The development of a modular integrated recirculating aquaculture system using Porphyra (nori) for the bioremediation of marine finfish effluent

Jennifer Pauline Day

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The development of a modular integrated recirculating aquaculture system using Porphyra (nori) for the bioremediation of marine finfish effluent

Abstract
It is crucial for the development of the fish aquaculture industry to be managed in a way that provides a reliable, long term source of products without negatively impacting the environment. The simplest form of integrated multi-trophic aquaculture (IMTA) uses a fed component (e.g. finfish) and an extractive component (e.g. seaweed) to remove the inorganic metabolites from finfish aquaculture effluent. In IMTA systems metabolic wastes become nutrients for the other cultured organisms and are incorporated into potentially valuable biomass. A demonstration-scale Modular Integrated Recirculating Aquaculture Systems (MIRAS) was constructed in a greenhouse adjacent to Great Bay Aquaculture, LLC (GBA), Newington, NH. The MIRAS consists of two independent demonstration scale modular systems each with four 4m³, 3600 L, white fiberglass tanks.

Ammonium production rates were determined for Atlantic cod (Gadus morhua L.) and nutrient uptake rates for Porphyra umbilicalis Kutzing and P. linearis Greville. The ammonium kinetic characteristics of the fish and seaweed were used to develop a model to predict the system ammonium dynamics. Integrated fish/seaweed trials were run in the MIRAS to test the predictive model using varied fish feed rates and seaweed biomass. The trials showed that the model may be used to predict the effect of various parameters on the system's nutrient levels. Thus, an aquaculture operation may use the model to maintain desired system nutrient levels that will meet the needs of both the finfish and Porphyra and meet production goals. Porphyra produced in the MIRAS was used to partially replace fish meal as a source of protein and omega-3 fatty acids in the cod fish diets. The net effect is to convert a greater portion of the system protein input into fish biomass and to discharge less nitrogenous waste.

Keywords
Agriculture, Fisheries and Aquaculture, Biology, Ecology

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THE DEVELOPMENT OF A MODULAR INTEGRATED RECIRCULATING
AQUACULTURE SYSTEM USING *PORPHYRA* (NORI) FOR THE
BIOREMEDIATION OF MARINE FINFISH EFFLUENT

BY

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DISSERTATION

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TABLE OF CONTENTS

CHAPTER I. INTEGRATED MULTI-TROPHIC AQUACULTURE ....................... 1

Sustainability in Fisheries and Aquaculture........................................ 1
Sustainable Aquaculture ......................................................................... 2
Integrated Multi-Trophic Aquaculture .................................................. 5
Multi-Trophic Integrated Recirculating Aquaculture ................................. 10

CHAPTER II. DESCRIPTION OF THE MODULAR RECIRCULATING
INTEGRATED AQUACULTURE SYSTEM (MIRAS) .................................. 14

INTRODUCTION .................................................................................. 14
MIRAS DESCRIPTION ........................................................................ 16

CHAPTER III. AMMONIUM DYNAMICS IN A MODULAR INTEGRATED
RECIRCULATING AQUACULTURE SYSTEM ........................................ 24

INTRODUCTION .................................................................................. 24
The Importance of Developing a Sustainable Aquaculture Industry ........ 24
Factors Influencing Ammonium Excretion of Marine Teloests ............... 27
The Fate of Excreted Nitrogenous Wastes ........................................... 29
Objectives .......................................................................................... 32
METHODS ......................................................................................... 32
Ammonium analysis ............................................................................. 33
Seaweed .............................................................................................. 33
Seaweed Ammonium Uptake ......................................................... 34
Fish .......................................................................................... 35
Fish Growth, Feed Conversion, Ammonium Production .......... 35
Black Seabass (Centropristis striata) ........................................... 36
Atlantic Cod (Gadus morhua) ...................................................... 37
Statistics ................................................................................... 38
RESULTS .................................................................................... 39
Black seabass: Ammonium Production, Growth and Feed Conversion .... 39
Atlantic cod: Ammonium Production ......................................... 39
Ammonium uptake ..................................................................... 40
DISCUSSION ............................................................................. 40

CHAPTER IV. AMMONIUM DYNAMIC MODEL FOR THE OPTIMIZATION OF A MODULAR INTEGRATED AQUACULTURE SYSTEM (MIRAS) .... 50
INTRODUCTION ............................................................................ 50
Objectives .................................................................................. 54
METHODS .................................................................................. 56
System Description ..................................................................... 56
Model Development ................................................................. 57
Model I: Ammonium concentration versus time ....................... 58
Model II: Ammonium concentration of the MIRAS at Equilibrium ... 60
Model Validation: ................................................................. 61
Ammonium recovered ............................................................. 63
Ammonium analysis ................................................................. 64
LIST OF FIGURES

Figure 2.1: Recirculating mode: Diagram describes one modular system where the chiller is included in the recirculation loop. Minimal water replacement is indicated to account for evaporation and other losses............................................. 20

Figure 2.2: Flow-through mode: diagram describes one modular system set to a high water replacement rate. The outflow lines that feed directly into the outgoing sump may be used to rapidly drain tanks. The chiller and cartridge filters are not used when the system is in flow-through mode. ...................... 21

Figure 2.3: Static mode: Diagram describes one modular system with no water replacement. The chiller and filters are not used when the system is run in static mode................................................................. 22

Figure 2.4: Aeration system: Diagram is simplified to include one fish tank and one seaweed tank. Dashed supply pipe lines indicate that the pipes continue to the remaining tanks of the modular system........................................... 23

Figure 3.1: Black seabass: feed conversion ratio (FCR) (A) and ammonium excretion expressed as % protein fed (B) for fish provided feed at rates equaling 3, 4, and 5% of their body weight per day. Error bars represent standard error. Means labeled with the same letter are not significantly different (α=0.05). .................................................................................. 45

Figure 3.2: Michaelis-Menten type relationship ammonium uptake rate of Porphyra umbilicalis and ammonium concentration. R² = 0.956. Error bars are ±SE. Maximum rate of ammonium uptake (V_max) = 12.72 μmol gFW⁻¹ hr⁻¹ and half saturation constant (K_s) = 61.33 μM...................................................... 48

Figure 3.3: Michaelis-Menten type relationship ammonium uptake rate of Porphyra linearis and ammonium concentration. R² = 0.932, Error bars are ±SE. Maximum rate of ammonium uptake (V_max) = 9.68 μmol gFW⁻¹ hr⁻¹ and half saturation constant (K_s) = 8.52 μM............................................................. 49

Figure 4.1: Predicted ammonium concentration (μM) in the MIRAS versus time (hours) when the system is recirculating with no water exchange. Curves represent ammonium predicted for the system with 10, 7.5 and 5 Kg cod (Gadus morhua) in the MIRAS fed at a rate of 1% fish biomass per day. .... 78

Figure 4.2: Predicted ammonium concentration (μM) in the MIRAS versus time (hours) when the system is recirculating with a low (30 L h⁻¹) water exchange rate. Curves represent ammonium predicted for the system with 10, 7.5 and 5 Kg cod (Gadus morhua) in the MIRAS fed at a rate of 1% fish biomass per day........................................................................................................... 79
Figure 4.3: Predicted ammonium concentration (µM) in the MIRAS versus time (hours). Curves represent ammonium predicted for the system with seaweed to fish feed ratios of 15, 20 and 30 (g FW seaweed: g feed d⁻¹). For the demonstration scale MIRAS the seaweed biomass:feed ratios represent when the cod are fed 50, 75 or 100 g feed d⁻¹ and the biofilter has 1.5 Kg of Porphyra umbilicalis.

Figure 4.4: Predicted ammonium concentration (µM) in the MIRAS versus time (days). Curves represent predicted ammonium concentration for the fish tank and seaweed tanks when the flow rate into the tanks is set at 2520 L h⁻¹ (A) or 360 L h⁻¹ (B). The ammonium dynamic parameters used in the model are for cod and P. umbilicalis in the MIRAS.

Figure 4.5: Ammonium concentration at system equilibrium versus seaweed biomass to daily cod feed ratio. For the demonstration scale MIRAS the seaweed to feed ratios represent increasing seaweed biomass while the fish are fed 100g feed per day. The curves represent predicted ammonium levels when Porphyra umbilicalis and P. linearis are used as the biofilter and cod are used as the N source.

Figure 4.6: Ammonium concentration at system equilibrium versus seaweed biomass for cod fish feed rates of 50, 100, 200, 300 and 400 grams per day. The curves represent predicted ammonium levels when Porphyra umbilicalis is used as the biofilter.

Figure 4.7: Ammonium concentrations from integrated trials using Porphyra umbilicalis and P. linearis compared to ammonium levels predicted by the predictive model. Results are presented based on seaweed biomass to cod fish feed ratio.

