condition (Appendix F). The modification required increasing by 2.6 times the concentration of all the nutrients and vitamins used in Chapter 3.

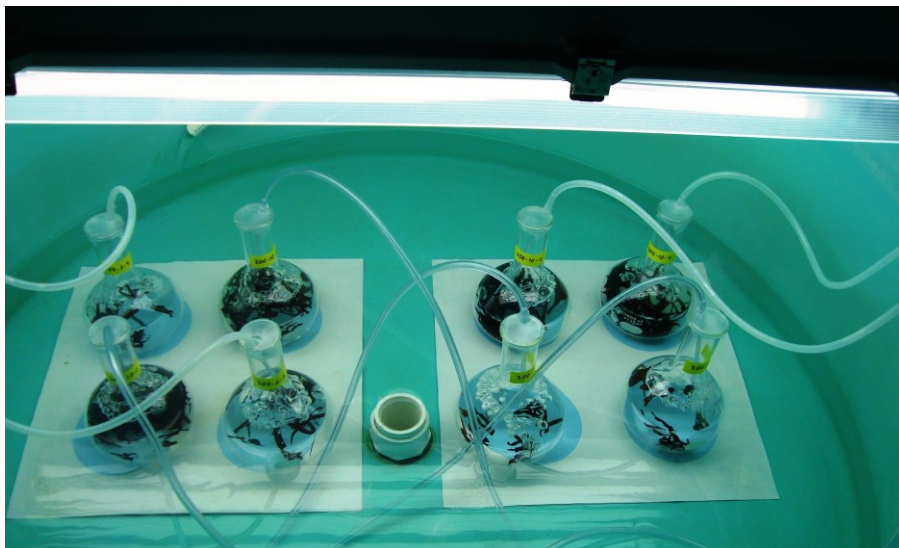


Figure 4.1. Randomized set up of the one-litre Florence flasks stocked with *Chondrus crispus* at 2 and 10 g L⁻¹ FW. Tubing from the right fed a mixture of air and carbon dioxide to the flasks stocked at 10 g L⁻¹ while the tubes from the left fed the flasks at 2 g L⁻¹.

A light fixture (132 cm L x 37 cm Wi) with up to six 6500K fluorescent tubes (F54W/T5/865/ECO, General Electric, China) was suspended a set height (11 to 78 cm) above the flasks to achieve the experimental irradiance levels (PPFFR of 50, 100, 200, 300, 400, and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The spectral composition of the fluorescent tubes included Photosynthetically Active Radiation (PAR, 400 - 700 nm) and a fraction of the UV-A spectrum (350 - 400 nm, Fig. 4.2). To avoid possible interference among the light treatments, each circular tank was isolated with a double black plastic curtain (01-0305, Polytarp Products, ON, Canada). Photosynthetically Active Radiation was logged every 15 min on consecutive days in every tank, using a spherical quantum sensor (LI-193SA, LI-COR, NE, USA). Due to the shape of the Florence flask and the size of the spherical light sensor, it was not possible to measure the irradiance inside the flasks, but the sensor was set up at the same height as the flasks. This sensor measures the total number of photons incident on a point from all directions, denominated Photosynthetic Photon Flux Fluence Rate (PPFFR, $\mu\text{mol m}^{-2} \text{s}^{-1}$). To compare with experiments under natural light, Daily Photon Dose (DPD, $\text{mol m}^{-2} \text{d}^{-1}$) and Total Photon Dose (TFD, $\text{mol m}^{-2} 26 \text{d}^{-1}$) were calculated, integrating the PPFFR during the time that the lights were on (Sagert and Schubert 2000). Photoperiod was controlled with a digital timer (052-8817-6, Noma, China) and set at the maximum daylength at the latitude of Nova Scotia, 16:8 L:D.

The effect of the flask glass on irradiance transmittance was assessed with a three-litre beaker (1000-3L, Pyrex, MA, USA) which had the similar optical properties of the flasks. Both are borosilicate glass code 7740 but have a different shape. When the beaker was inverted above the spherical light sensor, the PPFFR was reduced by 3 %. It was not possible to determine the effect of seawater on irradiance transmittance but it

could be lower than the flasks because the seawater refractive index, 1.33 (Quan and Fry 1995) is lower than the refractive index of the borosilicate glass, 1.47 (CORNING 2008).

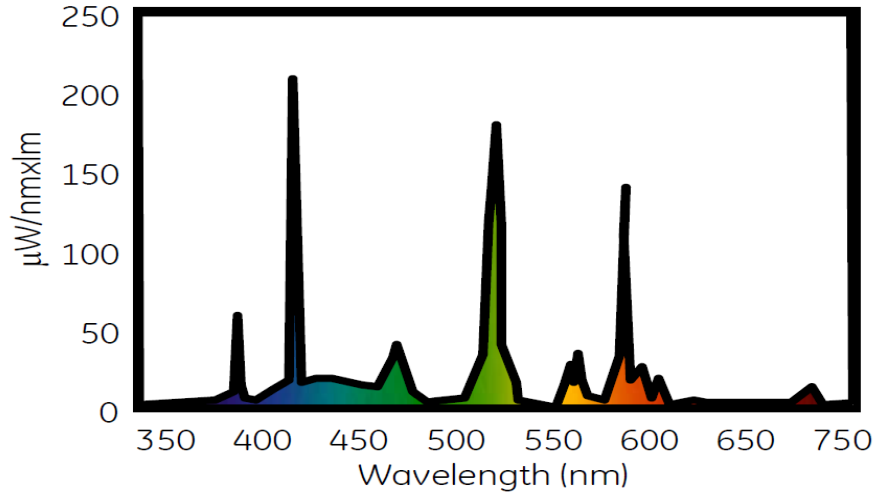


Figure 4.2. Spectral power distribution of the 6500K fluorescent tubes F54W/T5/865/ECO. Power measured as microwatt per nanometer of wavelength per lumen ($\mu\text{W} / \text{nm} \times \text{lm}$). It includes peaks in the violet, green, and orange portions of the spectrum.

http://www.gelighting.com/LightingWeb/na/images/28345_Starcoat_T5_Ecolux_tcm201-21063.pdf.

To maintain the flasks at a constant temperature, the fibreglass holding tanks were used as a water bath, each supplied with constant flow-through of 10 °C-groundwater. Water temperature was logged every 10 min (Minilog 8-bit, Vemco, NS, Canada). To achieve the tumble culture, each flask was supplied with air via a rigid clear acrylic tube (4.76 mm OD, 16007, Dynamic Aqua-Supply Ltd., BC, Canada) connected to a 1/2" PVC tubing manifold by translucent silicone tubing (4.78 mm ID, Silastic 515-013, Dow Corning, MI, USA). Each manifold had four brass valves (VBR1, Dynamic Aqua-Supply Ltd., BC, Canada) to control the aeration of each flask. Each manifold was connected to a two-litre Florence flask (4060-2L, Pyrex, MA, USA) with silicone tubing, where the air was mixed with carbon dioxide (CO_2). The CO_2 supply to each two-litre

flask was controlled manually with a brass valve. The CO₂ requirement was assessed indirectly by monitoring the pH of the culture medium twice per day with a hand-held meter (Electrode 13-620-AP50 and Accumet AP61, Fisher Scientific, Singapore). If the pH of a specific stocking density - light condition was above 8.5, CO₂ was added to this mix flask. The CO₂ supply from the cylinder was controlled by a high-pressure solenoid valve (Series 8223, ASCO, ON, Canada). The solenoid valve, controlled in turn by a digital timer, opened one hour after the lights were turned on and closed one hour before lights were turned off. Air (4 psi) was generated by a positive displacement blower (Sutorbilt GABL DPA, Gardner Denver, ON, Canada). The air input to each two-litre flask was controlled by a 1/2" stop valve (SCL-0500-T, King Brothers Industries, CA, USA).

Sampling protocol: At each exchange of the culture medium, the temperature and pH were recorded inside each flask. Two seawater samples were taken from each flask to determine ammonium and orthophosphate absorption using the same methodology as described in Chapter 3 for water nutrient analysis. In addition, fronds were evaluated and cleaned of epiphytes using a scalpel.

Each week, fronds were photographed, dried with paper towel and weighed (0.001 g precision balance), after which, whole segments were removed to return each flask to the original stocking density. The initial number of segments in each flask was *ca.* 25 and 125 at low and high stocking density, respectively. The number was decreased depending on the growth at each treatment and replicate. Specific growth rate was expressed as percent per day for each replicate flask (Eq. 3.1, Evans 1972).

At the start and end of the experiment, tissue samples (1 to 4 g FW) were dried at 60 °C to analyze carbon and nitrogen using the same methodology described in Chapter 3 for tissue nutrient analysis. These samples also were utilized to calculate the fresh weight/dry weight ratio (FW/DW ratio). In addition, tissue samples (*ca.* 1 g FW) were frozen at -20 °C for subsequent analysis of the pigments content (chlorophyll *a* and phycobiliproteins).

Net photosynthetic rate (NPR) and dark respiration rate (DRR): At the end the experiment, the NPR and DRR of a sample of segments from each treatment was determined using the methodology described in Chapter 3. Dark respiration rate (mg C g⁻¹ FW h⁻¹) was determined with Eq. 4.1 (Thomas 1988).

$$\text{Eq. 4.1} \quad \text{DRR} = [(\text{IB} - \text{DB}) \times 375.9] / (\text{T} \times \text{RQ}) / (\text{FW} \times 3333)$$

where: IB is the initial oxygen level, in mg L⁻¹; DB is the final oxygen level in the dark bottles, in mg L⁻¹; 375.9 is the factor to standard volume (1 m³); T is incubation time in h; RQ is the respiration quotient (1.0 was assumed); FW is the fresh weight of the segments in g and 3333 is the factor to standardize to a litre basis.

Tissue pigments analysis: Pigment concentrations were analyzed with a modification of the methods described for phycoerythrin (PE) and phycocyanin (PC), and chlorophyll *a* (Chl *a*) by Beer and Eshel (1985) and Chapman (1988), respectively. Samples of frozen tissues (100 mg FW, 0.001 g precision balance) were ground with fine sand in a 50-mL porcelain mortar (60310, CoorsTek, China) using a porcelain pestle (60311, CoorsTek,

China). To extract phycobiliproteins 4 mL of orthophosphate buffer (0.1 M, pH = 6.6) was gradually added during the grinding, and then the homogenate was transferred to a 10-mL translucent polypropylene test tube (149567A, Fisherbrand, Mexico). To avoid pigment degradation, the grinding was done in a dark room under dim red light (3 lux, Light Meter 840022, Sper Scientific, AZ, USA). Samples were kept overnight in the dark at 4 °C and then centrifuged at 4,912 g (6,500 rpm, maximum speed) for 30 min at 4 °C (Harrier 18/80 refrigerated, Measuring and Scientific Equipment, UK). The supernatant was transferred to a polystyrene cuvette with a 10-mm pathlength (GD14955125, Fisherbrand, China), also under dim red light, and absorbance was measured at 455, 564, 592, 618, and 645 nm with a visible and near infrared spectrophotometer (DR2700, Hach, Germany). To extract Chl *a*, the resulting centrifugation pellet was ground with 4 mL of 90 % buffered acetone with magnesium carbonate 1 %, and centrifuged at 4,912 g for 20 min at 4 °C. The supernatant was collected in a 10-mL test tube and the new pellet was ground again with the acetone buffer and centrifuged at the same conditions. The new supernatant was combined with the previous and then the absorbance was measured at 665 nm.

Pigment concentration was calculated using a modification of the equations used for phycoerythrin (PE) and phycocyanin (PC; Beer and Eshel 1985), and for Chl *a* (Chapman 1988), with equations 4.2, 4.3 and 4.4, respectively:

$$\text{Eq. 4.2} \quad \text{PE (mg g}^{-1} \text{ FW)} = \{[(A564 - A592) - (A455 - A592) * 0.20] * 0.12\} * (V / g)$$

$$\text{Eq. 4.3} \quad \text{PC (mg g}^{-1} \text{ FW)} = \{[(A618 - A645) - (A592 - A645) * 0.51] * 0.15\} * (V / g)$$

$$\text{Eq. 4.4} \quad \text{Chl } a \text{ (mg g}^{-1} \text{ FW)} = (A655 / 74.5) * (V / g)$$

where A is the absorbance at each wavelength; 0.12 and 0.15 are the absorption coefficients derived from the specific absorbance of PE and PC, respectively; V is the extraction volume in mL (4.0 mL of orthophosphate buffer or 8.0 mL of acetone buffer) and g is the fresh weight in grams of the sample used.

Culture conditions: The nominal irradiance showed some variation with respect to measurements made with the sensor. Regardless, irradiances were still significantly different among treatments (Appendix G, Table G.1). After the lamps switched on, they emitted *ca.* 20 % more irradiance for the initial 1.5 h then stabilized; this could have affected the mean PPFRR. The temperature in the water bath tanks was stable, 10.1 ± 0.0 °C. Nevertheless, the short distance between the light fixtures and the flasks to meet the highest irradiance levels, affected the temperature inside the flasks. The mean temperature inside the flasks ranged between 10.1 to 10.7 °C, with an overall mean of 10.4 ± 0.0 °C (Appendix G, Table G.2). pH of the culture media was also affected by the light treatment, requiring the addition of higher flow CO₂ to help maintain a low pH in most treatments. However, at high stocking density and PPFRR of 200 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the mean pH was 7.2 ± 0.3 and 8.6 ± 0.1 , respectively (Appendix G, Table G.2), with pH 8.5 being the upper limit for optimum photosynthesis of *C. crispus* (Craigie and Shacklock 1995).

Statistical analysis: To evaluate the light saturation points, the equation for light saturation curves of Jassby and Platt (1976) was used (Eq. 4.5). The slope of the initial phase of the saturation curve was calculated with a linear regression without an intercept, by the REG procedure of SAS (SAS 9.3, Institute Inc., NC, USA). Outliers were identified running the Univariate procedure of SAS and then inlaying the residual through the Anderson-Darling normality test (Anderson and Darling 1952) in Minitab 16 (Minitab Inc., State College, PA, USA). Mean and standard error were calculated in the Surveymeans procedure of SAS.

$$\text{Eq. 4.5} \quad R = R_m * \tanh * [(\alpha * I) / R_m]$$

where R is the response variable at a specific irradiance, R_m is the higher value of the response variable, \tanh is the hyperbolic tangent function, α is the initial slope of saturation curve and I is the irradiance level.

For nutrient uptake and specific growth rate, a repeated measures analysis of variance was conducted to include the effect of the different sampling periods. Different types of covariance structure were tested, and those with a lower Akaike's Information Criterion (AIC) that met the normality requirement were selected to run the Mixed procedure (Elliot and Woodward 2010). The Anderson-Darling normality test (Anderson and Darling 1952) was conducted in Minitab 16, using the residuals of the Mixed procedure of SAS statistical software. Orthophosphate uptake, phycobiliproteins, and respiration rate data were square transformed to meet the normality criterion. For tissue nutrients and pigments, and their ratios, a single time was analyzed. Least-squares means

analysis ($p=0.05$) was used as a *post hoc* test to determine pairwise relationships among all combinations.

In a factorial design (irradiance x stocking density), the interaction between both factors can be analyzed by first determining the regression of effect of irradiance at each stocking density. Then a predicted curve is computed using the regression model at low stocking density but using the initial value of the regression at high stocking density. The difference between the predicted curve and the actual curve at 10 g L^{-1} is the effect of the interaction between stocking density and irradiance (Bhujel 2008). To determine the effect of stocking density on the response to irradiance, quadratic regressions were computed in the GLM procedure of SAS. Graphs were created in Excel 2010 (Microsoft Corporation, WA, USA) with the data obtained from the regression models of SAS.

4.4 RESULTS

At low stocking density (2 g L^{-1}), *C. crispus* consumed a maximum of $888 \text{ } \mu\text{M NH}_4^+$ at a PPFFR of $500 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ in three to four days (Fig. 4.3a). This amount represented an uptake of 68 % of the available ammonium. The light saturation point was at a PPFFR of $386 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Table 4.1). At high stocking density (10 g L^{-1}) the highest ammonium uptake was $1,271 \text{ } \mu\text{M NH}_4^+$ (98 %) at $200 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ with a saturation point at $190 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$. Because at high stocking density *C. crispus* removed almost all the ammonium available in the flasks, was not possible to determine the effect of the stocking density on ammonium uptake. The predicted ammonium uptake at high stocking density was higher than was available, *ca.* $1,500 \text{ } \mu\text{M NH}_4^+$ (Fig. 4.3b). The highest orthophosphate uptake at 2 g L^{-1} was $44 \text{ } \mu\text{M PO}_4^-$ at the highest

irradiance (PPFFR of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$), which corresponded to 56 % of the available orthophosphate (Fig. 4.4a). The saturation point was $267 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4.1). At 10 g L^{-1} , the highest orthophosphate uptake was $76 \mu\text{M PO}_4^{3-}$ (97 %) at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. At the highest irradiance, the uptake decreased to $68 \mu\text{M PO}_4^{3-}$ (87 %). The saturation point at the high stocking density was $217 \mu\text{mol m}^{-2} \text{s}^{-1}$. The predicted orthophosphate uptake indicated a positive effect of increasing the stocking density, it was 20 % lower than the actual orthophosphate uptake at 10 g L^{-1} . However, the predicted value at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ coincided with the actual value, then at high irradiance the effect of stocking density decreased (Fig. 4.4b). Nutrient uptake was significantly affected by the experimental factors, irradiance and stocking density (Appendix H, Table H.1). According to the slopes of the initial phase of the saturation curves, *C. crispus* used irradiance more efficiently to uptake nutrients at 10 g L^{-1} than at 2 g L^{-1} (Table 4.1).

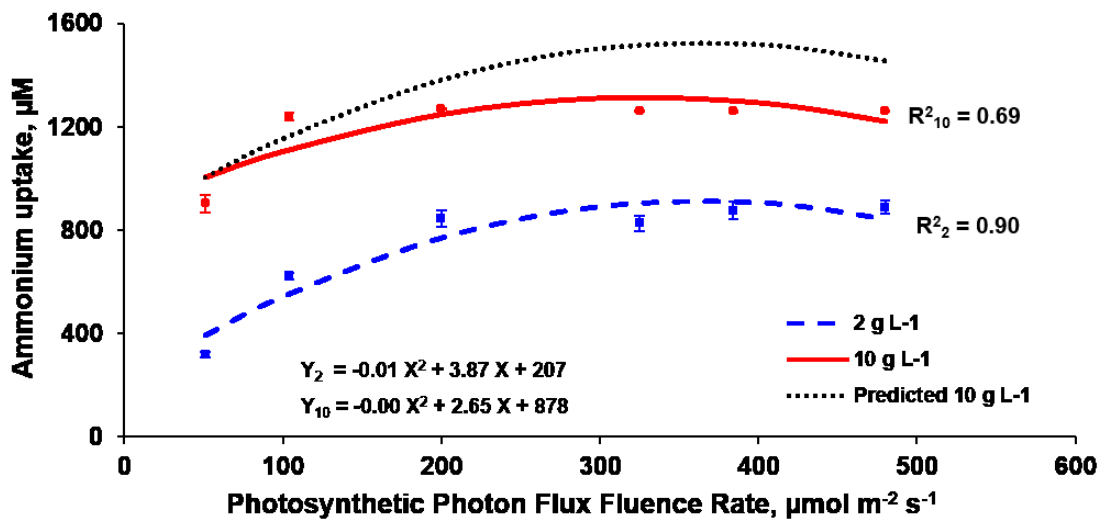
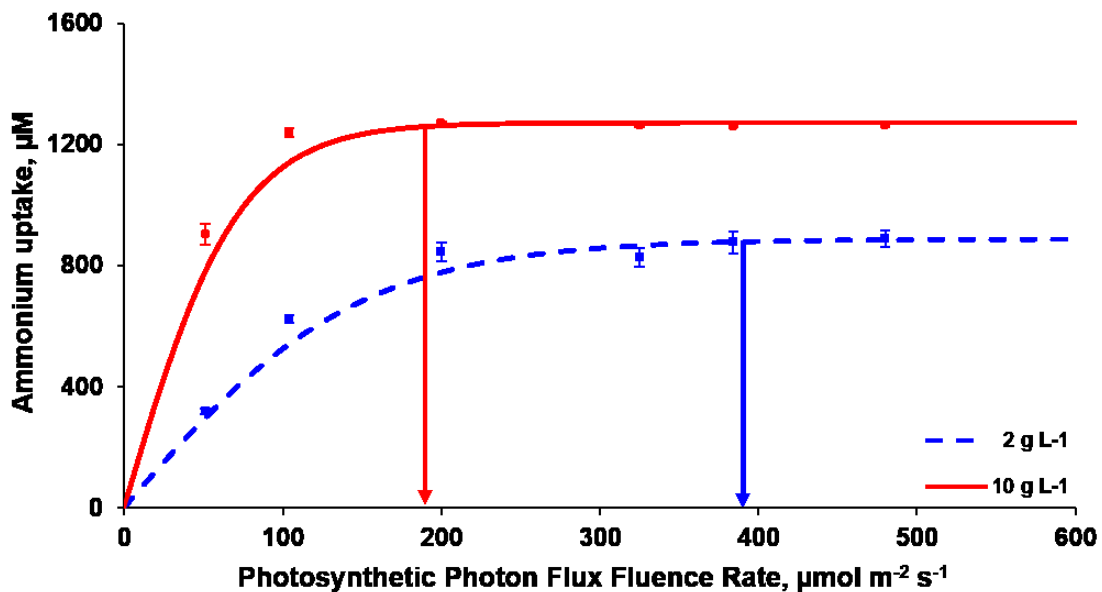


Figure 4.3. Effect of irradiance and stocking density on ammonium uptake efficiency of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. A) Light saturation curves. B) Effect of stocking density on ammonium uptake. Dashed lines represent low stocking density (2 g L^{-1} FW). Solid lines the high stocking density (10 g L^{-1} FW). Dotted lines the predicted curve, the difference between the solid line and the dotted line represents the effect of stocking density. Error bars = SE, n = 16, four samples x four replicates.

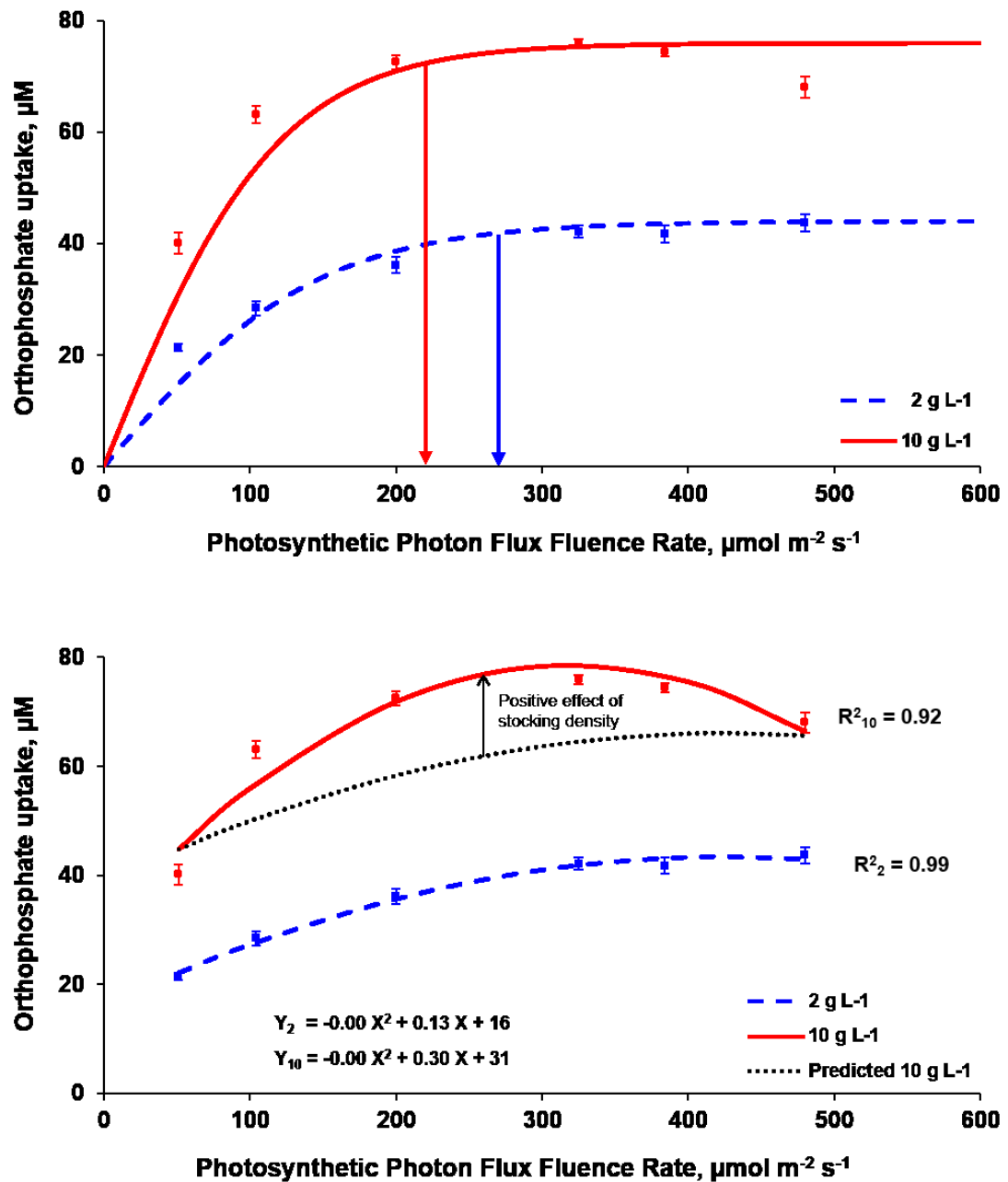


Figure 4.4. Effect of irradiance and stocking density on orthophosphate uptake efficiency of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. A) Light saturation curves. B) Effect of stocking density on orthophosphate uptake. Dashed lines represent low stocking density (2 g L⁻¹ FW). Solid lines the high stocking density (10 g L⁻¹ FW). Dotted lines the predicted curve, the difference between the solid line and the dotted line represents the effect of stocking density. Error bars = SE, n = 16, four samples x four replicates.

Table 4.1. Parameters of the light saturation curves of *Chondrus crispus* cultured at 10 °C under six light irradiances, Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

	<i>Slope</i>		<i>Maximum value of response variable</i>		<i>Saturation point, $\mu\text{mol m}^{-2} \text{s}^{-1}$</i>	
	<i>2</i>	<i>10</i>	<i>2</i>	<i>10</i>	<i>2</i>	<i>10</i>
<i>Stocking density, g L⁻¹</i>						
<i>Response variable</i>						
Ammonium uptake, μM	6.0	17.7	888	1271	386	190
Orthophosphate uptake, μM	0.3	0.6	44	76	267	217
Specific growth rate, % d ⁻¹	0.05	0.03	6.8	5.3	234	335
Net photosynthetic rate, mg C g ⁻¹ FW h ⁻¹	0.002	0.001	0.15	0.07	249	186

The light saturation point for growth at low stocking density was at a PPFFR of 234 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with a maximum SGR of 6.8 % d⁻¹ (Fig. 4.5a). At high stocking density, the highest SGR was 5.3 % d⁻¹, with a saturation point for growth at 335 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4.1). The predicted SGR showed a positive effect of stocking density, it was 10 % lower than the actual SGR, mainly from 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to higher irradiances (Fig. 4.5b). Specific growth rate was significantly affected by the irradiance and stocking density treatments (Appendix H, Table H.1).

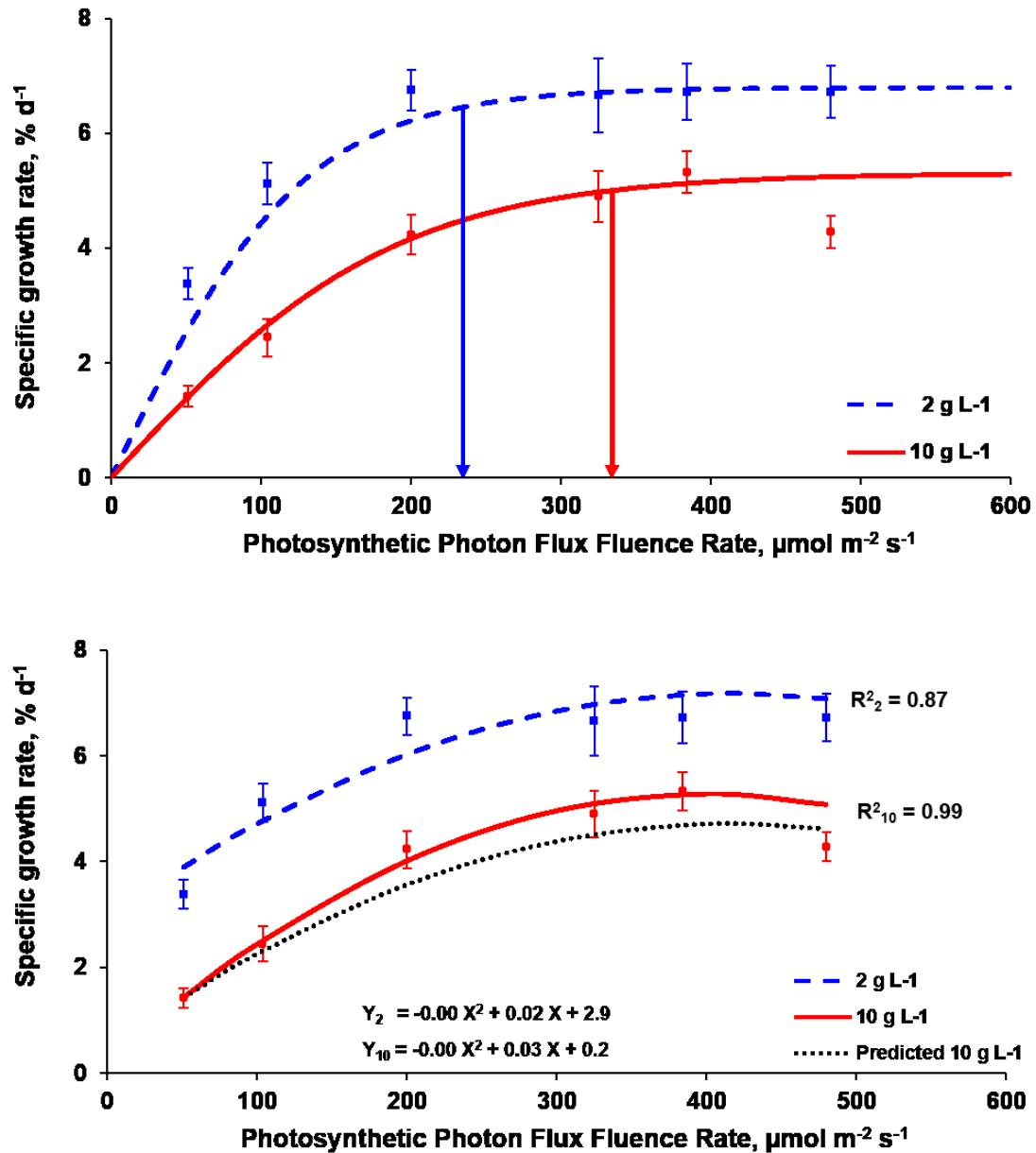


Figure 4.5. Effect of irradiance and stocking density on specific growth rate of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. A) Light saturation curves. B) Effect of stocking density on specific growth rate. Dashed lines represent low stocking density (2 g L⁻¹ FW). Solid lines the high stocking density (10 g L⁻¹ FW). Dotted lines the predicted curve, the difference between the solid line and the dotted line represents the effect of stocking density. Error bars = SE, n = 12, three samples x four replicates.

The Net Photosynthetic Rate (NPR) at the high stocking density was independent of irradiance (Fig. 4.6a; Appendix H, Table H.2). It was lower than the NPR at low stocking density at every irradiance tested because the photosynthetic rate was expressed in terms of the fresh weight (Fig. 4.6a). The effect of the stocking density was not clear, the predicted curve was lowest at the lowest irradiances and highest at the highest irradiances. At the highest NPR, the light saturation points for photosynthesis were at a PPFFR of 249 and 186 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at low and high stocking density respectively (Fig. 4.6b).

The dark respiration rate (DRR) of the fronds exposed to lowest irradiances (PPFFR of 50 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was equal at both stocking densities, and increased as irradiance up to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ where a plateau was observed (Fig. 4.7; Appendix H, Table H.2). However, the maximum respiration rate was higher at 2 than at 10 g L^{-1} because it was expressed as in term of the fresh weight. At the low stocking density, the respiration rate was inversely related to the irradiance to which the fronds were acclimated, while at the high stocking density it was independent of photoacclimation.

Tissue carbon and nitrogen content and the molar C/N ratio, at low stocking density remained close to their initial values (Table 4.2). In contrast, at high stocking density the tissue carbon and nitrogen content decreased as irradiance increased up to a PPFFR of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. However, the decrease in nitrogen content was an order of magnitude greater than the decrease in carbon content, 40 % and 3 %, respectively. Consequently, the molar C/N ratio increased up to a maximum of 12 at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. All the tissue nutrient variables were affected significantly by the experimental factors (Appendix H, Table H.3).