Figure 4.8: Porphyra umbilicalis that was produced in the MIRAS spread out on a drying rack in the greenhouse (A) and a close up of the seaweed (B).
LIST OF TABLES

Table 3.1: Feed excreted as NH$_4$ (% feed) and ammonium production rates (g NH$_4$ hr$^{-1}$) for Atlantic cod (Gadus morhua) in the MIRAS............................... 46

Table 3.2: Ammonium uptake rates (µmol g$^{-1}$ hr$^{-1}$) of Porphyra umbilicalis and P. linearis at six ammonium levels. *data excluded, complete cloud cover for the entire day................................................................. 47

Table 4.1: Results of Integrated trials listed by Porphyra species and the date associated with the beginning of the system equilibrium period................ 86
ABSTRACT

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CHAPTER I. INTEGRATED MULTI-TROPHIC AQUACULTURE

Sustainability in Fisheries and Aquaculture

Using fisheries beyond their capacity will eventually result in reduced supply and perhaps irreversible damage to fish populations (Dey et al. 2006, FAO 2006, 20076, Troell et al. 2003). When the demand for fish products exceeds what wild fisheries can provide, it must either be met through other means, such as farming, or ultimately go unmet. Many people in Asia and Africa depend on fish for protein, yet per capita intake is decreasing due to depleted freshwater fisheries (Dey et al. 2006, Edwards 1999, Edwards et al. 1997, FAO 1999, 2006). Marine fisheries are also being exploited at their maximum sustainable yield (FAO 2007). As these natural resources reach their limit, the rapidly growing aquaculture industry is serving to meet the increased worldwide demand for fish products. However, current monoculture methods may not be sustainable (Neori et al. 2007).

It is vital to manage fisheries and aquaculture development in a manner that protects marine ecosystems (FAO 2006). Indeed, the security of the global food supply may well hinge on the development of sustainable fisheries and aquaculture management. The goals of aquaculture include a reduction of human impact on wild populations of fish and the provision of a steady supply of fish products (FAO 2006, Salin and Mohanakumaran 2006). Concurrent with the rapid growth of aquaculture, there is increasing awareness that the development

*Sustainable Aquaculture*

Modern agriculture generally practice monoculture, producing only one product, which may lead to increased disease or pest infestation, poor soil, and increased nutrient loading of local waters (FAO 1999). In Bangladesh, the production of carp lead to the exclusion of many local fish species (Gal et al 2007). In some cases reduced product diversity can threaten the nutritional security of local people, which rely on highly nutritious indigenous fish to provide micronutrients. Polyculture, where multiple organisms are grown together, has been successfully used for a long time in many parts of the world. The co-culture of rice and fish, livestock and fish, and mulberry, fish and silkworms have been practiced since the first century B.C. in China (Beveridge et al. 2002, FAO 2001). Historically, polyculture has been used by small scale farmers to diversify production and increase efficiency of effort and capital (Beveridge et al. 2002). Efforts are now underway to encourage small farmers to reintroduce several species of native fish in Bangladesh (Gal et al. 2007).

The concept of polyculture may well provide a way to mitigate the negative effects of nutrient loading from intensive aquaculture. Research institutions and aquaculture industries are actively investigating methods to increase economic
and environmental sustainability (Buschmann et al. 2008, Chopin et al. 2008, Neori et al. 2004, Ridler et al. 2007, Ruyter et al. 2006, Sindilariu 2007, Stickney and McVey 2002, van Rijn and Barak 2002, Wang et al. 2006). Environmental pollution and eutrophication of marine water impacts all sectors of fish production. Other sustainability concerns include depletion of wild stocks, bioaccumulation of heavy metals, and increased levels of harmful algal blooms (HABs). Integrated Aquaculture is a form of polyculture where organisms from more than one trophic level are grown together. The main advantage of integrated aquaculture is that the byproducts of one species are used to enhance the productivity of another (Edwards 1998). Such systems essentially recycle nutrients from the higher trophic level (e.g. fish) to the lower trophic level (e.g. seaweed) rather than releasing these nutrients into the environment (Buschmann et al. 2008, Chopin et al. 2008).

Another challenge to sustainability is that the success of an aquaculture operation depends on the supply of protein from fish meal. Aquaculturists seek to maximize fish growth and produce fish that has high nutritional quality and the composition of fish feed (especially its protein and fatty acid (FA) profiles) is one of the most important factors affecting aquaculture productivity (Jobling 1996). The production of high value omnivorous and carnivorous fishes relies on fish meal and oil, primarily from anchovies and sardines, to supply protein and omega-3 fatty acids (Bolton 2006, Wang et al. 2006). However, the inclusion of fish meal is not economically or environmentally sustainable as it is expensive and limited by supplies from fisheries (Naylor and Chiu 2006, Naylor 2005). The
supply of fish meal depends on anchovy and sardine fisheries, and since these fisheries are currently harvested at their maximum sustainable level, many studies have focused on finding alternate protein and fatty acid sources for finfish diets (Cho and Bureau 1997, Chopin et al 2001, FAO 2006, Folke and Kautsky 1992, 1989, Lin and Yi 2003, Tacon and Forster 2003). Some of these studies seek to match fish feed formulations and fish nutritional requirements to reduce wastes and thereby reduce the amount of fish meal and oil used in feed. Other studies seek to reduce fish meal use by supplementation with alternative sources of protein and n-3 FAs, such as terrestrial plants, microalgae, or seaweed (Furuya et al 2004, Ruyter et al. 2006, Wang et al. 2006).

Attention has also focused on discharged effluent from fish aquaculture that contains metabolic wastes and dissolved nutrients including carbon dioxide (CO$_2$), ammonium (NH$_4^+$), nitrate (NO$_3^-$), nitrite (NO$_2^-$), and phosphate (PO$_4^{3-}$) (Hargrave et al. 1993, Ip et al. 2001, Lobban and Harrison 1994). Intensive aquaculture can alter the water quality of the surrounding area as nutrients from fish metabolism increase the occurrence of red tides and contribute to the growth of weeds and blooms of other nuisance and/or harmful algae (Cuomo et al. 1993, 1995, Muir 1998, Yardley 2008). Thus, metabolites in aquaculture effluent can contribute to the pollution of coastal waters. As ecologically concerned nations impose more stringent regulations regarding the limits on nutrient discharges, fish production will become limited.

Marine fish are often grown out to market size in coastal net-pens where metabolites such as ammonium are diluted by currents (Ashe et al. 1996, Chopin
and Yarish 1998, 1999, Foss et al. 2004, Meade 1985). Land-based recirculating aquaculture systems (RAS) are often used as hatcheries to supply fish for grow out, but may also be used to raise fish to market size (Foss et al. 2004, Malone et al. 2000, Montagne 2006). Recirculating aquaculture systems reduce the quantity of land and water used, and allow for a controlled environment. The low volume of water coming into RASs results in low volume of water that has high concentrations of metabolites (Mozes 2003, Van Gorder and Jug-Dujakovic 2005). Conventional biofilters used in RAS convert NH$_4^+$ to nitrate but do not remove nitrogen from the system. Nitrate can build up to higher concentrations than ammonium without harming the fish; however, it eventually leaves the system as water is replaced and enters the surrounding ecosystem (Chopin et al. 2001b). While coastal net-pen operations rely on the ecosystem to absorb nutrient concentrations and keep the fish healthy, these farms have large footprints and the input of dissolved and particulate wastes contribute to the nutrient enrichment of the surrounding marine environment (Bッシュmann et al. 2001, Chopin and Yarish 1999, Costa-Pierce 1996). Furthermore, ammonium is also a source of nitrogen and its presence in aquaculture effluent may lead to the negative environmental effects described above.

*Integrated Multi-Trophic Aquaculture*

Sustainable aquaculture fish production requires that freshwater and coastal ecosystems are conserved since aquaculture relies on the health of these ecosystems. One way to increase sustainability is to increase recycling of
resources such as water and nutrients through integrated multitrophic aquaculture (IMTA) which uses organisms from different trophic levels to increase the productivity of each organism (Chopin et al. 2001a, Neori et al. 2004, 2007). Integrating the culture of multiple organisms effectively reduces dependency and impact on the ecosystems, thereby increasing the long term sustainability of the system. The simplest form of IMTA combines the culture of an animal (e.g. finfish) and a plant (e.g. seaweed) such that the plant takes up and utilizes the animal metabolites for increased growth and biosynthesis (Buschmann et al. 2008, Chopin et al. 2008, 2001a, Mathieson 1981, Neori et al. 2004). In land based seaweed/fish integrated aquaculture, the seaweed can utilize the CO₂, NH₄, NO₂/NO₃, and PO₄ produced by the fish. Additionally, integrated aquaculture may be beneficial due to product diversification and may increase profits, especially if farms are required to compensate for environmental nutrient loading (Buschmann et al. 2001, Chopin et al. 2001a, Dey et al. 2006, FAO 2006, Neori et al. 2004).