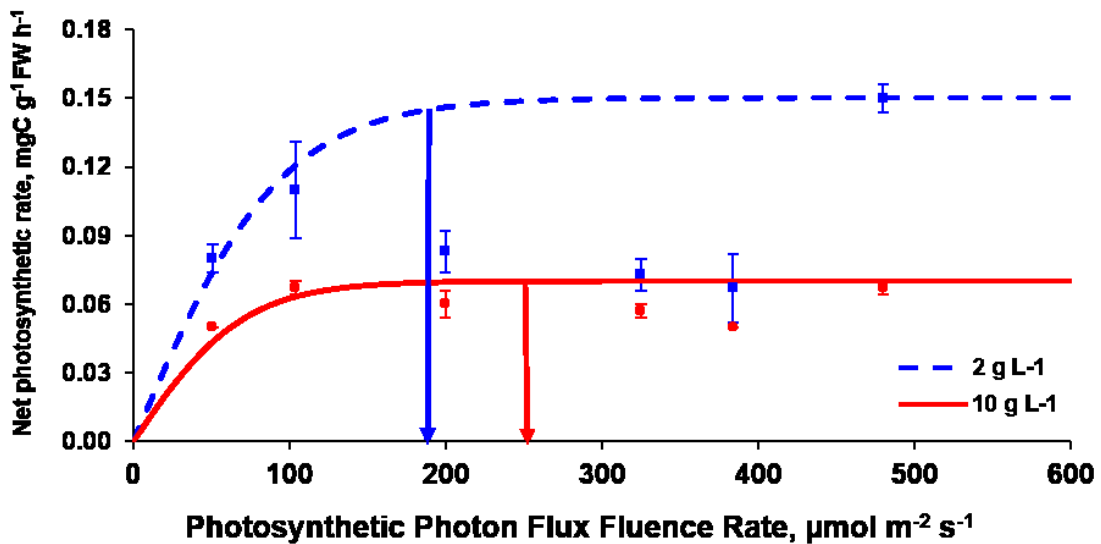
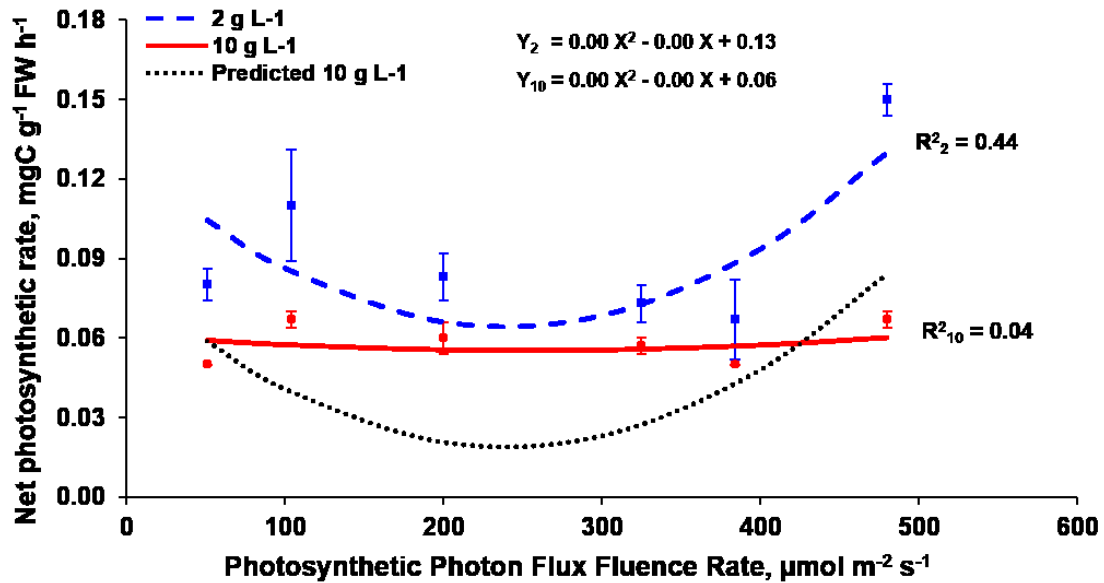


Figure 4.6. Effect of irradiance and stocking density on the photosynthetic rate of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 μmol m⁻² s⁻¹) with a photoperiod of 16:8 (light:dark) at 10 °C. A) Effect of stocking density on photosynthesis. B) Light saturation curves. Dashed lines represent low stocking density (2 g L⁻¹ FW). Solid lines the high stocking density (10 g L⁻¹ FW). Dotted lines the predicted curve, the difference between the solid line and the dotted line represents the effect of stocking density. Error bars = SE, n = three replicates.

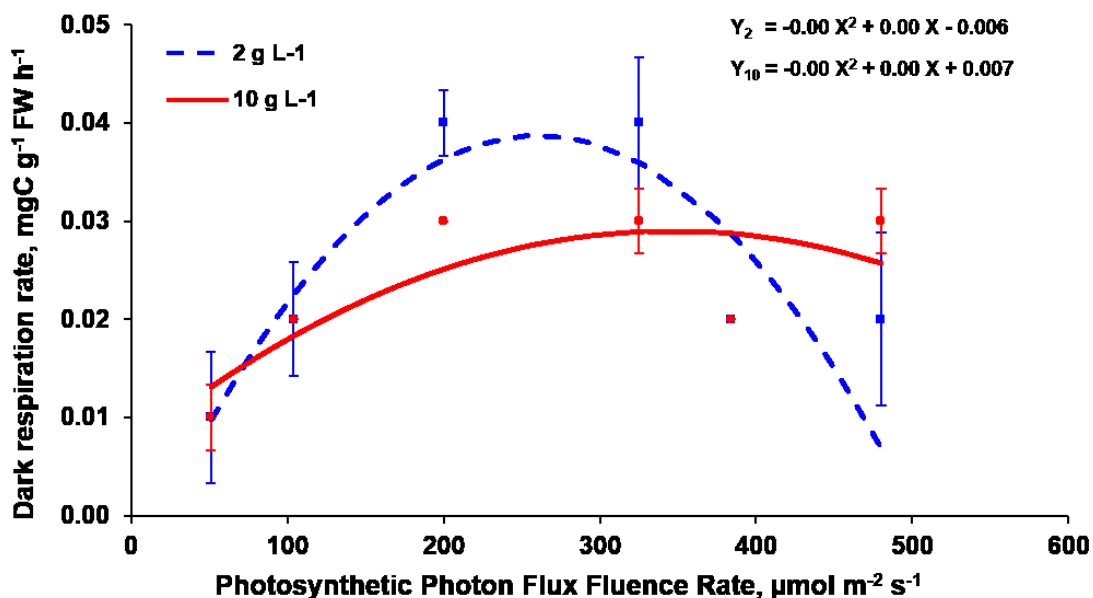


Figure 4.7. Dark respiration rate of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. Dashed line represents low stocking density (2 g L^{-1} FW). Solid line the high stocking density (10 g L^{-1} FW). Error bars = SE, n = three replicates.

Table 4.2. Tissue nutrient content of *Chondrus crispus* cultured for 26 days at two stocking densities (2 and 10 g L^{-1} FW) under six irradiances (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at 10 °C. Percent of carbon in dry weight (% C), percent of nitrogen in DW (% N) and molar ratio of carbon to nitrogen (C/N ratio). Mean \pm SE, n = 4. Means sharing the same letter are not significantly different (Least-square means test, $p > 0.05$).

	% C		% N		C/N ratio	
Initial	31.0		4.7		7.7	
Stocking density, g L^{-1}	2	10	2	10	2	10
Irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$						
50	31.3 \pm 0.2 ^{ab}	31.9 \pm 0.1 ^a	5.2 \pm 0.1 ^{BCD}	4.9 \pm 0.0 ^{BCD}	7.1 \pm 0.1 ^z	7.6 \pm 0.0 ^z
100	30.8 \pm 0.4 ^{bcd}	31.7 \pm 0.1 ^a	5.1 \pm 0.1 ^{BCD}	5.0 \pm 0.1 ^A	7.1 \pm 0.1 ^z	7.5 \pm 0.1 ^y
200	31.2 \pm 0.3 ^{abc}	30.3 \pm 0.1 ^{def}	5.2 \pm 0.1 ^{BCD}	3.4 \pm 0.1 ^{CDE}	7.1 \pm 0.1 ^z	10.5 \pm 0.2 ^y
300	31.3 \pm 0.3 ^{abc}	29.9 \pm 0.3 ^f	5.2 \pm 0.3 ^{BC}	3.3 \pm 0.1 ^{DE}	7.0 \pm 0.3 ^z	10.6 \pm 0.3 ^y
400	30.9 \pm 0.4 ^{bcd}	30.1 \pm 0.0 ^{ef}	5.0 \pm 0.3 ^{BCD}	2.9 \pm 0.0 ^E	7.4 \pm 0.4 ^z	12.0 \pm 0.2 ^x
500	31.3 \pm 0.3 ^{ab}	30.6 \pm 0.1 ^{cdef}	5.4 \pm 0.2 ^B	3.5 \pm 0.1 ^{CDE}	6.8 \pm 0.2 ^z	10.2 \pm 0.3 ^y

Tissue pigment content at both stocking densities was inversely related to irradiance up to a PPFRR of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4.8). Above 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ pigment content was independent of irradiance. At 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, pigment content increased from their initial concentrations of 1.86 mg g^{-1} FW of phycoerythrin (PE), 0.29 mg g^{-1} FW of phycocyanin (PC) and 0.74 mg g^{-1} FW of chlorophyll *a* (Chl *a*). This effect of irradiance on pigment content was more evident at 10 than at 2 g L^{-1} , where phycobiliproteins (PE + PC) decreased by 70 % while Chl *a* decreased 50 %. At the lowest irradiance, the ratio of PE/Chl *a* and ratio of PE+PC/Chl *a* also increased from the original ratio 2.5 and 2.9, respectively. The pigment ratios were lowest at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ when *C. crispus* was cultivated at 2 g L^{-1} , and was 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 10 g L^{-1} (Table 4.3). The effect of the treatment factors was more evident in Chl *a* than in phycobiliproteins (Appendix H, Table H.4).

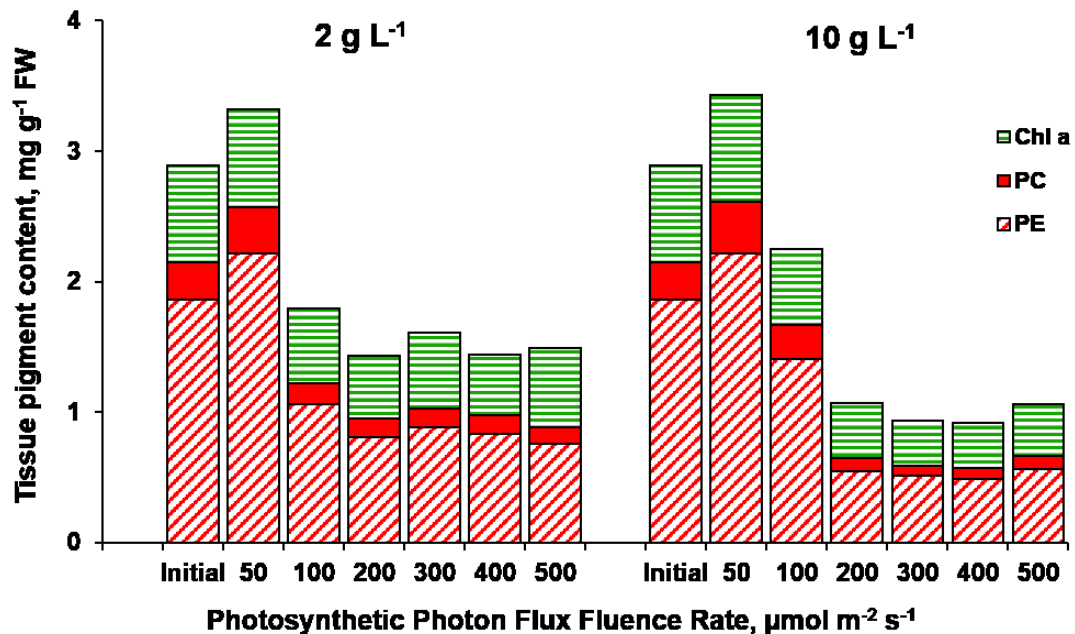


Figure 4.8. Tissue pigment content in *Chondrus crispus* cultured at two stocking densities (2 and 10 g L^{-1} FW) under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. Horizontal hatch pattern corresponds to chlorophyll *a* (Chl *a*), solid to phycocyanin (PC) and diagonal hatch pattern to phycoerythrin (PE).

Table 4.3. Ratio between pigments in tissue of *Chondrus crispus* cultured at two stocking densities (2 and 10 g L⁻¹ FW) under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 μmol m⁻² s⁻¹) with a photoperiod of 16:8 (light:dark) at 10 °C. Phycoerythrin (PE), phycocyanin (PC) and chlorophyll *a* (Chl *a*). Mean ± SE; n = 4. Means sharing the same letter are not significantly different (Least-square means test, p >0.05).

	<i>PE / PC</i>		<i>PE / Chl a</i>		<i>PE + PC / Chl a</i>	
Initial	6.5		2.5		2.9	
<i>Stocking density,</i> <i>g L⁻¹</i>	<i>2</i>	<i>10</i>	<i>2</i>	<i>10</i>	<i>2</i>	<i>10</i>
<i>Irradiance,</i> <i>μmol m⁻² s⁻¹</i>						
50	6.1±0.3 ^{AB}	6.1±0.5 ^{AB}	3.7±0.8 ^a	2.7±0.3 ^{ab}	4.3±0.9 ^w	3.1±0.4 ^{wx}
100	6.6±0.1 ^A	5.6±0.6 ^{AB}	1.9±0.3 ^{bcd}	2.4±0.3 ^{abc}	2.2±0.4 ^{xyz}	2.9±0.3 ^{xy}
200	6.2±0.2 ^{AB}	5.7±0.1 ^{AB}	1.7±0.3 ^{bcd}	1.3±0.1 ^d	2.0±0.3 ^{xyz}	1.5±0.2 ^z
300	5.9±0.2 ^{AB}	5.8±0.3 ^{AB}	1.6±0.4 ^{cd}	1.6±0.3 ^{cd}	1.8±0.5 ^{yz}	2.1±0.5 ^{xyz}
400	5.6±0.1 ^{AB}	6.2±0.5 ^{AB}	1.9±0.4 ^{bcd}	1.4±0.3 ^d	2.2±0.5 ^{xyz}	1.6±0.3 ^z
500	5.4±0.1 ^B	5.9±0.3 ^{AB}	1.2±0.0 ^d	1.4±0.3 ^d	1.4±0.1 ^z	1.6±0.3 ^z

4.5 DISCUSSION

According with the literature, increasing stocking density requires irradiance to be increased to maintain nutrient uptake, because of self-shading. However, in bath culture system self-shading was evident only at the light saturation point for growth. The light saturation point for photosynthesis of *C. crispus* at high stocking density (10 g L⁻¹) was a little less than at low stocking density (2 g L⁻¹), maybe because the carbon dioxide limitation in the small Biochemical Oxygen Demand bottles (300 mL). Light saturation point for nutrients uptake also was lower at 10 than at 2 g L⁻¹ because the high nutrient uptake at this high stocking density; almost all the nutrients available in the one-litre Florence flasks (1,300 μM NH₄⁺ and 78 μM PO₄³⁻) were consumed between each media

exchange. The ammonium uptake efficiency at high stocking density was higher than the uptake expected from the preliminary assessment, where 500, 1,300, 2,100 and 2,900 μM NH_4^+ were evaluated (Appendix F). In the assessment, the time for total consumption of 1,300 μM NH_4^+ was 5.3 days; therefore it was in the range of the media exchange (3 or 4 days). According with the predicted curve developed from the quadratic regression at low stocking density, the maximum ammonium uptake could be 1,500 μM NH_4^+ , an intermediate value between the evaluated concentrations.

The light saturation point for growth of *C. crispus* at 10 g L^{-1} was 2.5 to 7.5-fold higher than the values reported in the literature for strains from Nova Scotia (Bird et al. 1979; Enright and Craigie 1981; Table 4.4). The range indicated as the saturation point for growth increases further if studies from other geographic areas are considered, but they were still lower than the values found in the present study (Table 4.5). The highest value found in the literature was in plants acclimated to high irradiance, while the lowest was in shade acclimated plants (Strömngren and Nielsen 1986; Greene and Gerard 1990; Table 4.5). Despite photoacclimation, increasing the size of the specimen used in the experiment tended to increase the saturation point, indicating that self-shading affected the results. In the case of my experiments, the self-shading was a consequence of the stocking density. In an experiment within this NSERC project, *C. crispus* cultured in Atlantic halibut effluent at 10 g L^{-1} and 17 °C, was not saturated for growth up to 322 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (S. Caines, pers. comm. May 2012). This value was consistent with that obtained in my laboratory experiment at the same stocking density. However, Caines used a flat sensor (LI-192SA), which in my laboratory conditions registered only the 60 % of the irradiance registered by the spherical sensor. In ecological studies and in tank

culture of macroalgae it is normal to use a flat sensor because the light only comes from the surface (Ramus 1985). Nevertheless, in laboratory studies with glass flasks, it is more realistic to use a spherical sensor because irradiance reaches the macroalgae from all directions (Chopin et al. 1995; Ferreira et al. 2006; Corey et al. 2012, 2013). Unfortunately, few studies report the type of light sensor used, and while some studies indicate the light meter; this can be connected to different sensors. The lowest light saturation point for growth of *C. crispus* was determined with a flat sensor (Table 4.5; Strömngren and Nielsen 1986). If this value is extrapolated to values measured with a spherical sensor, it could be $75 \mu\text{mol m}^{-2} \text{s}^{-1}$, similar to that found in another study with *C. crispus* from the same area (Table 4.5; Fortes and Lüning 1980). However, it is not possible to predict precisely difference in light readings of the two sensors in these experimental conditions.

Table 4.4. Light saturation points for growth of *Chondrus crispus* conducted in Canada. Irradiance indicates the saturation point and the values in brackets the range studied.

<i>Irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$</i>	<i>Temperature, $^{\circ}\text{C}$</i>	<i>Specimen size, cm</i>	<i>Source and method</i>	<i>Light sensor and original unit</i>	<i>Reference</i>
50 – 60 (5 - 150)	15	0.5	Lower Gulf of St. Lawrence. 30 segments 250 ml glass flasks	Unknown. Light meter LI-185, $\mu\text{E m}^{-2} \text{s}^{-1}$	Bird et al. (1979)
114 (24 - 188)	10	2 - 4	Clone T4 Nova Scotia. 2.6 g L ⁻¹ . 6.4-L flow-trough	Unknown. Light meter LI-185, $\mu\text{E m}^{-2} \text{s}^{-1}$	Enright (1978a, b)
115 (24 - 188)	5	2 - 4	Clone T4 Nova Scotia. 2.6 g L ⁻¹ . 6.4-L flow-trough	Unknown. Light meter LI-185, $\mu\text{E m}^{-2} \text{s}^{-1}$	Enright (1978a, b)
120 (24 - 188)	15	2 - 4	Clone T4 Nova Scotia. 2.6 g L ⁻¹ . 6.4-L flow-trough	Unknown. Light meter LI-185, $\mu\text{E m}^{-2} \text{s}^{-1}$	Enright (1978a, b)
121 (24 - 188)	20	2 - 4	Clone T4 Nova Scotia. 2.6 g L ⁻¹ . 6.4-L flow-trough	Unknown. Light meter LI-185, $\mu\text{E m}^{-2} \text{s}^{-1}$	Enright (1978a, b)
145 (Natural light and five reduced levels)	14 - 17	2 - 4	Clone T4 Nova Scotia. 2.6 g L ⁻¹ . 6.4-L flow-trough	Unknown. MJ m ² d ⁻¹ of PAR over 16 h	Enright and Craigie (1981)
234	10	3 – 4	Basin Head, PEI. 2 g L ⁻¹ in 1-L glass flasks	Spherical sensor LI-193, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Present study
335	10	3 – 4	Basin Head, PEI. 10 g L ⁻¹ in 1-L glass flasks	Spherical sensor LI-193, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Present study

Table 4.5. Light saturation points for growth of *Chondrus crispus* conducted in Europe and USA. Irradiance indicates the saturation point and the values in brackets the range studied.

<i>Irradiance,</i> $\mu\text{mol m}^{-2} \text{s}^{-1}$	<i>Temperature,</i> $^{\circ}\text{C}$	<i>Specimen</i> <i>size</i>	<i>Source and</i> <i>method</i>	<i>Light sensor</i> <i>and original</i> <i>unit</i>	<i>Reference</i>
46 - 91 (natural light and four reduced levels)	15 - 17	2 - 3 cm	North Sea. Subtidal (10 - 12 m). 6-L outdoor aquaria	Unknown. Meteorologic radiometer, W m^{-2} over 14 h	Strömgen and Nielsen (1986)
70 (0 - 250)		2 cm	North Sea. Individual segment. Growth in area	Unknown. LI- COR quantameter, $\mu\text{E m}^{-2} \text{s}^{-1}$	Fortes and Lüning (1980)
198 (13 - 198)	8 to 20	Whole thalli	English Channel. 100- L tanks in recirculation system	Flat sensor LI- 192SB, $\mu\text{E m}^{-2}$ s^{-1}	Braud and Delépine (1981)
224 (8 - 490)	12	3 - 4 cm	New York, USA. Subtidal (1 - 2 m). Six segments in 7- L Plexiglas tanks	Spherical sensor, Biospherical Instruments, $\mu\text{mol quanta}$ $\text{m}^{-2} \text{s}^{-1}$	Greene and Gerard (1990)

This is the first report of light saturation point for photosynthesis for *C. crispus* from Canada. There are photosynthetic studies, but they deal with seasonal fluctuations of the photosynthetic activity, not the light saturation curves evaluated here (Bidwell et al. 1985; Juanes and McLachlan 1992a, b). The values in the present study were within the range indicated as saturating light points for strains of *C. crispus* from both sides of the Atlantic Ocean (Table 4.6). At 10 g L^{-1} , the saturation point for photosynthesis was close to that reported for plants from the subtidal zone (-12 m, Mathieson and Norall 1975). In addition, the fronds at the high stocking density were exposed to a high irradiance; however, because of self-shading they were acclimated to a low light

irradiance. At 2 g L^{-1} , the saturation point for photosynthesis was close to the upper range indicated in the literature based on the oxygen evolution method (Brachignac and Andre 1984). The highest light saturation points have been reported when either carbon evolution or electron transport rate methods have been used (Table 4.6). The difference in the saturation point for photosynthesis measured as oxygen evolution and electron transport rate could be the results of photorespiration at high irradiances (Cabello-Pasini et al. 2000). There is a wide range of light saturation points reported for the oxygen evolution method. This is due to differences in instrumentation, the extent of photo- and thermo-adapted specimens, the diverse nutritional conditions of the media and the plants themselves, as well as the different light sensors used. This could explain the overlap between the upper values indicated as light saturation with the irradiance indicated as photoinhibitory to *C. crispus* (Table 4.7). For this reason, it is important to describe in detail the experimental conditions to allow a comparison of the results.

Enright and Craigie (1981) indicated that the clone T4 of *C. crispus* increased its respiration rate as irradiance increased up to $180 \mu\text{mol m}^{-2} \text{ s}^{-1}$. In my study, the same trend was observed in the same range of irradiances, but at mid and high irradiances, the respiration rate was stable when *C. crispus* was cultured at 10 g L^{-1} . At low stocking density, respiration rate was lower when fronds were previously exposed to high irradiance. However, the dark respiration rate of *C. crispus* from the English Channel evaluated at similar irradiances (50 to $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was relatively constant (Brachignac and Andre 1984). Regrettably, the authors did not give details of their acclimation conditions and specimen size and this precludes a direct comparison. The response of red macroalgae to irradiance (depth) varies depending on their

photoacclimation strategies (Marquardt et al. 2010). They studied three species *Mazzaella laminarioides* (Bory de Saint-Vincent) Fredericq and *Sarcothalia crispata* (Bory de Saint-Vincent) Leister were consistent with my results, in that the dark respiration rate increased with increasing irradiances. However, the dark respiration rate of the third species, *Gracilaria chilensis* Bird, McLachlan and Oliveira, was independent of irradiance, indicating a restricted physiological acclimation ability (Marquardt et al. 2010). A similar result was found in *Ahnfeltia tobuchiensis* (Kanno and Matsubara) Makienko (Cherbadgy et al. 2010). Unfortunately, in these studies, the highest irradiance was *ca.* 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$; therefore, it is not possible to know if higher irradiances would result in photoinhibition. In addition, both studies used different type of sensors, which increase the difficulty to interpret the results, Marquardt et al. (2010¹⁶) used a spherical sensor (PPFFR) and Cherbadgy et al (2010¹⁷) used a flat sensor (PPFR).

¹⁶ Spectroradiometer SR-9910, Macam Photometrics Ltd. Livingston, Scotland with spherical sensor

¹⁷ Underwater quantum sensor (likely LI-192 a flat light sensor)

Table 4.6. Light saturation points for photosynthesis of *Chondrus crispus*. Irradiance indicates the saturation point and the value in brackets the range studied.

<i>Irradiance,</i> $\mu\text{mol m}^{-2} \text{s}^{-1}$	<i>Temperature,</i> $^{\circ}\text{C}$	<i>Specimen</i> <i>size</i>	<i>Source and method</i>	<i>Light sensor and</i> <i>original unit</i>	<i>Reference</i>
116 (7 - 585)	5	1 - 2 cm	Subtidal (12 m). New Hampshire (NH), USA. O ₂ evolution	Unknown. Light meter LI-185, μE	Mathieson and Norall (1975)
182 (7 - 585)	10	1 - 2 cm	Intertidal and subtidal (12 m). NH, USA. O ₂ evolution	Unknown. Light meter LI-185, μE	Mathieson and Norall (1975)
182 (7 - 585)	15	1 - 2 cm	Subtidal (12 m). NH, USA. O ₂ evolution	Unknown. Light meter LI-185, μE	Mathieson and Norall (1975)
200 (0 - 800)	17	---	Baja California, Mexico but originating from Maine, USA. 5-mL chamber. O ₂ evolution	Likely spherical sensor, Biospherical Instruments, μmol quanta $\text{m}^{-2} \text{s}^{-1}$	Cabello- Pasini et al. (2000)
216 (40 - 1080)	5 and 15	0.6 cm \varnothing disc	NH, USA. Oxygen evolution	Unknown, foot- candle	Mathieson and Burns (1971)
224 (8 - 490)	12	1.5 cm \varnothing disc	New York, USA. Subtidal (1 - 2 m). 5-mL chamber. O ₂ evolution	Spherical sensor, Biospherical Instruments, μmol quanta $\text{m}^{-2} \text{s}^{-1}$	Greene and Gerard (1990)
250 - 300 (0 - 570)	17	---	English Channel. Oxygen evolution	Likely spherical sensor Megatron, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Breignac and Andre (1984)
263 (7 - 585)	5	1 - 2 cm	Tetrasporic and carposporic plants. Subtidal (6 m). NH, USA. O ₂ evolution	Unknown. Light meter LI-185, μE	Mathieson and Norall (1975)
>270 (40 - 270)	10 - 12	Whole thalli	New York, USA. Carbon evolution	Unknown. Gossen light meter, foot- candle	Brinkhuis and Jones (1974)
>450 (0 - 450)	17	---	Baja California, Mexico but originating from Maine, USA. Electron transport rate	Likely spherical sensor, Biospherical Instruments, μmol quanta $\text{m}^{-2} \text{s}^{-1}$	Cabello- Pasini et al. (2000)
186 (50 - 500)	10	3 - 4 cm	Basin Head, PEI. 10 g L ⁻¹ in 300-mL flasks. O ₂ evolution	Spherical sensor LI-193, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Present study
249 (50 - 500)	10	3 - 4 cm	Basin Head, PEI. 2 g L ⁻¹ in 300-mL flasks. O ₂ evolution	Spherical sensor LI-193, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Present study

Table 4.7. Photoinhibition of photosynthesis of *Chondrus crispus*. Irradiance indicates the photoinhibition point and the brackets the range studied.

<i>Irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$</i>	<i>Temperature, $^{\circ}\text{C}$</i>	<i>Specimen size</i>	<i>Source and method</i>	<i>Light sensor and original unit</i>	<i>Reference</i>
324 (27 – 338)	6 to 7	Whole thalli	New York, USA. Carbon evolution	Unknown. Gossen light meter, foot-candle	Brinkhuis and Jones (1974)
400 – 500 (0 – 2000)	17	---	Baja California, Mexico but originating from Maine, USA. Effective quantum yield	Likely spherical sensor, Biospherical Instruments, $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$	Cabello-Pasini et al. (2000)
570 (0 – 570)	17	---	English Channel. Oxygen evolution	Likely spherical sensor Megatron, $\mu\text{mol m}^{-2} \text{s}^{-1}$	Brechignac and Andre (1984)

Light saturation curves are traditionally used to analyze the photosynthetic activity of macroalgae. Since the main purpose of my research was to determine the best irradiance for nutrient uptake to be implemented to bioremediate a fish farm effluent, I adopted the same approach to evaluate its effect on nutrient uptake, the first study to do so. At low stocking density, the light saturation points for orthophosphate and ammonium uptake efficiency were in the same range as for net photosynthetic rate. In a preliminary assay, where *C. crispus* was cultured at a lower nutrient level (30 $\mu\text{M PO}_4^{3-}$), the light saturation point for orthophosphate was similar to high orthophosphate that was found in my experiment, a PPFRR of 272 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This indicates that despite the orthophosphate concentration, the light saturation point for orthophosphate uptake efficiency at low stocking density is constant. In the case of ammonium, at low stocking density, the light saturation point was a little higher than the other parameters because the

highest uptake efficiency was at the highest irradiance; however, the uptake was similar from 200 to 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. At the high stocking density the light saturation point for orthophosphate uptake efficiency was a little lower than at the low stocking density, possibly because of the higher uptake efficiency at the lowest irradiance. In turn, the light saturation point for ammonium uptake was lower because it was consumed quickly even at low irradiance. The light saturation points for orthophosphate and ammonium uptake efficiency were lower at 10 than at 2 g L^{-1} , but in the range of their particular light saturation point for net photosynthetic rate.

The predicted curve for ammonium uptake efficiency was higher than the actual curve, while predicted curve for orthophosphate uptake efficiency was lower than the actual curve. This indicates that *C. crispus* at different stocking densities needed different ammonium to orthophosphate ratios. Chopin and Wagey (1999) indicated that the concentration of nitrogen and phosphorus is more important rather than their ratio, because the same ratio can be reached by different nutrient concentrations. However, they reported that *C. crispus* from NB was saturated at 4.5 to 6.0 $\mu\text{M PO}_4^{3-}$, a concentration 6 to 14-fold lower than the nutrient consumed in my experiment at 2 and 10 g L^{-1} , respectively. *Chondrus crispus* was cultured under similar conditions to my experiment (at 2 g L^{-1} , a PPFFR of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 13 to 14 $^{\circ}\text{C}$), but in 85-L tanks with a weekly medium exchange (Chopin et al. 1995; Chopin and Wagey 1999). This may be due to the difference in nitrogen concentration or source, as they used 12.5 to 56 $\mu\text{M NO}_3$, or due to the highest irradiances used in my experiment. Raven and Hurd (2012) indicated that as the irradiance increases; algae need more orthophosphate to synthesize the enzymes necessary for cellular proteins and photosynthetic structures.

Stocking density affected both the light saturation curves and the tissue nutrient and pigment concentrations. At low stocking density, the nutrient uptake efficiency was highest at the highest irradiance showing that the nutrient concentration was not a limiting factor. This means that the response was only affected by irradiance. The highest specific growth rate of 6.8 % d⁻¹ corresponds to the maximum potential of the species at these culture conditions. However, this value was about half that of the 13 % d⁻¹ reported by Greene and Gerard (1990). They achieved this very high growth rate by culturing 3 to 4-cm segments of *C. crispus* attached to the wall of a 7-L Plexiglass tank, in the tank's wall opposite the artificial light source. An irradiance, PPFFR, of 490 μmol m⁻² s⁻¹ was delivered intermittently with pulses every one second (1 Hz). At the same irradiance, but constant light delivery, the growth rate also was high, 9.7 % d⁻¹, mainly because of the low stocking density, 0.07 g L⁻¹. A similar value, *ca.* 9 % d⁻¹, was reported by Enright and Craigie (1981); however, the authors did not give details of the culture conditions. Other values similar to my result have been reported when very small segments (5 mm) were cultured at *ca.* 3.5 g L⁻¹ under 100 μmol m⁻² s⁻¹, 7.3 % d⁻¹, and in individual 2-cm segments cultured at 250 μmol m⁻² s⁻¹, 7.0 % d⁻¹ (Bird et al. 1979; Fortes & Lüning 1980). The latter experiments were conducted with smaller segments than I used. In addition, different growth rates are obtained depending on which approximation was used, discrete or continuous growth (Kain 1987; Yong et al. 2013). I selected the continuous growth approach because it fits better to the growth of *C. crispus*, since it grows during the night (Strömberg and Nielsen 1986). The specific growth rate of my data would have been overestimated up to 5 % if the discrete approach had been used. The growth rate also changes if the mean of the data is calculated before or after the

logarithmic transformation (Hoffmann and Poorter 2002). For these reasons, it is difficult to compare absolute values reported in the literature.

In *C. crispus*, growth has been associated with carbon assimilation (Greene and Gerard 1990). However, at low stocking density, the specific growth rate was independent of tissue carbon content, and there was an overlap in the carbon content among the light treatments. This indicates a high individual variability among fronds. Specific growth rate was also independent of tissue nitrogen content and hence the molar C/N ratio. However, nitrogen storage varied depending on the irradiance. At the lowest irradiance ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$), the phycobiliprotein content was 3-fold higher than at $\geq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$. A high phycobiliprotein concentration is expected at low irradiance (Häder and Figueroa 1997). However, in my experiment the nitrogen content was similar at all irradiances. In *C. crispus*, a possible nitrogen sink could be L-citrullinyl-L-arginine, which is normally found in winter conditions (Laycock and Craigie 1977). In the laboratory conditions, its synthesis was higher at 8 than at 15 °C, and at $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ than at $\leq 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Laycock et al. 1981¹⁸). However, the presence of this compound, at the highest irradiance, needs to be investigated.

Growth rate at low stocking density was affected by irradiance, and decreased at the highest irradiance ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$). Because tissue nutrients were stable and nutrient uptake highest at this irradiance, this decrease was clearly due to photoinhibition. At high irradiance, plants protect their photosynthetic apparatus by reducing their phycobiliprotein to chlorophyll *a* ratio (López-Figueroa 1992; Fig. 4. 9). The change in the pigments ratio was mainly due to the reduction of phycoerythrin that is easily cleaved from the phycobiliprotein complex (Cornish et al. 2013). This explains why the

¹⁸ Original irradiances 64 and $\leq 22 \text{ ly d}^{-1}$, respectively

phycoerythrin to phycocyanin ratio decreased at high irradiances, especially at low stocking density (Table 4.3). Photoacclimation to high irradiance ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) increased chlorophyll *a* concentration, and this allowed the fronds to achieve the highest photosynthetic rate. The high nitrogen content found in this irradiance could be related to the high chlorophyll *a* concentration (Chopin et al. 1995; Fig. 4.10).