Small-scale integrated fish farming is being promoted in Africa, India, Malaysia, Bangladesh, Indonesia, and Viet Nam as a way of improving nutrition and increasing the income of local people (Bolton 2006, Dey et al. 2006, FAO 2006). Abalone farming in South Africa developed in response to declining abalone populations. Abalone farms in this region are usually composed of gravity fed flow-through tank systems, although some are recirculating systems. Abalone feed primarily on kelps [*Ecklonia maxima* (Osbeck) Papenfuss]. Kelp beds in proximity to the abalone farms are being harvested close to the
maximum sustainable levels. Kelp is now grown successfully on the abalone effluent at two farms that have no access to wild harvested kelp. The farmed kelp is utilized as the primary abalone feed (Bolton 2006, Troell et al. 2006) in place of wild harvested kelp. The seaweed and abalone industries are economically important to South Africa as they provide export income and employ poor people in coastal areas (Dey et al. 2006). Countries including Israel, Chile, Portugal, Canada and the United States of America are seeking to develop large-scale integrated aquaculture to increase food security and the environmental, social and economic sustainability of the aquaculture industry (FAO 2006).

Marine capture fisheries and aquaculture provide Chile with one of its most important sources of income (Fernandez and Castilla 2005). Chile has a substantial salmon aquaculture industry and growing abalone and seaweed industries (SERNAPESCA 2003, Westermeier et al. 2006). Mass cultivation techniques of the carrageenophytes *Sarcothalia crispata* (Bory de Saint-Vincent) Leister and *Chondracanthus chamissoi* (C. Agardh) Kützing, as well as the agarophyte *Gracilaria chilensis* C.J. Bird, McLachlan & E.C. Oliveira, are being developed to ease the pressure on wild populations and meet the increasing demand for carrageenan and agar (Bulboa et al. 2005, Buschmann 2000, Buschmann et al. 2008, Romo et al. 2001). *Lessonia* and *Macrocystis* mariculture techniques are being investigated to provide a reliable food source for abalone and for export as human food (Buschmann et al. 2008, Edding and Tala 2003, Westermeier et al. 2006).
A number of studies have investigated seaweed and bivalves as biofilters for fish aquaculture. The red alga *Gracilaria* has been successfully used in Chile, China, and Portugal. Buschmann et al. (2000) grew oysters and *Gracilaria* near salmon farms in Chile and found that ammonium levels were significantly reduced. *Gracilaria* grown near fish farms in China grew rapidly and sequestered nutrients produced by fish (Zhou et al. 2006). In Portugal, Matos et al. (2006) demonstrated that combinations of red seaweed (*Chondrus crispus* (Stackhouse), *Gracilaria compressa* (C. Agardh) Greville and *Palmaria palmata* (Linnaeus) Kuntze) efficiently removed nitrogen from fish effluent and increased oxygen in the discharge water. 

Seaweed farms in close proximity to fish aquaculture sites also have increased productivity which makes seaweed an ideal partner for multi-trophic marine aquaculture (Buschmann et al. 2008, Chopin et al. 2008). *Gracilaria* and *Ulva* both grow better on abalone or fish effluent compared to natural seawater (Friedlander and Levy 1995, Hernandez et al. 2006, Njobeni 2006, Robertson-Andersson 2003). Moreover, multi-trophic aquaculture has been shown to produce seaweed that is higher in protein than monocultured seaweed (Brzeski and Newkirk 1997, Chopin et al. 1999, Folke and Kautsky 1992, Lüning and Pang 2003, Troell et al. 2003). Highly nutritious *Ulva*, *Gracilaria* and *Ecklonia* grown in integrated farms may be used in abalone feed to improve growth (Boarder and Shpigel 2001, Naidoo et al. 2006, Shpigel et al. 1999).

Marine finfish aquaculture is being promoted in the United States as an opportunity to compensate for the declining fish industry. However, in New
England crowded nearshore waters offer little opportunity for the development of aquaculture farms. The Open Ocean Aquaculture Demonstration Project (NOAA/UNH) avoids water access conflicts by growing organisms far from shore and below the surface (Langan and Horton 2005). Successful harvests of fish (cod and halibut) and mussels grown by the project demonstrated that these crops can be grown in the open ocean. Halibut (*Hippoglossus hippoglossus* L.) and cod (*Gadus morhua* L.) are grown in submerged cages (24 m) and mussels (*Mytilus edulis* L.) are grown on submerged long lines (12 m) adjacent to the fish cages. The fish are fed formulated feed, while the mussels filter out phytoplankton growing in the water column. Since the phytoplankton take up dissolved nutrients, and the mussels feed on the phytoplankton, total environmental nutrient loading is reduced (Langan and Horton 2005, www.ooa.unh.edu/publications).

A pilot scale project in New Brunswick, Canada is cultivating kelp (*Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl and G.W. Saunders and *Alaria esculenta* (L.) Greville) and mussels (*Mytilus edulis* L.) in close association to salmon (*Salmo salar* L.) pens (Chopin et al. 1999, 2008, Chopin and Yarish 1998, Ridler et al. 2007). The six year Canadian project demonstrated that the co-culture of fish, seaweed and mussels doubled the productivity of the seaweed and mussels due to the availability of nutrients with no accumulation of toxins (Chopin et al. 2008, Ridler et al. 2007). The inclusion of seaweed in the Canadian project increased environmental and economic sustainability by increasing nutrient remediation and product diversification. Another aspect of this
project was the partnership with industry (Cooke Aquaculture Inc in New Brunswick Canada, and Acadian Seaplants Limited of Dartmouth, Nova Scotia Canada) to study social and economic sustainability since these factors are critical to the long term success of an IMTA endeavor.

*Multi-Trophic Integrated Recirculating Aquaculture*

The concept of integrated aquaculture is based on an ecological approach where nutrient inputs and outputs are balanced. Let us, for simplicity, consider integrated farm with finfish and seaweed. While seaweed can be grown in proximity to open ocean or coastal fish farms, it is difficult to assess the degree to which nutrients are being removed. Since the fish effluent is diluted by currents and the fed and extractive components are separated spatially, only a small portion of nutrients may be taken up by the seaweed. Water treatment can be effectively controlled in semi-enclosed or recirculating systems as nutrient availability to the seaweed can be increased by adjustment of water flow rates and mixing (Buschmann et al. 2001, Neori et al. 2000, Schuenhoff et al. 2003). Currently recirculating systems are primarily used in hatcheries and are rarely used to grow fish to market size because of their high set up and operational costs. However, recirculating systems lend themselves to use in areas where the water supply or access to water ways is limited such as desert or urban areas.

Researchers in Israel have effectively developed a commercial-scale multi-trophic intergrated recirculating system (MIRAS) using seaweed to treat fish effluent and reduce the amount of replacement water required to maintain water
quality (Cohen and Neori 1991, Neori et al. 2003, 1996, Schuenhoff et al. 2003). The MIRAS uses the green macroalga *Ulva lactuca* L. to filter effluent from gilthead sea bream (*Sparus aurata* L.). High efficiency was achieved by balancing the fish and seaweed biomass such that the water quality was improved and could be recirculated back through the fish tanks. The seaweed removed the nitrogenous wastes, phosphorus and CO₂; stabilized the pH and dissolved oxygen; and decreased water usage. The system demonstrated high productivity for the sea bream and *Ulva*, scalability and economic feasibility that would improve if nutrient loading costs were internalized. Additionally, the *Ulva* produced was found to be a nutritious abalone/sea urchin feed (Neori et al. 2000, Schuenhoff et al. 2003).

Fish health and productivity depend on high water quality (Timmons et al. 2001), so it is essential to maintain a stable water quality by balancing the biofilter capacity and fish metabolism (Neori et al. 1996). While fish metabolites include CO₂ and PO₄, protein in fish feed is metabolized primarily as ammonia (NH₃) (Wood 2001). Ammonia concerns aquaculturists as high levels can increase fish stress and mortality (Ip et al. 2001, King and Berlinsky 2006, Tomasso 1994). In marine effluent with a pH of 8 and temperature of 25°C 95-96% of ammonia is present as ammonium (NH₄⁺) (Ip et al. 2001, Bower and Bidwell 1978). Thus, it is vital to use a seaweed biofilter with a high NH₄⁺ removal capacity, which is determined by the seaweed biomass and nutrient uptake rate. The research of Neori and others (1996, 2003, 2004) showed that when the ammonium concentration was close to half saturation for maximal uptake, the
seaweed biofilter buffered the system against spikes in ammonium. Efficiency of the *Ulva* biofilter was increased by using three successively smaller seaweed ponds in series, each stocked with an equal seaweed density; thus, the concentration of ammonium decreased as it flowed through each biofilter tank. Additionally, *Ulva* yield and protein content were dependent on ammonium supply (Neori et al. 2003). If the seaweed is considered a secondary product then it is important to maintain nutrient levels that support high productivity and nutritional value.

Seaweed mariculture techniques generally involve seeding ropes or nets that are placed in estuarine or coastal areas for grow out. The use of tank or pond culture is generally limited to the culture of spores, juvenile sporophytes, or gametophytes since capital investment for tank culture is high. However, the potential to control environmental factors and increase productivity is also high. Tank culture may also be an attractive method in areas where pollution interferes with coastal or open ocean cultivation. Currently tank culture of seaweed species is being investigated in Israel, Chile, the Canada and the United States of America (Bidwell et al. 1985, Buschmann et al. 2005, Capo et al. 1999, Carmona et al. 2006, Israel et al. 2006, Neori et al. 2004, Ryther et al. 1979, Yarish 2004).