At high stocking density (10 g L^{-1}), the situation was different from at 2 g L^{-1} . Ammonium uptake efficiency was almost 100 % at a PPFFR $\geq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and orthophosphate at $\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Consequently, the specific growth rate was similar at $\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$. However, in spite of the fact that the nutrients were quickly consumed, the fronds did not experience nutrient limitation. Tissue nitrogen level was always higher than the value indicated as critical for *C. crispus* (2.5 % N, Chopin et al. 1995; Table 4.2). The same value, 2.5 % N, was reported by Holdt (2009) for *C. crispus* growing in a low nutrient concentration effluent from sea trout.

Investigators have proposed various ways to assess whether or not nitrogen is limiting, these include the relationship between specific growth rate to tissue nitrogen content (DeBoer 1981); the relationship between specific growth rate to molar C/N ratio (Lapointe and Duke 1984), and the relationship between nitrogen to phycobiliprotein content (Lapointe 1981; Mizuta et al. 2002). According with these criteria, in my experiment nitrogen was not a limiting factor because specific growth rate increased as tissue nitrogen decreased (Fig. 4.11). That specific growth rate increased as molar C/N ratio increased indicates there was light limitation, not nutrient limitation (Fig. 4.12). It is expected that nitrogen and phycobiliprotein content decreases as irradiance increases. The relationship between nitrogen and phycobiliprotein content at high stocking density

decreased with irradiance, but the projected critical nitrogen value was still slightly higher than the critical value indicated above (Fig. 4. 13). This confirms that *C. crispus*, at high stocking density effectively used the nutrients absorbed to grow, taking advantage of the available irradiance. If more nutrients were available, *C. crispus* could uptake and store them similar to what occurred at the low stocking density treatment.

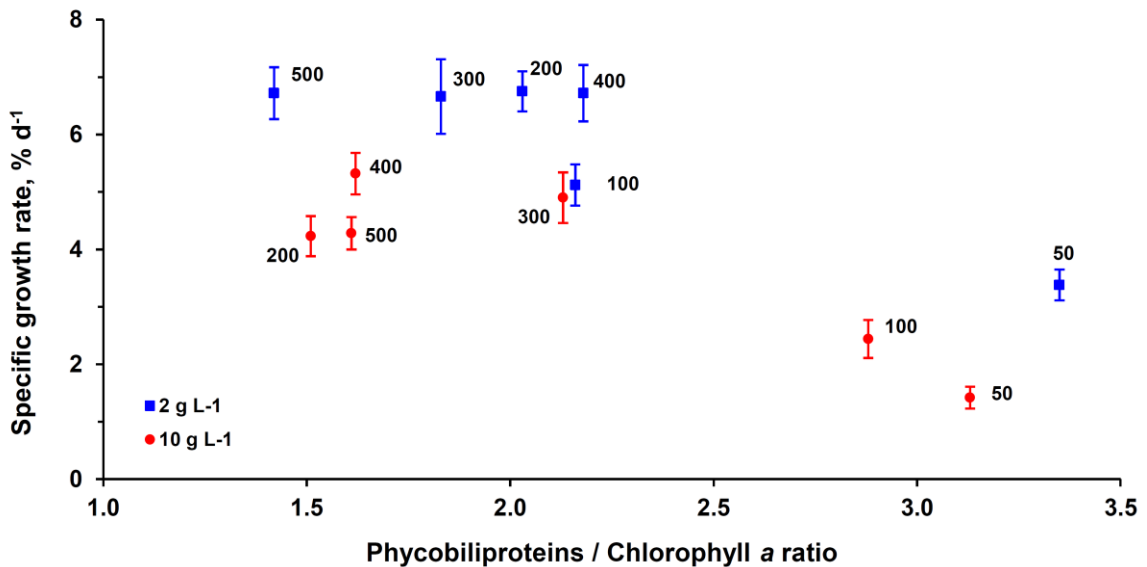


Figure 4.9. Relationship between the phycobiliproteins to chlorophyll *a* ratio and the specific growth rate of *Chondrus crispus* culture under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (L:D) at 10 °C. Squares represent low stocking density (2 g L⁻¹ FW) and circles the high stocking density (10 g L⁻¹). Error bars = SE of growth rate, n = 12. For pigments ratio n = 4.

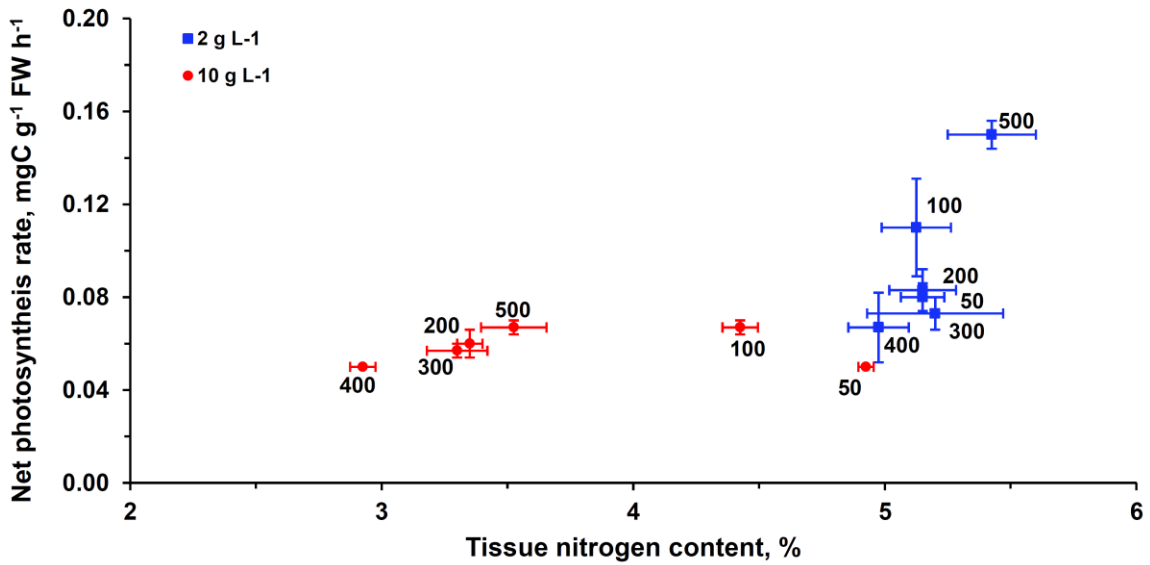


Figure 4.10. Relationship between the tissue nitrogen content ($n = 4$) and the net photosynthetic rate ($n = 3$) of *Chondrus crispus* culture under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (L:D) at 10 °C. Squares represent low stocking density (2 g L⁻¹ FW) and circles the high stocking density (10 g L⁻¹). Error bars = SE.

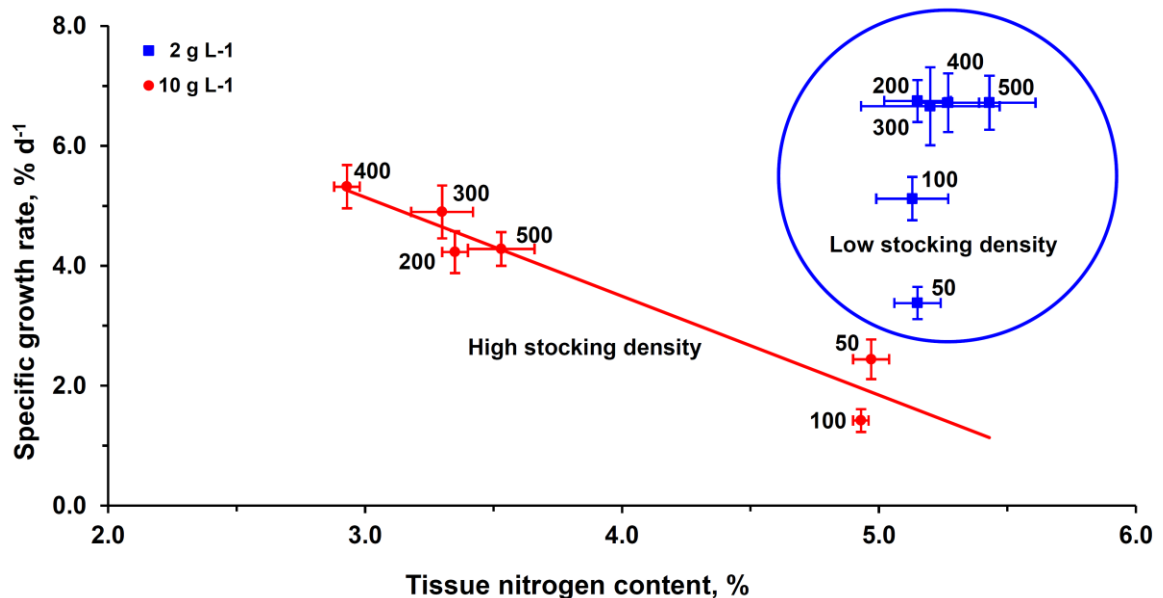


Figure 4.11. Relationship between tissue nitrogen content ($n = 4$) and specific growth rate ($n = 12$) in *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (L:D) at 10 °C. Squares represent low stocking density (2 g L⁻¹ FW) and circles high stocking density (10 g L⁻¹). Error bars = SE.

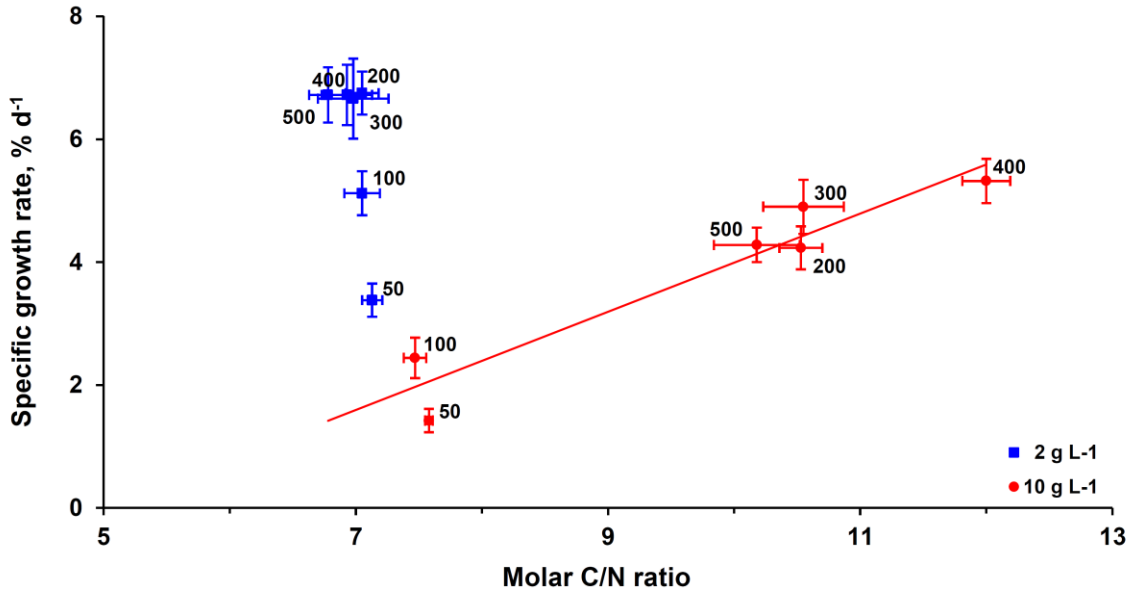


Figure 4.12. Relationship between tissue nitrogen content ($n = 4$) and specific growth rate ($n = 12$) of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (L:D) at 10 °C. Squares represent low stocking density (2 g L⁻¹ FW) and circles high stocking density (10 g L⁻¹). Error bars = SE.

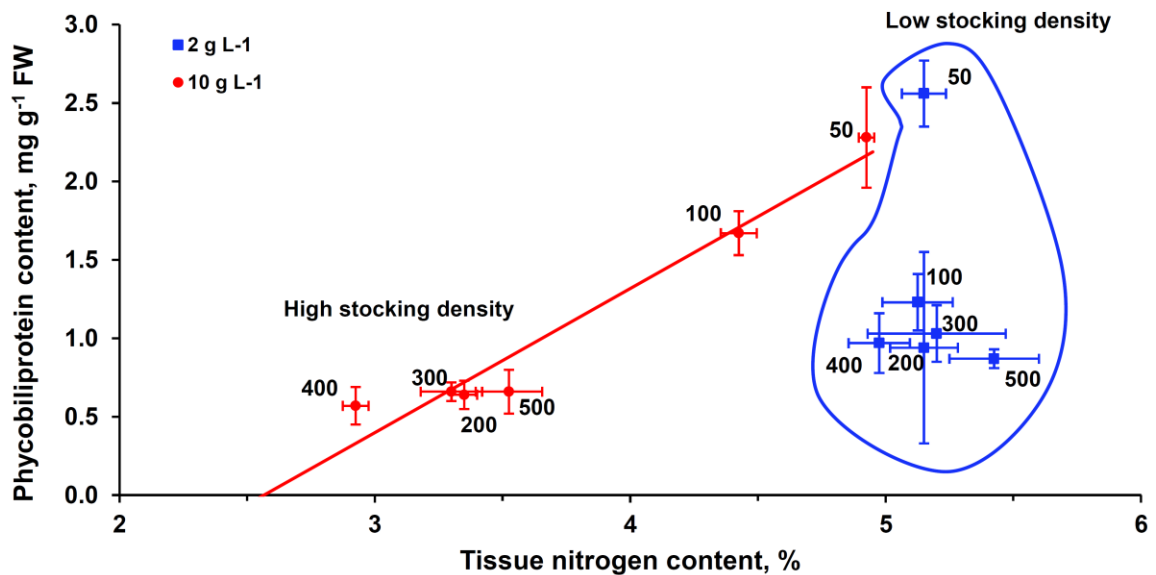


Figure 4.13. Relationship between nitrogen content and phycobiliproteins (phycoerythrin + phycocyanin) content in the tissue of *Chondrus crispus* cultured under artificial light (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a photoperiod of 16:8 (light:dark) at 10 °C. Squares represent low stocking density (2 g L⁻¹ FW) and circles the high stocking density (10 g L⁻¹). Error bars = SE, $n = 4$.

Dark respiration rate influenced the specific growth rate, because it supply much of the energy and carbon skeleton required for biosynthesis (Falkowski and Raven 2007). At high stocking density, the highest growth rate was at a PPFRR of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas the respiration rate was lower at irradiances $\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$. This means that carbon and nitrogen were used for growth and that storage was lowest. Presumably, the decrease in growth at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ was an effect of the high pH at this light treatment (Appendix G, Table G.2), since *C. crispus* growth at pH 8.5 was 20 % lower than at pH 7.5 (Simpson et al. 1978b). However, at both irradiances, the pigment content were similar and the ratios of phycobiliprotein pigments to green pigments were almost identical. This indicates that nutrient storage at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ was not as pigments.

Consequently, optimization of *C. crispus* growth when stocked at 10 g L^{-1} should use a PPFRR of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ at $10 \text{ }^\circ\text{C}$. Higher temperatures will need a higher illumination because the saturation point increases with temperature, but its quantification was beyond the scope of this study. In my experiment, *C. crispus* grew well at this irradiance in a batch system, using all the nutrients available ($1,300 \mu\text{M NH}_4^+$, $78 \mu\text{M PO}_4^{3-}$). In a recirculation system, with constant nutrients supply and using this irradiance, a higher growth rate can be expected than the 1 \% d^{-1} found at a PPFRR of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the Atlantic halibut effluent (Corey 2011). After the present study, it will be necessary to re-evaluate the optimum stocking density to culture *C. crispus* and *P. palmata* in Atlantic halibut effluent. There is a high possibility that the 10 g L^{-1} ($= 2 \text{ kg m}^{-2}$) proposed by Kim et al. (2013) as optimum to balance productivity and nutrient uptake was underestimated. The studies were conducted at a PPFRR of $140 \mu\text{mol m}^{-2} \text{s}^{-1}$, therefore the performance at 10 g L^{-1} and higher was affected by the low irradiance. My

data indicated that higher stocking densities require higher irradiances. A factorial study (stocking density x irradiance) will be necessary to determine the appropriate combination of both factors. With the appropriate irradiance, the surface-area for algal rearing could be reduced, increasing the stocking density to more than 10 g L⁻¹.

CHAPTER 5 CONCLUSIONS

The present study, together with the results from other investigations within the larger NSERC Strategic Project, have shown that red macroalgae are good candidates for land-based integrated culture with Atlantic halibut. They have high nutrient uptake efficiency and can remove a high percentage of the nitrogen and phosphorus contained in the effluent. Laboratory scale experiments allows one to isolate the experimental factor(s) of interest and reveal the potential of the species investigated. For *Chondrus crispus* and *Palmaria palmata* in batch culture systems at 10 g L⁻¹ and in the range of temperature recommended for juvenile Atlantic halibut growth, 10 to 14 °C, the best irradiance under natural illumination was a Daily Photon Dose (PDP) of 17 mol m⁻² d⁻¹. While under artificial illumination and highest nutrient concentration, the best irradiance for *C. crispus* will be 23 mol m⁻² d⁻¹. The artificial illumination is equivalent to a Photosynthetic Photon Flux Fluence Rate (PPFFR) of 400 μmol m⁻² s⁻¹ with a photoperiod of 16:8 L:D.

A simultaneous evaluation of *C. crispus* and *P. palmata* at this temperature range at the laboratory scale under natural irradiance established that *P. palmata* had a higher potential for integration with Atlantic halibut culture than *C. crispus*. At 10 g L⁻¹, *P. palmata* had a higher nutrient uptake efficiency (7 %) and growth rate (225 %) than *C. crispus*. Under natural light, a DPD of 8 mol m⁻² d⁻¹ was sufficient to allow *P. palmata* absorb, in three days, 500 μM of ammonium and 30 μM of orthophosphate with laboratory scale culture conditions. This irradiance is equivalent to a PPFFR of 140 μmol m⁻² s⁻¹ over 16 h. Growth continued at the highest DPD up to 17 mol m⁻² d⁻¹ (PPFFR of

295 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16:8 L:D) using nutrient reserves. In Atlantic halibut effluent, with a constant supply of nutrients *P. palmata* could grow at this daily photon dose without using its nutrient reserves.

Under equivalent nutrient and temperature culture conditions, *C. crispus* grew better under artificial light than under natural light, mainly at a DPD of 8 $\text{mol m}^{-2} \text{d}^{-1}$. At 17 $\text{mol m}^{-2} \text{d}^{-1}$ the difference between the light sources was lowest. *Palmaria palmata* also grew better under artificial light (Morgan et al. 1981); however, this was not evaluated in the present study. Nutrient uptake and growth rate of *C. crispus* can be improved using artificial light and high nutrient concentrations. A PPFFR of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 h per day is recommended for growth of *C. crispus* at 10 g L^{-1} , equivalent to a DPD of 23 $\text{mol m}^{-2} \text{d}^{-1}$. Using optimal culture conditions allows determination of the maximum tissue nitrogen content for species. The maximum nitrogen in *C. crispus* culture at 2 g L^{-1} was 5 % N, regardless of irradiance, nutrient uptake, or specific growth rate. Therefore, it is possible to establish 5 % N as a target of optimal culture conditions. If tissue nitrogen is lower than this target, then culture conditions can be further optimized.

The seasonal change in culture conditions at the Wood's Harbour farm makes it impossible to apply the optimal culture conditions determined from laboratory scale experiments to a farm scale (Fig. 5.1). The DPD determined as optimum under natural illumination is not available in winter conditions, according to measurement conducted in Halifax, NS (Craigie and Shacklock 1995¹⁹). In accordance with the same source, the DPD determined in the artificial illumination experiment only could be reached between April and September. The maximum photoperiod at the farm is 15 h 28 min, at the

¹⁹ Original irradiance measured as total radiation MJ m^{-2} by pyranometer

summer solstice (NRC 2013). Hence it would be necessary to supplement with artificial light to meet the 16:8 L:D recommended for both species.

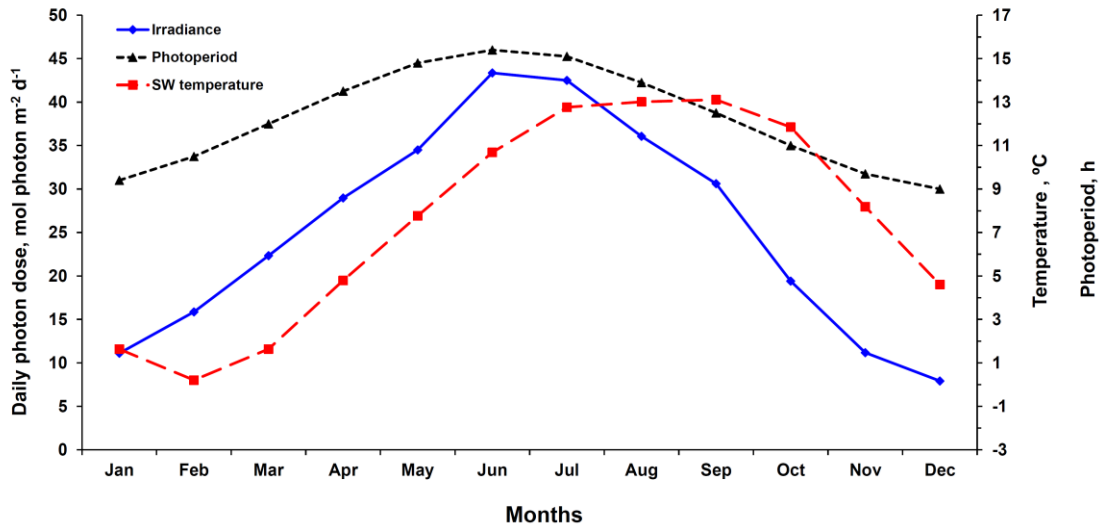


Figure 5.1. Annual fluctuation of abiotic variables that affects the culture of red macroalgae at the Atlantic halibut farm, Wood’s Harbour, NS. Irradiance, dotted line, corresponds to the mean daily values for total irradiance (1980-1984) at Halifax, NS (Craigie and Shacklock 1995). Photoperiod, solid line, corresponds to the mean of the daylength in 2013 at Yarmouth (NRC 2013). Seawater temperature, dashed line, corresponds to the mean daily values for temperature recorded in the inflow to the halibut farm (2002 – 2011; P. Corey, pers. comm.).

The mean monthly temperature for ten years of daily records of the seawater pumped onshore to the halibut farm varied from 0 to 13 °C, with a maximum range from -2 to 17 °C (P. Corey, pers. comm.). However, from November 2009 to October 2010 the temperature in the effluent ranged from 0 to 19 °C indicating that the recirculating system increases the temperature by *ca.* 2 °C (Corey 2011). Assuming this increase of 2 °C, the temperature recommended to juvenile Atlantic halibut culture (10 to 14 °C) was achieved only 33 % of the time, four months per year. Accordingly, for six months the effluent was below, and for two months above the target temperature range. Therefore, it is

necessary to evaluate if this increment in temperature is constant, and to monitor the effluent throughout the year.

The temperature fluctuations also affect nutrient concentrations, because the feed rate of the halibut is reduced at both low and high temperatures. The nitrogen concentration in the effluent was highest in summer conditions (mean 245 $\mu\text{M N}$), following by fall, winter and spring conditions (P. Corey, pers. comm.), while ambient seawater had the highest nitrogen concentration in winter conditions (mean 116 $\mu\text{M N}$). Phosphorus concentration in the effluent was highest in fall conditions (mean 11 $\mu\text{M P}$), following by summer, spring and winter conditions, while in the ambient seawater it was highest at winter conditions (3 $\mu\text{M P}$) and equally low in the other seasons. In addition, the ammonium excretion rate by the fish is not constant during the day, and is dependent upon the feeding regimen. Hence, due to seasonal and daily variation in nutrient concentration, composite sampling design is recommended, where the number of samples and their frequency depends on the flow (Spellman 2011). This monitoring should consider physical and chemical variables, such as flow, temperature and salinity in the former, and pH, un-ionized ammonium, ammonia ion, nitrate, nitrite, orthophosphate, carbon dioxide, bicarbonate, iron and manganese in the latter.

Standardization of the effluent sampling design is important to allow comparison with other studies, and these should reflect the variables and analytical techniques utilized. For the fish farm point of view, it is important to report the results of the nutrient analysis as a concentration because the removal percentage does not show if the water that is returned to the fish still has toxic levels of un-ionized ammonia. From the perspective of the algae culture, the concentration of the different nutrients affects the

absorption of other nutrients and the growth performance. It is more important to report the nutrient concentration than the ratio between the nutrients. There are many techniques and equipment to analyze nutrients and the other physiological variables, such as photosynthetic and respiration rates, and irradiance, which are difficult to standardize. However, it is important to describe the details of the techniques and equipment to facilitate comparison with other studies. In addition, it is difficult to standardize the size of the specimen used in the experiments, as it depends on the vessel size. It is important to report the size of the thalli to allow a better interpretation of the results. This is especially important for the comparison of the specific growth rates, as algae grow at different rates depending on their size, and fragments or whole organisms.

In this study, 10 g L^{-1} was used as the high stocking density condition based on a screening study with Atlantic halibut effluent conducted at the beginning of the NSERC project in 2009 by Kim et al. (2013). Ten grams per litre was determined to be the optimum stocking density to balance productivity and nutrient uptake by both *C. crispus* and *P. palmata*. However, the study was conducted at a PPFRR of $140 \mu\text{mol m}^{-2} \text{ s}^{-1}$, assuming that this irradiance exceeded the light saturation point. Nevertheless, there is a high probability that the optimum stocking density proposed by Kim et al. (2013) was underestimated. My research found, the light saturation point for growth of *C. crispus*, at this stocking density, was a PPFRR of $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Kim et al.'s results were highly biased towards lower stocking densities affected by the low light intensity. It is expected that with a higher irradiance, the performance at higher stocking densities could be significantly better. For this reason, it seems possible to further increase the stocking density, and thereby reduce the surface-area rearing space and the number of tanks

necessary to treat the Atlantic halibut effluent. Therefore, it is recommended that future studies determine this new optimum stocking density in a factorial study with both higher stocking densities and irradiance. In one-litre glass flasks, it was not possible to culture either *C. crispus* or *P. palmata* at 20 g L⁻¹. There were problems with the high carbon dioxide consumption and the low capacity of movement of the algal segments in this vessel type at this stocking density. It would be better to conduct this study directly at a pilot scale, in tanks that meet the size proportions indicated by Craigie and Shacklock (1995), specifically a length to width ratio of 2:1 or 3:1, and 0.9 m depth.

To remove 50 % of the nitrogen produced by 100 t of Atlantic halibut cultured in winter conditions and a feeding rate of 0.4% per day, Corey (2011) estimated that in the sump tanks, at a stocking density of 1.1 g L⁻¹, 98.8 t of macroalgae and a rearing surface-area of 34,000 m² would be necessary. However, if this biomass is cultured at this stocking density in 1-m depth tanks, as it is recommended for *C. crispus* (Bidwell et al. 1985), the rearing surface-area would be 89,818 m². If this biomass were cultivated at 10 g L⁻¹, as is proposed in my study, the surface-area required would be 9,680 m². Corey (2011) determined this algal biomass under a Daily Photon Dose of 12.6 mol m⁻² d⁻¹, a lower irradiance than that recommended by my study (23 mol m⁻² d⁻¹). Extrapolating the results from the artificial light experiment, increasing the stocking density we could reduce the biomass needed to achieve the same nutrient uptake by 43 %, this means only 56.3 t of macroalgae would be needed. Increasing irradiance from 12.6 to 23 mol m⁻² d⁻¹, could reduce the algal biomass needed by a further 7%. Then, by cultivating at 10 g L⁻¹, the tank space might be reduced to 5,240 m². Of course, the accuracy of these estimates

depend on how well the data derived from experiments in glass flasks can be scaled up to farm operation. These assumptions need to be tested in future studies.

The main conclusion of my study is that the cultivation of *C. crispus* and *P. palmata* can continue to be optimized. Both species can be grown at a stocking density greater than 10 g L⁻¹ if the correct irradiance is used. A starting point for such experiment would be 10 g L⁻¹ *C. crispus* saturating at a PPFFR of 400 μmol photon m⁻² s⁻¹. The more the stocking density can be increased, the smaller the rearing surface-area will be needed. The rearing surface-area will determine the quantity of lamps that will be needed, and their installation cost; and the target irradiance and the lamp type will determine the operational costs. Whatever improvements can be made it is still uneconomic to culture red macroalgae in a land-based IMTA system, with current markets for the algal product.

5.1 FUTURE OF LAND-BASED INTEGRATED AQUACULTURE

Land-based integrated aquaculture has a promising future. The pharmaceutical and food industries require a constant supply of raw macroalgal material, both in quantity and quality, to be able to develop their products (Fleurence et al. 2012; Hafting et al. 2012; Ibañez and Cifuentes 2012). Wild macroalgae, IMTA in the open ocean and IMTA in land-based flow-through systems cannot guarantee this constant production, which can only be met by land-based recirculation system with a high control of their culture conditions (Stengel et al. 2011; Hafting et al. 2012). Due to the high production costs of land-based macroalgal monoculture, integrated aquaculture has been seen as the only option to satisfy this demand (Figuroa et al. 2011; Pereira et al. 2013). While this will

increase production costs, if the correct species are selected, the product can pay for these costs. Kang et al. (2013) proposed an index to select macroalgal species for inclusion in an IMTA system. This index is based on physiological criteria and has only a general economic variable. However, because of the expected high operational costs, it is necessary to include more economic criteria to make a complete evaluation. These must include production costs of the seed stock (spores vs. vegetative propagules), operational costs (controlling the culture variables to meet fish and macroalga requirements), as well as the market price of products that can be obtained from each algal species. In the case of *C. crispus* and *P. palmata*, most of the values for the physiological parameters included in Kang et al. (2013)'s index can be found in the literature. However, as observed in my research, there is a high variation in results between studies depending on the growth conditions, in the wild or culture with seawater. Therefore, it is necessary to reassess all these variables in the actual effluent conditions. *Palmaria palmata* performed better in my study conditions. However, in the third year of its cultivation at Wood's Harbour, it deteriorated badly because of the high summer temperatures, up to 18 °C. Therefore, it might be necessary to propagate *P. palmata* from spores. There are different approaches with laboratory scale cultivation (Le Gall et al. 2004; Pang and Lüning 2006; Edwards and Dring 2011). Nevertheless, the technology required to control *P. palmata* reproduction at a commercial scale is not yet developed (Watson and Dring 2011). However, if the temperature is controlled between 10 to 14 °C, as was suggested in my conclusions, *P. palmata* could be propagated by vegetative reproduction. In contrast, the Basin Head strain of *C. crispus* used in this project was successfully maintained in temperatures up to 20 °C using vegetative reproduction. It was maintained

in culture for several years at the Marine Research Station of the National Research Council, Ketch Harbour, Nova Scotia. Therefore, commercial cultivation of *C. crispus* is feasible in controlled conditions without requiring costly and uncertain reproduction from spores.

The high cost of producing macroalgae from spores (hatchery) makes it necessary to find a niche in the market that generates large dividends (Dring et al. 2013). An alternative is human consumption and selling directly to restaurants; however, in the Western markets this strategy is limited (Edwards et al. 2012; Dring et al. 2013; Tacon and Metian 2013). To export to the Asian markets it is necessary for at least a limited processing, such as drying, as carried out by Acadian Seaplants Ltd. with their sales of *C. crispus* to Japan (McHugh 2003). The control of culture conditions is very important for edible macroalga because of the seasonal variation in their physical characteristics and chemical composition. This is particularly important because some culture conditions may result in elevated levels of heavy metals or other compounds potentially detrimental to human health (Holdt and Kraan 2011; Mouritsen et al. 2013). However, only France has regulations for acceptable concentrations of harmful compounds in algal tissues (Fleurence et al. 2012; Mouritsen et al. 2013). In Canada, the Canadian Food Inspection Agency made a specific notice to avoid the consumption of Hijiki seaweed [*Sargassum fusiforme* (Harvey) Setchell] because of its high arsenic content; however, there are no regulations relating to the consumption of macroalgae generated in IMTA systems (Chopin and Robinson 2004). *Palmaria palmata* has a high protein content, essential amino acids and minerals (Morgan et al. 1980b; Galland-Irmouli et al. 1999; Mouritsen et al. 2013). However, the digestibility by humans of the proteins present in *P.*

palmata is controversial, because the anti-nutritional effect of the polysaccharides (Galland-Irmouli et al. 1999; Fleurence et al. 2012; Mouritsen et al. 2013).

Another possibility is indirect human consumption via extraction of the compounds present in the macroalgae. The chemical composition of both *P. palmata* and *C. crispus* has been studied with respect to compounds potentially relevant to human, animal and plant health. These compounds are secondary metabolites generated by macroalgae as a defense against excessive irradiance, mainly in the UV spectrum (Yuan et al. 2005; Logan et al. 2006; Figueroa et al. 2008). These secondary metabolites can be produced with less than 24 h exposure to stress conditions, hence their production can be stimulated artificially (Figueroa et al. 2011). Some of the properties studied in *P. palmata* are antiproliferative (Yuan and Walsh 2006; Yuan et al. 2009), antioxidant (Cornish and Garbary 2010; Liu and Pang 2010; Li et al. 2012), antimicrobial (Cox et al. 2010), antiviral (Jiao et al. 2012), antihypertensive (Fitzgerald et al. 2012), and antidiabetic (Harnedy and FitzGerald 2013). *Palmaria palmata* is also an important source of commercial phycoerythrin (Galland-Irmouli et al. 2000; Wang et al. 2002; Dumay et al. 2013). Phycoerythrin and other phycobiliproteins are commodities used as pigments in a wide range of industries, such as food, cosmetic and paint, and for fluorescent contrast in medical assays (Galland-Irmouli et al. 2000; Sekar and Chandramohan 2008; Dumay et al. 2013). *Palmaria palmata* also has been studied as a source of methane for energy production (Jard et al. 2012). So far, *C. crispus* has been less studied, but antioxidant and antimicrobial properties have been described (Plaza et al. 2008; Cornish and Garbary 2010; Cox et al. 2010; Sangha et al. 2013). It should be noted that antioxidant and antimicrobial activities depend on the extraction procedures, and

with ethanol and acetone extractions the antimicrobial activity was higher in *C. crispus* than *P. palmata* (Wang et al. 2009; Cox et al. 2010). Extracts of *C. crispus* also have potential as antifouling agents (Chambers et al. 2011) or antibiotics (Liao et al. 2003).