Recently, *Porphyra* (nori, purple laver) was successfully grown via outdoor tank culture in Israel (Israel et al. 2006) and may be well suited for bioremediation. Numerous studies have investigated the suitability and performance of *Porphyra* species as a biofilter (Carmona et al. 2006, Chopin et al. 1999, Chung et al. 2002, Day 2003, Kim 2007, Kraemer et al. 2003, Kraemer
and Yarish 1999, Pereira et al. 2008, 2006, Yarish et al. 1998, 1999). Some of the most important characteristics of *Porphyra*, in terms of bioremediation, are its nutrient uptake capacity and economic importance. The thin (1-2 cells thick) sheet-like thallus of *Porphyra* lends itself to rapid nutrient uptake (Littler 1981). *Porphyra* species have been shown to have rapid, sustained uptake which is vital to an integrated aquaculture application (Carmona et al. 2006, Day 2003, Kim 2007, Pereira et al. 2006, 2008).

*Porphyra* has been cultured for hundreds of years in Japan, South Korea and China as a highly nutritious food and is one of the most economically valuable seaweeds (FAO 2006, Sahoo et al. 2002). Additionally, the protein pigments (phycobilins) from *Porphyra* are sought for use as a fluorescent marker in biotechnology and microbiology (Mumford and Miura 1988). Since *Porphyra* is rich in protein and omega-3 fatty acids, this seaweed is also being investigated as a protein source to partially replace fishmeal in fish diets (Walker et al. In press). The *Porphyra* biofilter essentially recaptures part of the nutrient input that would otherwise be lost to the system. The net effect is that the protein input of the system is used more efficiently. Developing an efficient biofilter is a critical component of a multi-trophic integrated recirculating aquaculture system. The secondary economic value or usefulness of the seaweed produced will increase the profitability, and therefore economic sustainability, of integrated aquaculture (Buschmann et al. 2001, Chopin et al. 2008, 2001, Neori et al. 2007).
CHAPTER II. DESCRIPTION OF THE MODULAR RECIRCULATING INTEGRATED AQUACULTURE SYSTEM (MIRAS)

INTRODUCTION

Integrated multi-trophic aquaculture (IMTA) systems utilize herbivorous fish, shellfish and/or plants to mitigate the negative environmental effects of finfish aquaculture effluent. The primary advantage of these integrated systems is that metabolic wastes of fish become nutrients for other cultured organisms and are incorporated into valuable biomass (Buschmann et al. 2008, Chopin et al. 2008, Chopin and Yarish 1999, Neori et al. 2004, 2007). Raising shellfish and/or seaweed in proximity to fish also counteracts nutrient loading of the surrounding environment with intensive finfish aquaculture.

Large-scale integrated aquaculture is being developed in Israel, Chile, Portugal, Canada and the United States of America (FAO 2006). Studies have shown that seaweed used downstream of fish effluent reduce the nutrient loadings of the effluent (Boarder and Shpigel 2001, Buschmann et al. 2000, 2008, Chopin et al. 2008, Cohen and Neori 1991, Matos 2006, Naidoo et al. 2006, Schuenhoff et al. 2003, Shpigel et al. 1999, Troell et al. 2006). The green seaweed Ulva has been successfully used in a recirculating aquaculture system (RAS) to treat sea bream (Sparus aurata L.) effluent, and clean water is pumped back to the fish tanks (Buschmann et al. 2001, Neori et al. 2000, Schuenhoff et al. 2003).
Great Bay Aquaculture, LLC, Portsmouth, NH, USA, is a hatchery that produces Atlantic Cod (*Gadus Morhua* L.) for grow out. A Modular Integrated Recirculating Aquaculture System (MIRAS) has been constructed to develop an experimental integrated multi-trophic aquaculture system that combines the hatchery production of cod and seaweed. Two *Porphyra* species, the aseasonal annual *Porphyra umbilicalis* (L.) Kützing and the winter-spring species *P. linearis* Greville, were employed in the MIRAS so that a system could run year round. These *Porphyra* species exhibit characteristics that indicate it will be an efficient biofilter, including rapid growth, high ammonium uptake rates and high protein contents (Carmona et al. 2006, Kim et al. 2007, Kraemer et al. 2004, Neori et al. 2004, Pereira et al. 2006). It is also important that the seaweed employed is a native species with potential market and ecological value. Several species of the red alga *Porphyra* which are some of the most valuable cultured seaweeds, are native or common to New England. (FAO 2006, He and Yarish 2006, Mathieson et al. 2008, Yarish et al. 1999).

The modular system was used to quantify the nitrogen dynamics of the organisms used. Understanding the nitrogen kinetics of the system, predominantly ammonium production and uptake, was essential to the development of an efficient recirculating system (Ellner et al. 1996, Neori et al. 1996). Ammonium production was examined by measuring the quantities produced by fish with different feed rates, and evaluating optimum feed rate and feed conversion efficiency of fish in the MIRAS. The nutrient uptake capacity of the system was ascertained by evaluating seaweed nutrient uptake rate and
biomass production. Ammonium uptake parameters were measured over a range of ammonium concentrations that bracketed expected levels in the recirculating systems.

The fish ammonium production rates, seaweed ammonium uptake rates and system parameters were used to generate two computer models of the MIRAS. The models may be used by the operator to predict optimum operational conditions or to examine the effects of parameter changes on the ammonium concentration of the system or individual tanks. Further, the models may be used to determine the optimum seaweed/fish balance for reduction of ammonium in the MIRAS effluent and production of fish and seaweed. Trials were conducted using Atlantic cod and Porphyra to test the predictive models and demonstrate continuous operation of the MIRAS.

**MIRAS DESCRIPTION**

The MIRAS was housed within a greenhouse adjacent to Great Bay Aquaculture, LLC (GBA) in Newington, New Hampshire, USA (40°05'58.57"N, 70°47'35.51"W. The 6 x 11 meter gothic style greenhouse (TekSupply, South Windsor, CT) was covered with 2 layers of 6 mil polypropylene (PPE) and air was pumped between the PPE layers to provide insulation. A fan and louvered vent system was connected to a thermostat (Phason, AEC-2) and a shade cloth was used in the summer. A propane heater was connected to a second control (Honeywell, CT87).
Two demonstration scale systems, which were modeled after the system described by Neori et al. (1996), were constructed within the greenhouse at GBA (figure 2.1-3). Each system consisted of four 2x2x1m, 3600 L white fiberglass tanks (Marine Biotech, Beverly, MA). The volume of water in filled tanks was typically 3150 L, however, the volume was dependent on flow rate due to head pressure at the stand pipes. Polyvinylchloride (PVC) pipes were used for the plumbing and aeration systems. Each tank had a 3 inch diameter drain in the center of the tank bottom and 2 inch stand pipes set the water level for each tank. The first tank in each system was used to grow fish and the other three were employed for seaweed culture (See figure 2.1). The fish tanks were shaded by tarps attached to PVC frames. The shades were also used to cover seaweed tanks when not in use.

The fish tank drains were covered by screens flush with the bottom of the tank to allow passage of uneaten feed. The seaweed tank drains were covered by a 61 cm high cylindrical 1.27 cm mesh screen to prevent clogging. Three sump tanks were situated below ground level and each system had a separate recirculating tank and a shared outgoing sump. A cast iron submersible pump (Zoeller, M57) was used in each sump tank. Large particles were filtered by a parabolic filter (FIAP Aquaculture, AN2875) and smaller particles were filtered by two 10-25 micron polymicro cartridge filters. A 330 Watt Ultraviolet sterilizer (Tropical Marine Centre Ltd., UV 180) was used to sanitize water prior to recirculation back to the tanks.
New saltwater, originating from the Piscataqua River, was supplied from GBA's storage tanks and the water flow into the greenhouse was controlled by a one-way valve and measured by a flow meter. The maximum flow rate into the system was 2483 L h$^{-1}$. Two valves were installed beyond the flow meter to provide flow rate control. Valves placed throughout the system allowed the flow rates and patterns to be adjusted to allow for maximum flexibility in system usage. The system could be set to run in one of three modes: recirculating, flow-through and static. When the system was set to recirculating mode, water flowed into each tank and was then filtered through the parabolic filter, mixed in the recirculating sump tank, and then returned to the tanks after passing through the cartridge and UV filters (Figure 2.1). The system turnover rate was one system volume every three hours and the water replacement rate equaled the rate of incoming water. The system was operated at an incoming water flow rate of 38 L h$^{-1}$. Twice a week the drain pipes coming from the tanks were purged by opening the valves in sequential order to remove uneaten food and other debris. Subsequent to each drain purge the incoming water flow rate was increased to refill the system. When drain line purging was considered the average incoming water flow rate was 145 L hr$^{-1}$. When the system was running in flow-through mode, the water replacement rate was increased and the recirculating sump partially bypassed so that some of the water from the system flowed out directly via the outgoing sump (Figure 2.2). Static mode was set by turning off the flow into the system or an individual tank such that no water was removed or replaced.
(Figure 2.3). Furthermore, the flow rate to individual tanks was adjusted or turned off so 1, 2, 3 or 4 tanks were included in the recirculating loop.