In conclusion, *C. crispus* and *P. palmata* grow well in fish effluent, but to be commercially viable the inclusion into a land-based IMTA system needs to solidify the market demand for these valuable byproducts.

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APPENDIX A Conversion of irradiance units

Conversion of irradiance units (Thimijan and Heins 1983)

$\mu\text{E m}^{-2} \text{ s}^{-1}$	= 1.000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
ft-c (cool-white fluorescent)	= 0.135 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
ft-c (incandescent lamp)	= 0.216 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
W m^{-2} (natural light)	= 4.570 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
W m^{-2} (cool-white fluorescent)	= 4.590 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
ly d^{-1} (natural light)	= 2.212 $\mu\text{mol m}^{-2} \text{ s}^{-1}$
ly d^{-1} (cool-white fluorescent)	= 2.222 $\mu\text{mol m}^{-2} \text{ s}^{-1}$

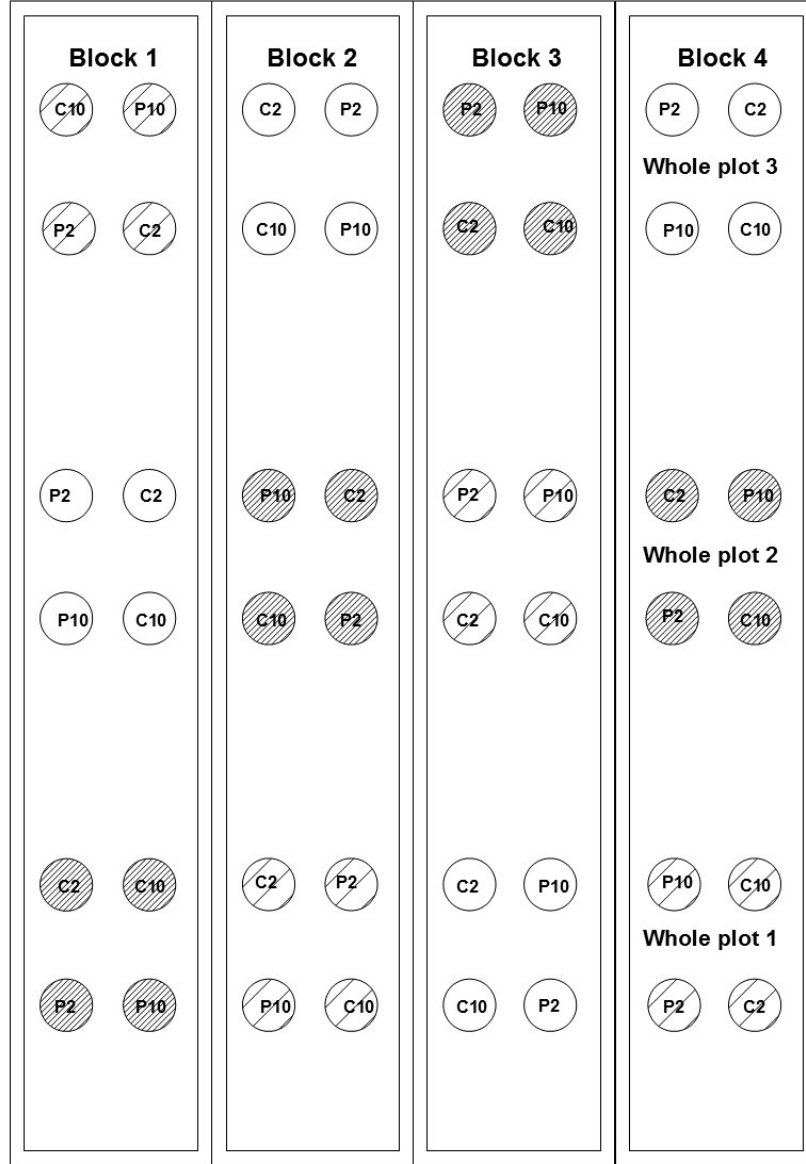
Conversion of Daily Photon Dose units (Ting and Giacomelli 1987)

$\text{MJ m}^{-2} \text{ d}^{-1}$ of PAR	= 2.0804 $\text{mol m}^{-2} \text{ d}^{-1}$
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Daily Photon Dose calculation from a constant Photosynthetic Photon Flux Fluence Rate (artificial light)

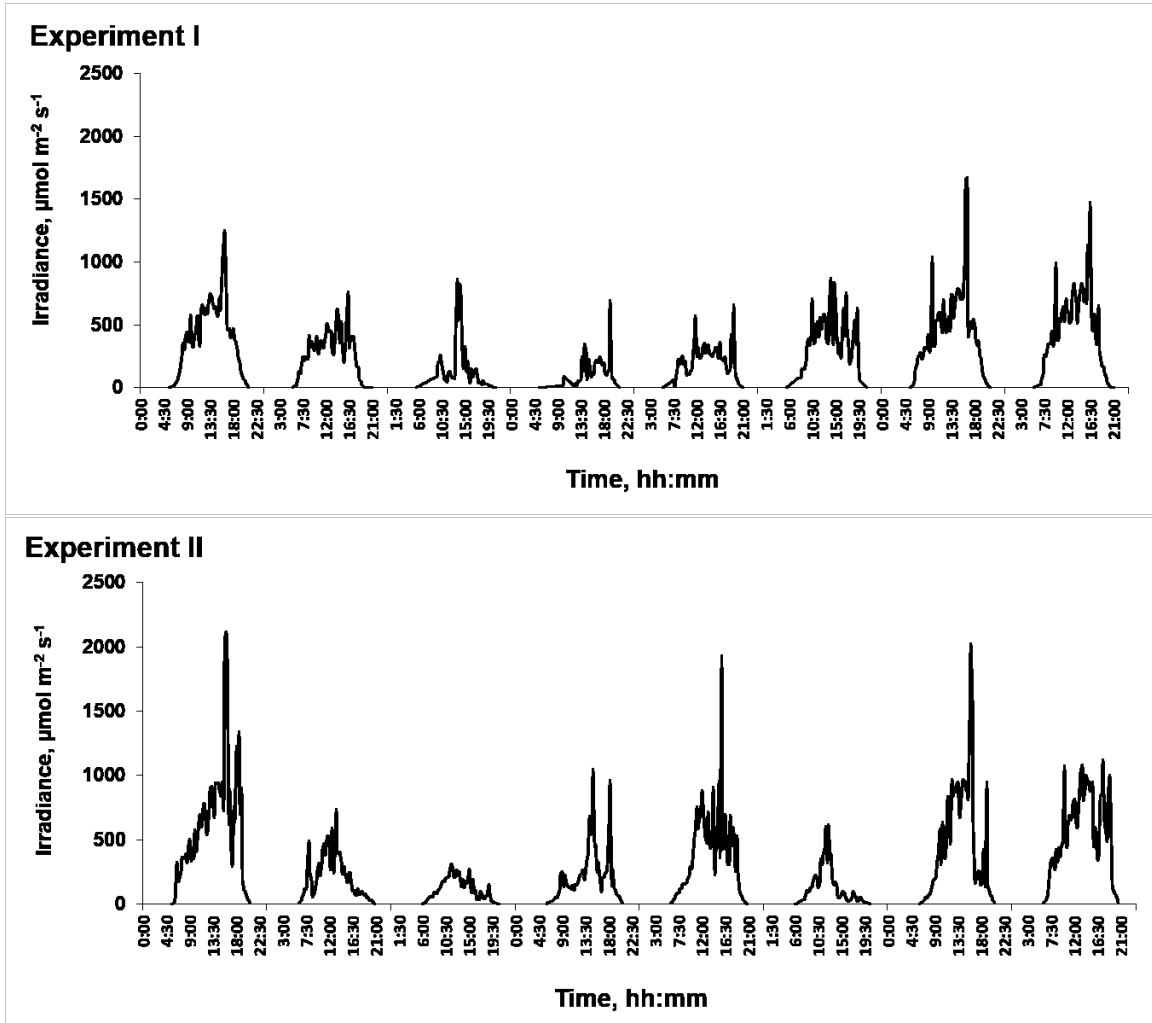
$\mu\text{mol m}^{-2} \text{ s}^{-1}$	= (Daylight, h * 0.0036) $\text{mol m}^{-2} \text{ d}^{-1}$
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**APPENDIX B Experimental design greenhouse experiments
(Chapter 3)**



Distribution of flasks in a Split – Plot- Factorial design. Four blocks, three whole plots (irradiance): 100 %, clear circles; 50 %, light patterns and 20 %, dark patterns. Subplot 2 x 2 factorial: Stocking density, 2 and 10 (2 and 10 g L⁻¹ FW, respectively) and species, C, *Chondrus crispus* and P, *Palmaria palmata*.

APPENDIX C Daily fluctuation of the irradiance inside the greenhouse (Chapter 3)



Examples of the daily fluctuation of the irradiance inside the greenhouse during one week of Experiment I, June 17 to 24, 2011 and one week of Experiment II July 24 to August 1st, 2011. A peak was observed in both experiments between 16:00 and 17:00 Coordinated Universal Time -4.

APPENDIX D Culture conditions during experiments in the greenhouse (Chapter 3)

Table D.1. Daily Photon Dose ($\text{mol m}^{-2} \text{d}^{-1}$) to which *Chondrus crispus* and *Palmaria palmata* were exposed to in each experiment during the nutrient uptake-sampling period (mean \pm SE). The sampling period corresponds 3 or 4 days between culture medium exchanges, twice a week. Means sharing the same letter within each experiment are not significantly different (Tukey's test, $p > 0.05$).

Nutrient sampling period	Experiment I			Experiment II		
	Light treatment, %			Light treatment, %		
	100	50	20	100	50	20
1	11.5 \pm 1.17 ^{ab}	5.7 \pm 0.58 ^{bcd}	2.3 \pm 0.23 ^d	13.0 \pm 2.10 ^{ABC}	6.5 \pm 1.05 ^{ED}	2.6 \pm 0.42 ^D
2	7.8 \pm 1.52 ^{bcd}	3.9 \pm 0.76 ^{cd}	1.6 \pm 0.30 ^d	17.9 \pm 3.26 ^{AB}	8.9 \pm 1.63 ^{CD}	3.6 \pm 0.65 ^D
3	12.2 \pm 2.37 ^{ab}	6.1 \pm 1.19 ^{bcd}	2.4 \pm 0.47 ^d	19.7 \pm 3.41 ^A	9.8 \pm 1.70 ^{BCD}	3.9 \pm 0.68 ^D
4	17.3 \pm 2.56 ^a	8.7 \pm 1.28 ^{bc}	3.5 \pm 0.51 ^{cd}	14.2 \pm 2.17 ^{ABC}	7.1 \pm 1.08 ^{CD}	2.8 \pm 0.43 ^D

Table D.2. Daily Photon Dose ($\text{mol m}^{-2} \text{d}^{-1}$) to which *Chondrus crispus* and *Palmaria palmata* were exposed in experiment during the growth rate assessment (mean \pm SE). The sampling period corresponds to the days between each weighing which happen one a week. Means sharing the same letter within each experiment are not significantly different at each growth period (Tukey's test, $p > 0.05$).

Growth sampling period	Experiment I			Experiment II		
	Light treatment, %			Light treatment, %		
	100	50	20	100	50	20
1	13.0 \pm 1.13 ^a	6.5 \pm 0.56 ^{cd}	2.6 \pm 0.23 ^d	19.4 \pm 1.99 ^A	9.7 \pm 1.00 ^{BC}	3.9 \pm 0.40 ^{CD}
2	11.4 \pm 1.67 ^{ab}	5.7 \pm 0.84 ^{cd}	2.3 \pm 0.33 ^d	15.4 \pm 2.16 ^{AB}	7.7 \pm 1.08 ^{CD}	3.1 \pm 0.43 ^D
3	14.7 \pm 1.74 ^a	7.4 \pm 0.87 ^{bc}	2.9 \pm 0.35 ^d	16.9 \pm 2.07 ^A	8.5 \pm 1.04 ^{CD}	3.4 \pm 0.41 ^D

Table D.3. Temperature and pH inside culture flasks of *Chondrus crispus* and *Palmaria palmata* recorded twice a week in Experiment I and Experiment II in each light treatment (mean \pm SE). Means sharing the same letter within each experiment are not significantly different (Least-square means test, $p > 0.05$)

Experiment	Temperature, °C			pH		
	Light treatment, %			Light treatment, %		
	100	50	20	100	50	20
I	10.1 \pm 0.07 ^a	10.0 \pm 0.08 ^a	10.0 \pm 0.07 ^a	8.2 \pm 0.06 ^a	8.0 \pm 0.05 ^a	7.7 \pm 0.04 ^b
II	14.4 \pm 0.09 ^B	14.3 \pm 0.09 ^B	14.2 \pm 0.07 ^B	8.3 \pm 0.05 ^C	8.1 \pm 0.04 ^{CD}	7.7 \pm 0.04 ^D

Table D.4. pH at each treatment combination of species (*Chondrus crispus* and *Palmaria palmata*), stocking density (2 and 10 g L⁻¹ FW) and light treatment (100, 50 and 20 % incident light) in Experiment I and Experiment II. Values indicate means \pm SE; means sharing the same letter within each experiment are not significantly different (Tukey's test, $p > 0.05$).

Stocking density, g L ⁻¹	Species	Experiment I			Experiment II		
		Light treatment, %			Light treatment, %		
		100	50	20	100	50	20
2	<i>C. crispus</i>	8.2 \pm 0.06 ^{ab}	8.1 \pm 0.04 ^{ab}	7.9 \pm 0.04 ^{bcd}	8.2 \pm 0.07 ^{ABC}	8.0 \pm 0.05 ^{BCD}	7.8 \pm 0.03 ^{CD}
10	<i>C. crispus</i>	8.0 \pm 0.14 ^{abc}	7.8 \pm 0.12 ^{bcd}	7.6 \pm 0.08 ^d	8.4 \pm 0.11 ^A	8.2 \pm 0.10 ^{ABC}	7.6 \pm 0.08 ^D
2	<i>P. palmata</i>	8.2 \pm 0.10 ^{ab}	8.1 \pm 0.09 ^{abc}	7.8 \pm 0.06 ^{bcd}	8.0 \pm 0.08 ^{BCD}	8.0 \pm 0.07 ^{BCD}	7.7 \pm 0.06 ^D
10	<i>P. palmata</i>	8.3 \pm 0.11 ^a	8.2 \pm 0.11 ^{ab}	7.7 \pm 0.10 ^{cd}	8.4 \pm 0.14 ^{AB}	8.2 \pm 0.10 ^{AB}	7.7 \pm 0.11 ^D

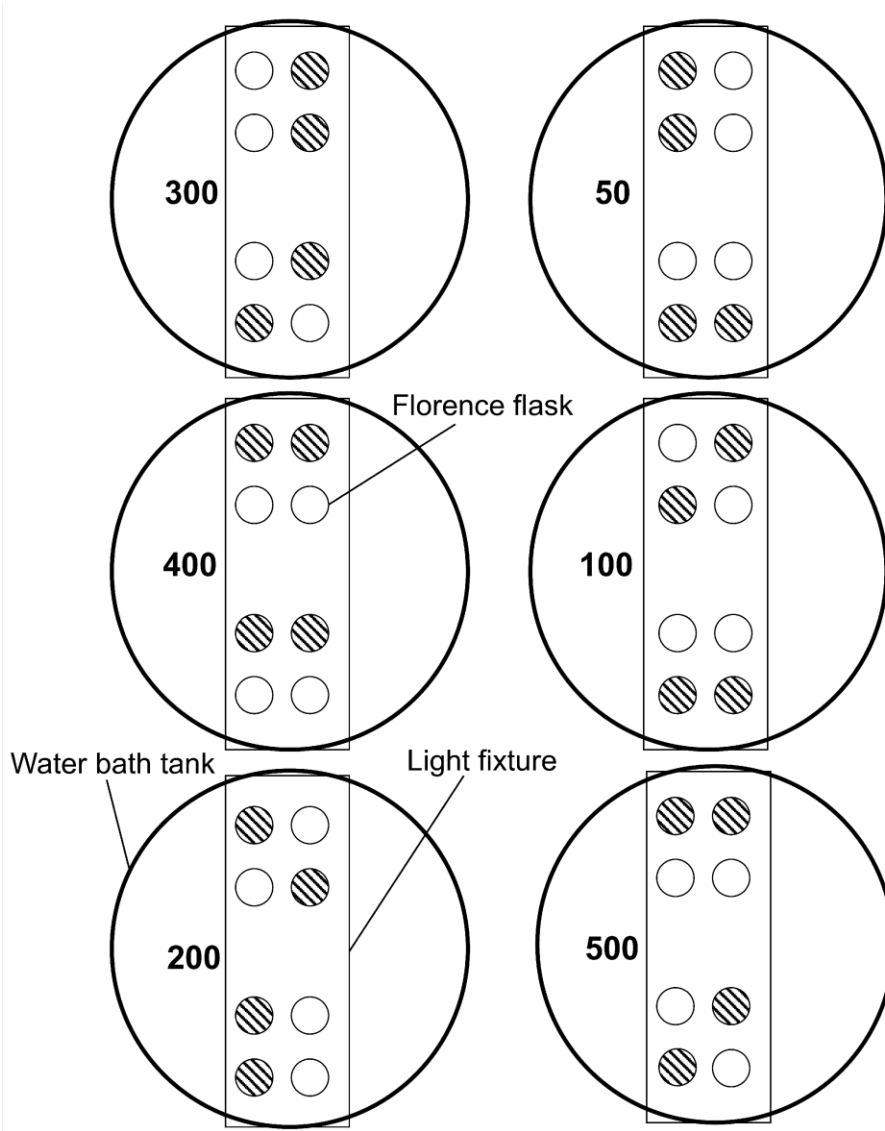
Table D.5. Experiment I. Irradiance conditions for photosynthesis rate measurement. Photosynthetic Photon Flux Fluence Rate (PPFFR) recorded every one minute with a spherical quantum sensor (LI-193SA). Values indicates mean \pm SE; means sharing the same letter are not significantly different (Tukey's, $p>0.05$).