The addition of a 24,000 BTU multi-temperature chiller (Aqualogic Inc., MT8) to one of the systems cooled the water and allowed the use of the recirculating mode in the summer. The chiller was placed outside the greenhouse and the water was pumped from the recirculating sump, through the cartridge filters, chiller and UV filter, and then returned to the tanks (Figure 2.1-3). During the winter months, when cooling was unnecessary, the chiller was bypassed by shutting off the valve between the cartridge filters and the chiller.

Aeration was provided by a 3.5 horsepower air compressor (Sweetwater, S63) that was vented to the outside of the greenhouse (Figure 2.4). The air compressor was connected to PVC supply lines that ran down opposite sides of each system. The fish tank had tubing connected to an air stone on each side of the tank. Each of the seaweed tanks had a PVC frame with three ¾ inch pipes that had 1cm holes drilled every inch. The drilled PVC pipes ran perpendicular to the supply lines and the center pipe surrounded the drain cover. Airflow was adjusted by a valve near the compressor and another valve on each of the tanks. The design of the aeration frames in the seaweed tank served to evenly circulate the seaweed thalli throughout the tank and provide equal light exposure.
Figure 2.1: Recirculating mode: Diagram describes one modular system where the chiller is included in the recirculation loop. Minimal water replacement is indicated to account for evaporation and other losses.
Figure 2.2: Flow-through mode: diagram describes one modular system set to a high water replacement rate. The outflow lines that feed directly into the outgoing sump may be used to rapidly drain tanks. The chiller and cartridge filters are not used when the system is in flow-through mode.
Figure 2.3: Static mode: Diagram describes one modular system with no water replacement. The chiller and filters are not used when the system is run in static mode.
Figure 2.4: Aeration system: Diagram is simplified to include one fish tank and one seaweed tank. Dashed supply pipe lines indicate that the pipes continue to the remaining tanks of the modular system.
CHAPTER III. AMMONIUM DYNAMICS IN A MODULAR INTEGRATED
RECIRCULATING AQUACULTURE SYSTEM

INTRODUCTION

The Importance of Developing a Sustainable Aquaculture Industry

Fish represent an important source of food for human populations throughout the
world. Marine fisheries yield 85 million tons of fish per year and the demand for
fish is increasing in conjunction with increasing human population. Fisheries
clearly cannot supply future increases in demand as the sustainable harvest of
wild fish has already plateaued (FAO 2006). Aquaculture production is growing at
a rate of 8.8% per year and currently provides 43% of the fish supply for human
consumption (FAO 2006). Running an aquaculture business requires the
continual development of methods of raising fish that are efficient, cost effective
and provide a steady supply of high quality fish (Beveridge et al. 1997, Folke et
al. 1994, Naylor et al. 2000, Wu 1995). It is crucial to develop the fish
aquaculture industry in a way that provides a reliable, sustainable long term
source of products.

Any industry that is not managed well may be detrimental to the
environment upon which it relies. A major concern regarding aquaculture is the
dependence on capture fisheries to supply fishmeal and fish oil to feed
omnivorous and carnivorous fish. The high value and marketability of carnivorous
fish make them an excellent choice for aquaculture production. However, the availability of fishmeal is limited since fisheries are already being harvested at or near their maximum sustainable rate (Akpaniteaku et al. 2005, Deka et al. 2005, FAO 2006, Naylor and Chiu 2006). Another major environmental concern in aquaculture is the imbalanced nutrient cycling resulting from intensive monoculture of animals. Marine fish aquaculture is dependent on a reliable supply of clean, high quality water to prevent the build up of metabolic wastes (Foss et al. 2006, Jobling 1996, Schuenhoff et al. 2003). However, these metabolic wastes represent nutrient loadings to coastal waters. Further, while increased productivity is crucial for the economic success of a fish farm, increased culture intensity results in greater nutrient loading (Beveridge 1984, Chopin and Robinson 2006, Folke et al. 1994, Wu 1995). Thus, sustainable growth of the fish aquaculture industry depends upon minimizing nutrient loading and reducing dependence on fishmeal (FAO 2006).

Fish grown in intensive aquaculture rely on high quality feed for physical development, growth, and health (e.g. stress tolerance and disease resistance). Fish feed is high in protein and represents up to 50% of the recurring costs of aquaculture operations (Schneider et al. 2007, Wang et al. 2006). Research has been geared towards decreasing feed costs by replacing fish meal with plant proteins and tailoring the nutrient profile to meet specific fish requirements (Beveridge 1984, Lin et al. 2004, Naylor et al. 2000, Wang et al. 2006). However, even the most efficient fish aquaculture systems lose nutrients in the form of metabolites, including ammonia/ammonium (NH$_3$/NH$_4^+$), carbon dioxide (CO$_2$),
and phosphate (PO$_4^-$) (Jobling 1996, Ip et al. 2001). Not only do these metabolites represent wasted nutrients, but they compromise the environment by contributing to eutrophication, plus promoting red tides and blooms of nuisance algae (Chung 2002, El-Shafai 2007, Pagand et al. 2000). Nitrogen is generally considered the most limiting nutrient in marine waters while phosphorus is generally limiting in freshwater systems (Jobling 1996, Lobban and Harrison 1994).

Fish feed provides fish with C, N and P, and fish effluent is rich in these nutrients, in the form of CO$_2$, NH$_3$ and PO$_4^-$. (Beveridge 1984, Chopin et al. 2001a, Ip et al 2001, Kautsky et al. 1997, Refstie et al. 2006). Nutrient concentrations of Great Bay Aquaculture (GBA) effluent during 2005 were 194.79 µM NH$_3$-N (SE 92.58) and 32.96 µM PO$_4$-P (SE 5.73) and (Chemserve Environmental Analysts, Milford, NH). Albrektsen and others (2002) reported an N:P ratio of 6:1 for cod fish feed and aquaculture was reported to have a N:P ratio of 6:1, 3:1 and 1:1 (GBA, Lin and Yi 2003, Dosdat et al. 1995, respectively). The present study focuses on nitrogen dynamics since N inputs have the greatest potential for negatively impacting marine environments. Further, ammonia (as NH$_3$/NH$_4^+$) is a primary fish metabolite and it removal from the recirculating system is crucial to maintaining fish health (Ashe et al. 1996). The Redfield ratio of 30:1 reflects the nutrient content of macroalgae and infers a low P requirement relative to N (Lobban and Harrison 1994). The N:P ratios cited above indicate that P will not be a limiting nutrient for seaweed grown in fish effluent. While seaweed nutritional investigations often maintain consistent nutrient ratios, a recent study supports the idea that a Porphyra biofilter will not be P limited even if provided
with high levels of nitrogen. Carmona and others (2006) reported that *Porphyra* sp. have a lower % P removal compared to N removal (64% vs 87%). Further, P removal efficiency decreased with increasing P concentration indicating possible P uptake saturation (Carmona et al. 2006). Therefore we assumed that the cod in the MIRAS excreted phosphorus at sufficient rates to support seaweed growth and phosphorus was not considered to limit seaweed productivity.

Factors Influencing Ammonium Excretion of Marine Teloests

Protein and nucleic acids fed to fish is metabolized primarily into ammonia (NH$_3$) which is excreted as NH$_3$ or ammonium (NH$_4^+$). In the water column ammonia exists in equilibrium with ammonium and the ratio of NH$_3$ to NH$_4^+$ depends on pH and temperature (Handy and Poxton 1993, Ip et al 2001, Jobling 1996). The total concentration of NH$_3$ and NH$_4^+$ (TAN) is constantly monitored in aquaculture tanks and ponds (Albrektsen et al. 2006, Handy and Poxton 1993) because ammonia is toxic to fish. Chronic exposure to elevated ammonia reduces fish appetite, decreases growth (Hillaby and Randall 1979, Shilo and Rimon 1982). Exposure high concentrations of ammonia causes lesions on gills, leads to respiratory problems, may affect the central nervous system and cause mortality (Beveridge 1984, Chopin et al. 2001b, Foss et al. 2004). Further, Ammonia excretion represents a loss of nitrogen that originated from fish feed and a source of nitrogen that can lead to negative environmental effects (Dosdat et al. 1995). The rate of ammonium excretion is influenced by primarily by fish species, feed consumption rate and protein content, body weight, temperature,

Feed consumption rate is affected by fish body weight, environmental factors (e.g. temperature and pH), stocking density and stress (Ip et al. 2001, Lambert and Dutil 2001, Wood 2001, Wood et al. 1995). Ammonium excretion in Atlantic cod (*Gadus Morhua* L.) is directly associated with feed ration and frequency (Ramnarine et al. 1987). Stress inducing environmental factors such as high stocking density and NH$_3$/NH$_4^+$ exposure have been shown to effect fish feed efficiency, and therefore N excretion (Copeland et al. 2003, Holm et al. 1990, Lambert and Dutil 2001, Uddin et al. 2007, Webb et al. 2007). Once consumed, the protein in the feed is used to synthesize white muscle and increase whole-body protein at a rate that is temperature dependent (McCarthy et al. 1997). Nitrogen excretion generally increases with temperature, as does feeding, protein synthesis and metabolic rate. However, there is a critical temperature at which protein accretion rates drop and protein degradation increases (Dockray et al. 1996, Linton et al. 1998, 1997).

Ammonium excretion is also effected by the pH of the water. Marine fish excrete ammonia primarily via the kidneys and the NH$_3$ gradient between the blood and water determines the rate of ammonia excretion (Sayer and Davenport 1987, Wilson et al. 1994). Low water pH (e.g. via the excretion of H$^+$ by the fish) favors NH$_3$ excretion since NH$_3$ in converted to NH$_4^+$ in the water column (gradient increased). High pH (e.g. above 9) lowers the gradient, and since not

The Fate of Excreted Nitrogenous Wastes

While coastal and open ocean aquaculture systems rely on currents to provide adequate water exchange, land-based tank and pond aquaculture rely on a combination of filtration and water replacement to maintain water quality (Beveridge 1984, Chopin 2001, Cohen and Neori 1991, Primavera 2006). Bacterial biofiltration is commonly used to convert ammonium to nitrite and nitrate, which fish tolerate at higher concentrations than ammonium. Bacterial biofiltration allows the water replacement rate to be reduced; however, it does not decrease the amount of nitrogenous wastes that are released into the surrounding environment (Maeda 1994, Pagand et al. 2000, Schneider 2007).

If unchecked, high nutrient loads, particularly nitrogen, will degrade the quality of water in ecosystems surrounding aquaculture facilities. Thus, the discharge of nitrogenous wastes in aquaculture effluent limits the expansion and intensity of the aquaculture industry and contradicts the concept of sustainability. Integrated multi-trophic aquaculture (IMTA) is designed to reduce nutrient loading by growing organisms from different trophic levels (e.g. fish and seaweed) in close association. Studies have shown that seaweed biofilters can efficiently sequester metabolic wastes in aquaculture systems and use the metabolites as nutrients (Cohen and Neori 1991, Matos 2006, Neori et al. 2004, 2003, Schuenhoff et al. 2003, Troell et al. 2006). Furthermore, seaweeds can be

Large-scale IMTA is being developed in Israel, Chile, Portugal, Canada and the United States of America (FAO 2006). Studies have shown that seaweed used downstream of fish effluent can reduce the nutrient loading of the effluents (Boarder and Shpigel 2001, Buschmann et al. 2008, 2000, Chopin et al. 2008, Cohen and Neori 1991, Matos et al. 2006, Naidoo et al. 2006, Schuenhoff et al. 2003, Shpigel et al. 1999, Troell et al. 2006). The green seaweed *Ulva* has been successfully used in a recirculating aquaculture system (RAS) to treat sea bream (*Sparus aurata* L.) effluent, with the clean water being subsequently pumped back to the fish tanks (Buschmann et al. 2001, Neori et al. 2000, Schuenhoff et al. 2003).

Great Bay Aquaculture (GBA), Portsmouth, NH, USA, is a hatchery that produces Atlantic Cod (*Gadus Morhua* L.) for grow out. A Modular Integrated Recirculating Aquaculture System (MIRAS) has been constructed to develop an experimental unit that combines the hatchery production of cod and seaweed (Yarish 2004). It is important that the seaweed employed is a native species with potential market value. Several species of the red alga *Porphyra*, which is one of the most valuable cultured seaweeds, are native or common to New England (Mathieson et al. 2008, Bray et al. 2007). *Porphyra* was the seaweed of choice for the biofilter because it is commercially valuable, has high nutrient uptake rates, and there are suitable native or local species. One of the advantages of
using valuable seaweed such as *Porphyra* is that they become secondary products. *Porphyra* may be utilized for human food, as a fishmeal replacement in fish aquaculture diets or as a commercial source of the fluorescent pigment phycoerythrin, which is use as a tag in biotechnology. Two species were employed so the system could run year round, the aseasonal annual *Porphyra umbilicalis* (L.) Kützing and the winter-spring species *P. linearis* Greville (Yarish 2004).

Effective design of seaweed biofilters must consider their physiological requirements; including temperature, light, nutrition, and water chemistry. The complimentary metabolic processes of the fish and seaweed must be balanced such that water quality parameters including oxygen, carbon dioxide, and pH match the requirements of both organisms. Therefore, the efficiency of the seaweed biofilter depends on understanding the relationship between fish metabolite production and seaweed nutrient uptake (Chopin and Robinson 2006, Ellner et al. 1996, Neori et al. 1996). For example, while ammonium concentrations must be maintained within a safe range for the fish, the ammonium supply rate must be high enough to support seaweed growth and chemical composition (Ashe et al. 1996, Chopin and Wagey 1999, Chopin et al. 1995, Deboer et al. 1978, Foss et al. 2006, Kraemer and Yarish 2004, Pedersen et al. 2004).
Objectives

The goal of the present study was to quantify the ammonium dynamics of the MIRAS at GBA. Quantification of the ammonium kinetics of both the cod and seaweed components is an essential first step towards optimization of the system (Ellner et al. 1996, Neori et al. 1996). The two primary objectives are as follows:

Objective 1. To determine ammonium production rates, growth rates and feed conversion rates of black seabass (*Centropristis striata* L) and Atlantic cod (*Gadus morhua* L) over a range of feeding levels.

Objective 2. To determine the ammonium uptake rates of two native species of *Porphyra* (*P. umbilicalis* Kützing and *P. linearis* Greville) over a range of ammonium concentrations.

METHODS

The experiments were conducted in an experimental Modular Integrated Recirculating Aquaculture System constructed in a 6 x 11 meter greenhouse (TekSupply, South Windsor, CT) adjacent to Great Bay Aquaculture (GBA), Newington NH. The MIRAS was comprised of two independent demonstration scale modular systems each with four 2x2x1 m, 3600 L white fiberglass tanks
(Marine Biotech, Beverly, MA). The volume of water in filled tanks was dependent on the recirculation rate due to head pressure at the stand pipes and typically held 3150 L of water. The water flow system was designed such that the MIRAS could be run in three modes; static (no flow), flow through or recirculating. A more detailed description of the MIRAS can be found in Chapter 2 of this dissertation.

*Ammonium analysis*

Water samples were stored at 4°C until the end of the experiment and then at -20°C until analyzed. Ammonium concentration was measured via the salicylate method and the reagents from a salt water ammonium-N test kit, code 3304 (LaMotte Co, Chestertown, MD, USA). The method was modified for 1 ml water samples and concentrations were determined using a Helios Alpha UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Calibrations were run against ammonium standard at 0, 30, 80, 120 and 150 μM. Samples with concentrations of 150 μM and greater were diluted by 50% with filtered water (Millipore) prior to analysis.

*Seaweed*

Two native species of the *Porphyra* were used, *P. umbilicalis* and *P. linearis*. *Porphyra linearis* is a winter-spring species and was used in winter experiments. *Porphyra umbilicalis* is an aseasonal annual and was used in experiments conducted during the spring, summer and fall. When not available from cultures
at the University of Connecticut, *P. umbilicalis* was collected from Wallis Sands, Rye NH (43°01’40.68N",70°43'29.86"W) and *P. linearis* from Popple Cove, Gloucester MA (42°36'13.18N",70°30'02.09"W). At the time of collection *Porphyra* thalli that appeared to be healthy and in good condition were carefully chosen. Blades that had visible fouling with epiphytes or mussels or did not appear to be healthy were avoided. The seaweed was rinsed in seawater to remove debris before it was added to the MIRAS. When the blades in the tanks started to be covered with substantial epiphyte populations or deteriorate, they were removed from the tanks. Each validation trial was ended when the *Porphyra* thalli appeared to be in poor condition.

*Seaweed Ammonium Uptake*

Prior to each experiment *Porphyra* was collected and acclimated in the MIRAS tanks for at least 24 hrs. The experiments were conducted in the greenhouse to estimate the ammonium uptake of *Porphyra* in the conditions of the MIRAS. Eighteen 1 L Erlenmeyer flasks were set up in three water baths set just above one of the seaweed tank in the greenhouse and cooled with water from the tank. Ammonium levels in the flasks were adjusted by addition of 0, 50, 100, 150, 200 and 250 μM ammonium chloride (NH₄Cl) to new seawater. The flasks were aerated by air stones connected through a manifold to the MIRAS aeration system. The flasks were placed in the water baths in a randomized complete block design. One ml samples were taken at 0, 1, 4, 7 and 24 hrs and stored at 4°C until the end of the experiment. The samples were then stored at -
20°C until analysed. The uptake rate was calculated based on ammonium depletion.

The Michaelis-Menten type equation; \( V = V_{\text{max}} \times S / (K_s + S) \) was fit to the uptake rate measurements for *Porphyra umbilicalis* and *P. linearis*, where \( V \) is the uptake rate and \( S \) is concentration was used to estimate the maximum uptake rate (\( V_{\text{max}} \)) and half-saturation ammonium concentration (\( K_s \)). The uptake efficiency at low ammonium concentrations was defined as the initial slope of the uptake curve (\( V_{\text{max}}/K_s \)). Uptake efficiency was used to compare the buffering capacity of the two seaweed species.