<i>Species</i>	<i>Chondrus crispus</i>			<i>Palmaria palmata</i>		
<i>Light treatment, %</i>	<i>100</i>	<i>50</i>	<i>20</i>	<i>100</i>	<i>50</i>	<i>20</i>
Date	Jun. 28	Jun. 28	Jun. 29	Jun. 28	Jun. 28	Jun. 29
Initial time, hh:mm	11:48	15:48	10:51	12:05	16:06	11:08
Final time, hh:mm	13:14	18:24	14:21	13:32	18:05	14:08
PPFFR, $\mu\text{mol m}^{-2} \text{s}^{-1}$	699 ± 8^a	153 ± 4^b	123 ± 2^c	717 ± 9^a	149 ± 4^b	126 ± 3^c

Table D.6. Experiment II. Irradiance conditions for photosynthesis rate measurement. Photosynthetic Photon Flux Fluence Rate (PPFFR) recorded every one minute with a spherical quantum sensor (LI-193SA). Values indicate mean \pm SE; means sharing the same letter are not significantly different (Tukey's test, $p>0.05$).

<i>Species</i>	<i>Chondrus crispus</i>			<i>Palmaria palmata</i>		
<i>Light treatment, %</i>	<i>100</i>	<i>50</i>	<i>20</i>	<i>100</i>	<i>50</i>	<i>20</i>
Date	Aug. 18	Aug. 17	Aug. 17	Aug. 18	Aug. 17	Aug. 18
Initial time, hh:mm	9:40	12:39	17:37	12:34	15:11	16:23
Final time, hh:mm	11:24	14:31	20:14	14:11	16:54	18:41
PPFFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$	817 ± 45^b	575 ± 31^c	41 ± 3^e	1435 ± 71^a	369 ± 6^d	85 ± 4^e

APPENDIX E Experimental design experiment with artificial light (Chapter 4)



Distribution of Florence flasks in artificial light experiment with *Chondrus crispus* at two stocking densities. Clear circles correspond to 2 g L^{-1} . Patterned circles correspond to 10 g L^{-1} . Flasks were randomized distributed in six circle fibreglass tanks (water bath tank) under six irradiance levels (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$)

APPENDIX F Best initial nutrient concentration determination assay (Chapter 4)

Four nutrient levels were tested 500, 1300, 2100 and 2900 $\mu\text{M NH}_4^+$. Selection of these levels was based on the original nitrogen level of the VS medium (von Stosch 1964; 500 $\mu\text{M NO}_3^-$) and the modification proposed by Guiry and Cunningham (1984, 2500 $\mu\text{M NO}_3^-$). Orthophosphate and other compounds were maintained at the same molar proportion to nitrogen indicated by von Stosch (1964).

The time of nutrient uptake was determined using the multiple flasks method (Pedersen 1994). Fourteen one-litre Florence flasks were filled with each concentration level, four levels in duplicate. One pair of flasks of each level was sampled at time zero (March 29, 2012 at 7 pm) and the other six pairs were random set up in six circular light-isolated tanks. Two flasks of each nutrient level were removed every 10 h (only during illuminated periods) and a water sample was taken. This continued until completed six samples. Samples were frozen at $-20\text{ }^\circ\text{C}$ for further analysis.

The one-litre Florence flasks were stocked with 10 g^{-1} FW of 3.5 cm fronds of *C. crispus*. The light-isolated tanks were maintained at a Photosynthetic Photon Flux Fluence of $500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, a photoperiod of 16:8 L:D and temperature of $10\text{ }^\circ\text{C}$. The aeration distribution system was adapted to mix CO_2 with air before injection into the flasks. The CO_2 from the cylinder was controlled manually, opened one hour after lights were turned on and closed one hour before lights were turned off. The CO_2 required by the fronds was determined indirectly by the pH of the culture media, the latter was checked twice a day (Electrode 13-620-AP50 and Accumet AP61, Fisher Scientific, Singapore). If the pH in some flask exceeded 8.5, CO_2 flux was incremented in the flask.

Fronds of *C. crispus* at a stocking density of 10 g L⁻¹ FW, exposed to 500 μmol m⁻² s⁻¹ for 16 h per day at 10 °C and a mean pH of 7.8±0.06 (range 6.4 to 8.6) showed a similar ammonium consumption trend at the four nutrient levels (500, 1300, 2300 and 2900 μM NH₄⁺). Consumption was high in the first 40 h and then tended to stabilize (Fig. F.1). The slope of the decay phase increased with the increasing of the nutrient levels up to 2300 μM NH₄⁺. At the highest nutrient level (2900 μM NH₄⁺) the slope decreased again (Table F.1). The results confirmed that at 500 μM NH₄⁺, all the ammonium were quickly consumed by the macroalgae. An ammonium level of 1300 μM NH₄⁺ was selected to conduct the experiment because, according with the linear regression, it takes between 4.4 to 5.3 days to consume all the ammonium. At low stocking density the ammonium uptake is lower than at high stocking density; consequently, at a highest concentration of ammonium, the ammonium consumption could be too low to be determined analytically. In addition, high levels of ammonia could be toxic to algae; it has been reported that the presence of ammonium encourages green spot and green rotting diseases in *C. crispus* (Craigie and Correa 1996). Concordant with that, after 50 h in culture, fronds started to present white tips (bleaching) at all the nutrient levels, but the problem was greatest at 1300 μM NH₄⁺ or higher. The proportion of fronds with white tips increased with the time. At the end of the assay, the fronds were pooled to conduct the next experiment but after two days, most of the fronds the white damage were turned green because a secondary phyto-infection, which affected 60 % of the biomass.

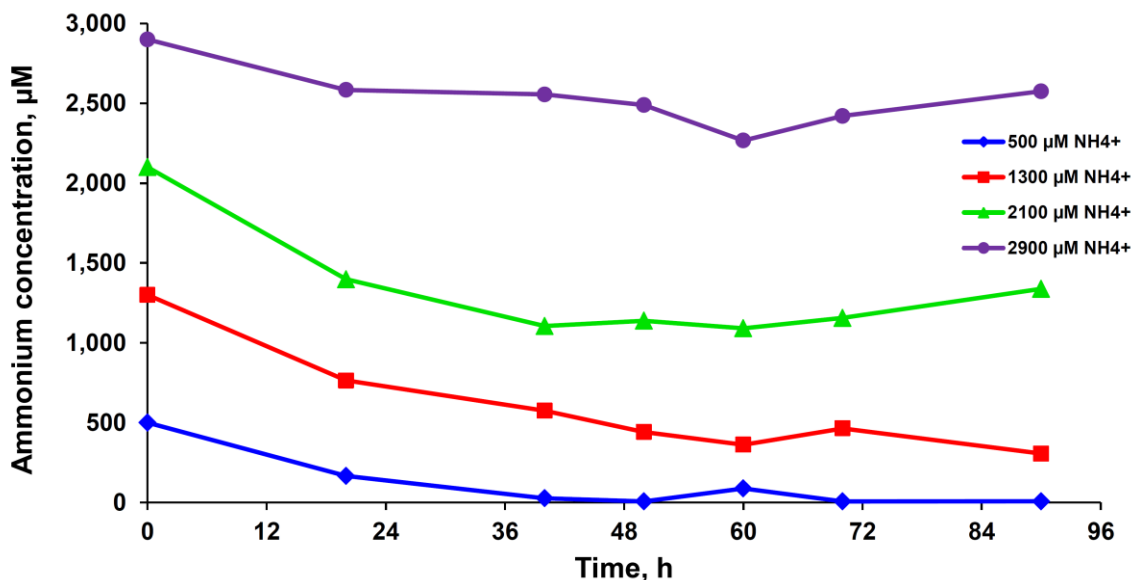


Figure F.1. Ammonium uptake by *Chondrus crispus* cultured at 10 g L⁻¹ under 500 µmol m⁻² s⁻¹ and 10 °C at four initial nutrient concentrations.

Table F.1. Estimate of the time (days) which *Chondrus crispus* cultured at 10 g L⁻¹ under a Photosynthetic Photon Flux Fluence Rate of 500 µmol m⁻² s⁻¹ and 10 °C took up all the ammonium of four culture media with different initial nutrient concentrations. Regressions correspond to curves in Fig.F.1.

Ammonium, µM	Slope	Intercept, µM NH ₄ ⁺	Time to total ammonium uptake, d	
			intercept	initial concentration
500	-4.80	341	3.0	4.3
1300	-10.19	1082	4.4	5.3
2300	-7.90	1705	9.0	12.1
2900	-4.18	2739	27.3	28.9

**APPENDIX G Culture conditions during determination of
saturation curves (Chapter 4)**

Table G.1. Photosynthetic Photon Flux Fluence Rate (PPFFR) measured with a spherical quantum sensor (LI 193). Daily Photon Dose (DPD) calculated by a photoperiod of 16:8 (light:dark). Mean \pm SE. Means which share the same letter are not significantly different (Tukey's test, $p>0.05$)

<i>Nominal</i>	<i>PPFFR, $\mu\text{mol m}^{-2} \text{s}^{-1}$</i>	<i>DPD, $\text{mol m}^{-2} \text{d}^{-1}$</i>
50	51 ± 0.07^f	3.0 ± 0.00
100	104 ± 0.13^e	6.0 ± 0.01
200	200 ± 0.61^d	11.5 ± 0.03
300	325 ± 0.21^c	18.7 ± 0.01
400	384 ± 2.26^b	22.1 ± 0.13
500	480 ± 1.17^a	27.6 ± 0.07

Table G.2. Temperature and pH of the culture medium inside the one-litre Florence flasks at each exchange water. Mean \pm SE. Means which share the same letter are not significantly different (Tukey's test, $p>0.05$)

<i>Nominal irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$</i>	<i>Stocking density, g L^{-1}</i>			
	<i>2</i>	<i>10</i>	<i>2</i>	<i>10</i>
	<i>Temperature, $^{\circ}\text{C}$</i>		<i>pH</i>	
50	10.1 ± 0.02^e	10.1 ± 0.01^e	$7.9 \pm 0.03^{\text{ABC}}$	$7.9 \pm 0.03^{\text{ABC}}$
100	$10.2 \pm 0.02^{\text{de}}$	$10.2 \pm 0.04^{\text{de}}$	$7.6 \pm 0.10^{\text{BCD}}$	$7.8 \pm 0.11^{\text{BCD}}$
200	$10.2 \pm 0.03^{\text{cd}}$	$10.2 \pm 0.03^{\text{cd}}$	$8.1 \pm 0.03^{\text{ABC}}$	$7.2 \pm 0.26^{\text{D}}$
300	$10.3 \pm 0.04^{\text{bc}}$	$10.4 \pm 0.04^{\text{b}}$	$8.2 \pm 0.02^{\text{AB}}$	$7.6 \pm 0.27^{\text{BCD}}$
400	$10.6 \pm 0.03^{\text{a}}$	$10.6 \pm 0.02^{\text{a}}$	$8.1 \pm 0.02^{\text{ABC}}$	$7.5 \pm 0.24^{\text{CD}}$
500	$10.7 \pm 0.02^{\text{a}}$	$10.7 \pm 0.03^{\text{a}}$	$8.0 \pm 0.05^{\text{ABC}}$	$8.6 \pm 0.13^{\text{A}}$

**APPENDIX H Significance of experimental factors during the
determination of saturation curves (Chapter 4)**

Table H.1. Significance of experimental factors alone and in combination. N uptake = Ammonium uptake, μM . P uptake = Orthophosphate uptake, μM . SGR = Specific Growth Rate, $\% \text{ d}^{-1}$. Values highlighted indicate no significant at $\alpha = 0.05$.

<i>Factor</i>	<i>N uptake</i>	<i>P uptake</i>	<i>SGR</i>
Irradiance (50 to 500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$)	<0.001	<0.001	<0.001
Stocking density (2 and 10 g L^{-1})	<0.001	<0.001	<0.001
Sampling period (1, 2, 3 and 4)	<0.001	<0.001	
Sampling period (1, 2 and 3)			<0.001
Irradiance x density	<0.001	<0.001	<0.001
Irradiance x sampling period	<0.001	<0.001	<0.001
Density x sampling period	<0.001	<0.001	<0.001
Irradiance x density x sampling period	<0.001	<0.001	0.0004

Table H.2. Significance of experimental factors alone and in combination for photosynthetic parameters. Values highlighted indicate no significant at $\alpha = 0.05$.

<i>Factor</i>	<i>Net photosynthetic rate</i>	<i>Dark respiration rate</i>
Irradiance (50 to 500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$)	<0.0001	<0.0001
Stocking density (2 and 10 g L^{-1})	<0.0001	0.3334
Irradiance x density	0.0626	0.1579

Table H.3. Significance of experimental factors alone and in combination for tissue analysis. % C = Tissue carbon content, %. % N = Tissue nitrogen content, %. C/N ratio = Tissue molar carbon to nitrogen ratio. Values highlighted indicate no significant at $\alpha = 0.05$.

<i>Factor</i>	<i>%C</i>	<i>%N</i>	<i>C/N ratio</i>
Irradiance (50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.0008	0.0072	0.0028
Stocking density (2 and 10 g L^{-1})	0.0130	0.0321	<0.0001
Irradiance x density	0.0003	0.0063	0.0057

Table H.4. Significance of experimental factors alone and in combination for pigment tissue analysis PE = phycoerythrin content. PC = phycocyanin. PE+PC = phycobiliproteins (addition PE and PC). Chl *a* = chlorophyll *a* content. Values highlighted indicate no significant at $\alpha = 0.05$.

<i>Factor</i>	<i>PE</i>	<i>PC</i>	<i>PE+PC</i>	<i>Chl a</i>
Irradiance (50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	<0.0001	<0.0001	<0.0001	<0.0001
Stocking density (2 and 10 g L^{-1})	0.0250	0.4182	0.0402	0.0012
Irradiance x density	0.2481	0.3856	0.2493	0.0069

APPENDIX I Changes in algal colour and size during the determination of saturation curves (Chapter 4)

Chondrus crispus cultured at six irradiances (Photosynthetic Photon Flux Fluence Rate of 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) during 26 days at 10 °C with a photoperiod of 16:8

L:D. Photograph scale 1:1.

Irradiance, PPF_{FR} 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$
Stocking density 2 g L⁻¹

April 22, 2012



May 17, 2012



Stocking density 10 g L⁻¹

April 22, 2012



May 17, 2012



Irradiance, PFFR $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$
Stocking density 2 g L^{-1}

April 22, 2012



May 17, 2012



Stocking density 10 g L^{-1}

April 22, 2012



May 17, 2012



Irradiance, PPFFR 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$
Stocking density 2 g L⁻¹

April 22, 2012



May 17, 2012



Stocking density 10 g L⁻¹

April 22, 2012



May 17, 2012



Irradiance, PPFFR $300 \mu\text{mol photon m}^{-2} \text{s}^{-1}$
Stocking density 2 g L^{-1}

April 22, 2012



May 17, 2012



Stocking density 10 g L^{-1}

April 22, 2012



May 17, 2012



Irradiance, PFFR $400 \mu\text{mol photon m}^{-2} \text{s}^{-1}$
Stocking density 2 g L^{-1}

April 22, 2012



May 17, 2012

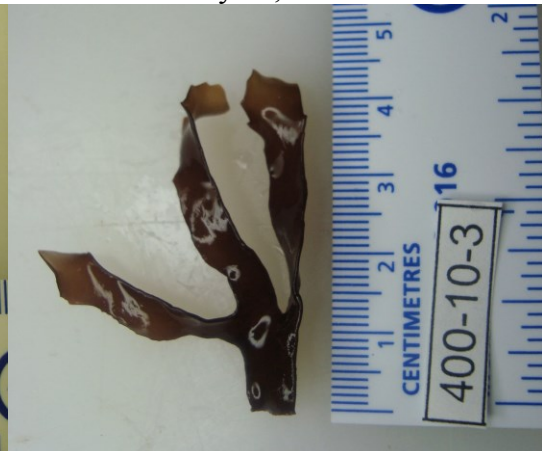


Stocking density 10 g L^{-1}

April 22, 2012



May 17, 2012



Irradiance, PFFR 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$
Stocking density 2 g L⁻¹

April 22, 2012



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Stocking density 10 g L⁻¹

April 22, 2012



May 17, 2012