**Fish**

Two fish species, black seabass (*Centropristis striata* L) and Atlantic cod (*Gadus morhua* L), were supplied by Great Bay Aquaculture (GBA). Preliminary studies were conducted with a total of 2,400 black seabass fingerlings with an initial average weight of 4.3 g per fish for a stocking density of 2.6 Kg m\(^{-3}\). When the integrated seaweed/fish trials began the fish tank was restocked with a total of 205 fingerling cod with a mean weight of 44.01 g per fish and an initial stocking density of 2 Kg m\(^{-3}\). Low stocking densities were used for both fish species to avoid complicating factors.

**Fish Growth, Feed Conversion, Ammonium Production**

Each species was investigated with separate experiments and the MIRAS was run in static mode with no water replacement. All fish feed rates represent
the amount of feed offered to the fish per day. The fish tank walls were cleaned daily and any uneaten particulates were removed via the drain on the tank bottom and subsequently filtered out through the parabolic filter. Black seabass growth and feed conversion rates were measured over a range of experimental conditions in order to evaluate the suitability of the MIRAS for growing fish. The ammonium production of Atlantic cod was used to develop a predictive model of ammonium dynamics. The ammonium production rate of the cod was calculated based on studies conducted throughout the range time of the validation trials to account for changes in fish metabolism (e.g. due to age, temperature, stress).

*Black Seabass (Centropristis striata)*

Six of the MIRAS tanks, 3 in each system, were stocked with 400 black seabass with an average weight of 4.3g per fish. The fish were fed INVE IDL 1.5 mm feed (52% protein, 15% fat) three times per day. Using a replicated 3 x 3 Latin square design, the tanks in each system were randomly assigned to total daily feed rates of 3, 4, or 5% of fish biomass. The rates were chosen based on the feed manufacturer’s recommendations based on a water temperature of 14 °C. Feed conversion rate (FCR) and growth rate were determined by weighing a sample of 20-30 fish at one week intervals. At the end of each three or four week trial, the feed rate treatment assignments were re-randomized. During the trials, the tanks were generally operated in flow-through mode. To determine ammonium production rates, the system was set to static mode (i.e. no incoming or outgoing water) for 12 hours. Water samples were taken at the beginning and
end of each period and the samples were stored at 4°C until the end of the experiment and then at -20°C until analyzed. Ammonium production was determined as increase in concentration over time divided by fish biomass. Due to a pump failure, the black seabass suffered 100% mortality. The fish tank was restocked with cod fingerlings which were subsequently used for further ammonium production studies.

Atlantic Cod (Gadus morhua)

Eight experiments were conducted periodically from April 15, 2004 to October 13, 2005, to measure the ammonium production of Cod in the MIRAS. One tank was used to grow the fish which allowed integrated trials to be run and ammonium production measurements to be taken over the course of the trials. During each ammonium production experiment tanks were set to static mode with no water exchange for 12 hours. Experiments were run during the day and night and water samples were collected every two hours. Ammonium excretion was determined as difference in ammonium concentration. The tank was initially stocked with 205 fingerling cod with a mean weight of 45g per fish so that the initial fish biomass was 9 Kg. Towards the end of the integrated trials the fish had a mean biomass of 456 g and the total biomass was 18 Kg.

The cod were larger than the black seabass and were fed Zeigler Marine Grower 55-15 Slow Sinking 8.0 mm pellets (55% protein, 15% fat) at a rate slightly lower than recommend by the feed manufacturer, 0.5 - 1% fish biomass per day (100g feed per day), to ensure most of the feed was consumed. Uneaten
feed was removed from the system on a daily basis via the drain pipe and the parabolic filter. While uneaten feed was not collected to determine actual feed consumption, the cod fish feeding activity and level of uneaten feed were closely observed. When the fish exhibited decreased appetite trials were suspended until feed rate returned to normal. Since ammonium exposure can reduce fish appetite and ammonium production, the fish were allowed to recover between trials. Dissolved oxygen (DO) was monitored periodically, pH and temperature were monitored daily.

Statistics

The effects of feed rate on black seabass feed conversion, N production, and growth measurements were analyzed via ANOVA (analysis of variance) in Systat (version 10, Systat Inc.). For each response variable and ANOVA was performed to look for effects of feed rate. When the ANOVA indicated significant effects of feed rate a Tukey’s HSD multiple comparison test was used to examine pairwise differences. The standard error in the text and figures are ±1 standard error based on the MS$_{error}$ from the appropriate ANOVA.

The mean seaweed nitrogen uptake rate for each ammonium concentration was calculated and the Michaelis-Menten equation was then fit to the means using the non-linear regression module of Systat (version 10, Systat Inc.). Error bars in the figures represent ±1 standard error.
RESULTS

Black seabass: Ammonium Production, Growth and Feed Conversion

The ammonium excretion of black seabass in the MIRAS was 5.72, 5.07, and 4.37 \% protein in the feed offered in the feed (±SE 0.27\%) for fish fed respective rates of 3, 4 and 5\% body weight per day, respectively (Figure 3.1). A smaller percentage of ammonium was excreted by fish fed at a rate of 5\% compared to 3\% (p=0.008). Similar results were found for ammonium excretion expressed as percent fish biomass 0.0711, 0.0855, 0.0898\% per day (±SE 0.004\%, p=0.013). The feed conversion ratio (FCR) was 1.01, 1.36, and 1.65 g feed per g wet weight gained (±SE 0.15) for fish fed at 3, 4 and 5\% daily feed rates (Figure 3.1). The feed conversion ratio (FCR) at the 5\% feed rate was significantly greater than at 3\% (p=0.021). The relative growth rate (RGR) of the fish was 2.83 (SD± 0.006) \% per day and was not affected by feed rate (p=0.79).

Atlantic cod: Ammonium Production

The ammonium excretion studies of Atlantic cod showed that 8.59\% (±SE 0.48) of protein in the feed was excreted as ammonium. Therefore, when the fish were given 50 g of feed per day the average daily ammonium production rate was 168.73 (±SE 9.33) \(\mu\text{moles day}^{-1}\). There was no difference between day and night ammonium production (p=0.12). Individual nitrogen production trial results are presented in terms of ammonium production rates (g \(\text{NH}_4\text{N hr}^{-1}\)) and \% feed
excreted as ammonium (Table 3.1). Periodic measurements showed stable
dissolved oxygen (DO) with a mean DO of 7.28 (SD ± 0.17) mg L⁻¹. The mean pH
throughout the cod N production studies was 7.8 (SD ± 0.24). The temperature
was maintained at 14.9 °C (SD ± 8.7) and 11.0 °C (SD ± 1.7) for the P.
*umbilicalis* and *P. linearis* trials, respectively.

**Ammonium uptake**

Both *Porphyra umbilicalis* and *P. linearis* increased the rate of ammonium
uptake with greater concentration (Table 3.1, Table 3.2). Michaelis-Menten
parameter estimates from the non-linear regression for *P. umbilicalis* were $V_{\text{max}} =
12.72 \mu\text{mol gFW}^{-1} \text{hr}^{-1}$ (ASE=1.78), and $K_s = 61.33 \mu\text{M}$ (ASE=28.06) ($R^2=0.956$).
Parameter estimates for *P. linearis* were $V_{\text{max}} = 9.68 \mu\text{mol gFW}^{-1} \text{hr}^{-1}$ (ASE=1.07),
and $K_s = 8.52 \mu\text{M}$ (ASE=12.23) ($R^2=0.932$).

**DISCUSSION**

The goal of this study was to separately measure the ammonium kinetics
of the fish and seaweed that would be integrated in the demonstration scale
recirculating system. The ammonium producers studied were black seabass and
Atlantic cod and the seaweed biofilters were *Porphyra umbilicalis* and *P. linearis*.
While these species have been studied in laboratory and/or commercial systems,
it was important to measure the nutrient kinetics for the specific MIRAS
conditions; this would allow development of predictive models to optimize the fish to seaweed biomass ratios and other operating parameters of the system.

The feed conversion ratio (FCR) of the black seabass grown in the MIRAS (1.01 – 1.65) was comparable to those found in the literature (Copeland et al. 2003, Refstie et al. 2006, Rosenlund et al. 2004, Schuenhoff et al. 2003). The black seabass converted feed to biomass less efficiently than the juvenile Atlantic cod (FCR=0.68, Foss et al. 2004; FCR= 0.74-0.88, Rosenlund et al. 2004) but more efficiently than 1-2 year Atlantic cod (1.26-1.37, Refstie et al. 2006) gilthead seabream (Sparus arata) (FCR = 1.98-2.66, Schuenhoff et al. 2003) and black seabass (FCR = 1.45-1.52) reported by Copeland et al. (2003). The temperature and pH of the MIRAS and size class of the fish were consistent throughout the black seabass NH$_4^+$ production studies, therefore the N excretion rates were directly correlated with feed ration and protein composition (Beamish and Thomas 1984, Wood 2001). A low FCR indicates an efficient use of nutrients since less feed is required per unit weight gain. The increased FCR and decreased ammonium production of the black seabass in our study indicated the fish were not consuming all of the feed when offered at 5% of their body weight and constant growth rate (Jobling 1996, Wood 2001) and indicates that a 4% feed rate would be appropriate.

The Atlantic cod appeared to be healthy and grew throughout the course of the cod N production studies. While size class is reported to influence Atlantic cod NH$_3$/NH$_4^+$ excretion (Refstie et al. 2006), the N excretion of the MIRAS cod was fairly consistent over time and the daily feed rate, feeding frequency and
environmental parameters were constant. The NH$_4$-N excretion rate of Atlantic cod in the MIRAS (0.833 mg N Kg$^{-1}$ feed$^{-1}$ h$^{-1}$) was higher than rates for adult Atlantic cod (0.14 mg N Kg$^{-1}$ h$^{-1}$, Chadwick and Wright 1999) and lower than rates for black sea turbot (*Psetta maerotica*) (5.1-7.5 mg N Kg$^{-1}$ h$^{-1}$, Yigit et al. 2003, 2005), stockeye Salmon (*Onchorhynchus nerka*) (14.5 mg N Kg$^{-1}$ h$^{-1}$, Brett and Zala 1975), trout (*Salmo gairdneri*) (18.33 mg N Kg$^{-1}$ h$^{-1}$, Rychly and Marina 1977), and gilthead seabream (*Sparus aurata*) (36-96 mg N Kg$^{-1}$ h$^{-1}$, Porter et al. 1987). The broad range of reported nitrogen excretion rates for fishes grown under different conditions demonstrate that it is important to use species and system specific excretion.

The nutrient uptake capacity and ability to maintain safe levels of ammonium are fundamental properties of a seaweed biofilter. Recently, Carmona and others (2006) reported uptake rates for *Porphyra umbilicalis* of that were much lower than in the present study. The higher uptake rates may possibly be due to the acclimation at lower ammonium levels in the greenhouse. The maximum uptake rate ($V_{max}$) of *P. umbilicalis* was higher than *P. linearis* which indicates that the former will take up ammonium at higher concentrations than the later. This is in contrast to the findings of Kim et al. (2007) who reported that *P. linearis* had a higher nitrogen uptake capacity than *P. umbilicalis*. However, the ammonium concentrations must be maintained below 50-100 µM to promote fish health and so nutrient uptake below 50 µM NH$_4^+$ low may be a more important indicator of biofilter performance. Therefore, the half saturation constant ($k_s$) may be more relevant to the actual function of the biofilter. When
ammonium concentrations are close to the $k_s$, an increase or decrease in ammonium concentration will result in a corresponding increase or decrease in seaweed uptake rate. The dynamic response of uptake rate may effectively buffer the system against spikes in ammonium concentration and safeguard the fish against ammonium toxicity. Kraemer and others (2004) suggested that the initial slope of the uptake curve ($V_{\text{max}}/k_s$) may be an important indicator of the buffering capacity of the seaweed biofilter. The uptake curve of $P. \text{linearis}$ has a steeper initial slope indicating a quicker response to changing ammonium levels and a greater buffering ability at lower concentrations.

In aquaculture systems, maintenance of stable, low ammonium levels is vital to the health and productivity of fish (Ashe et al. 1996, Foss et al. 2006, Halachmi 2006). Although the seaweed used in this study where acclimated to ammonium levels in the MIRAS, the results reported represent short-term uptake rates. Results from previous studies indicate that both $Porphyra$ species are able to sustain high rates of ammonium uptake at high (250 μM) ammonium concentrations for 2-3 weeks (Day 2003). A recent study by Kim and others (2007) found that $P. \text{umbilicalis}$ and $P. \text{linearis}$ maintained high uptake rates for 1-2 week periods. While the $Porphyra$ uptake rates reported by Carmona et al. (2006) were relatively low, they represent longer term (4 week) nitrogen uptake capacity. Further, $P. \text{linearis}$ demonstrated a greater degree of variability than $P. \text{umbilicalis}$, which may reflect the shorter day lengths and cloudy conditions encountered during winter months. The results of the ammonium production and uptake kinetic from this study can be used to develop an efficient seaweed
biofilter for the MIRAS. The success of the MIRAS hinges on maintaining water quality parameters within healthy limits for the fish. It is therefore important that nitrogen excretion and uptake rates be balanced within the system. The focus of subsequent research is to test the relationship between ammonium uptake and production by varying seaweed biomass, fish biomass, or fish feed rate in the system. Daily monitoring of ammonium concentrations in the MIRAS will test the efficiency and reliability of *Porphyra* biofilters.
Figure 3.1: Black seabass: feed conversion ratio (FCR) (A) and ammonium excretion expressed as % protein fed (B) for fish provided feed at rates equaling 3, 4, and 5% of their body weight per day. Error bars represent standard error. Means labeled with the same letter are not significantly different (α=0.05).
Table 3.1: Feed excreted as NH\textsubscript{4} (% feed) and ammonium production rates (g NH\textsubscript{4} hr\textsuperscript{-1}) for Atlantic cod (Gadus morhua) in the MIRAS.

<table>
<thead>
<tr>
<th>date</th>
<th>Duration (hr)</th>
<th>Increase in conc (µM)</th>
<th>N production rate (g NH\textsubscript{4} hr\textsuperscript{-1})</th>
<th>% feed excreted as NH\textsubscript{4}</th>
<th>( SE )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/25/2005</td>
<td>Day</td>
<td>12.2</td>
<td>38.71</td>
<td>0.138</td>
<td>6.63</td>
</tr>
<tr>
<td>9/15/2005</td>
<td>Day</td>
<td>9.0</td>
<td>20.98</td>
<td>0.102</td>
<td>4.89</td>
</tr>
<tr>
<td>10/11/2005</td>
<td>Day</td>
<td>9.7</td>
<td>22.34</td>
<td>0.099</td>
<td>4.77</td>
</tr>
<tr>
<td>10/25/2005</td>
<td>Day</td>
<td>12.0</td>
<td>23.70</td>
<td>0.086</td>
<td>4.12</td>
</tr>
<tr>
<td>8/25/2005</td>
<td>Night</td>
<td>13.2</td>
<td>38.61</td>
<td>0.127</td>
<td>6.11</td>
</tr>
<tr>
<td>10/12/2005</td>
<td>Night</td>
<td>14.2</td>
<td>22.52</td>
<td>0.069</td>
<td>3.29</td>
</tr>
<tr>
<td>10/13/2005</td>
<td>Night</td>
<td>10.3</td>
<td>14.93</td>
<td>0.063</td>
<td>3.01</td>
</tr>
<tr>
<td>10/26/2005</td>
<td>Night</td>
<td>11.0</td>
<td>25.94</td>
<td>0.102</td>
<td>4.92</td>
</tr>
</tbody>
</table>
Table 3.2: Ammonium uptake rates (μmol g⁻¹ hr⁻¹) of *Porphyra umbilicalis* and *P. linearis* at six ammonium levels. 
*data excluded, complete cloud cover for the entire day.*

<table>
<thead>
<tr>
<th>species</th>
<th>date</th>
<th>NH₄ level (μM)</th>
<th>0 ( SE )</th>
<th>50 ( SE )</th>
<th>100 ( SE )</th>
<th>150 ( SE )</th>
<th>200 ( SE )</th>
<th>250 ( SE )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. umbilicalis</em></td>
<td>5/17/2005</td>
<td>0.39 (0.00)</td>
<td>3.77 (0.11)</td>
<td>7.71 (0.06)</td>
<td>10.74 (0.14)</td>
<td>12.23 (0.09)</td>
<td>12.66 (0.09)</td>
<td></td>
</tr>
<tr>
<td><em>P. umbilicalis</em></td>
<td>6/8/2005</td>
<td>0.31 (0.02)</td>
<td>5.47 (0.08)</td>
<td>8.76 (0.09)</td>
<td>10.41 (0.08)</td>
<td>9.73 (0.19)</td>
<td>7.54 (0.20)</td>
<td></td>
</tr>
<tr>
<td><em>P. umbilicalis</em></td>
<td><em>11/22/2005</em></td>
<td>0.45 (0.02)</td>
<td>2.49 (0.03)</td>
<td>3.99 (0.02)</td>
<td>3.32 (0.02)</td>
<td>4.47 (0.24)</td>
<td>5.18 (0.06)</td>
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</tr>
<tr>
<td><em>P. umbilicalis</em></td>
<td>3/16/2006</td>
<td>0.16 (0.01)</td>
<td>6.07 (0.01)</td>
<td>7.41 (0.12)</td>
<td>9.36 (0.35)</td>
<td>8.60 (0.08)</td>
<td>6.99 (0.13)</td>
<td></td>
</tr>
<tr>
<td><em>P. linearis</em></td>
<td>12/21/2005</td>
<td>-0.07 (0.02)</td>
<td>4.31 (0.11)</td>
<td>6.06 (0.01)</td>
<td>7.52 (0.12)</td>
<td>7.11 (0.07)</td>
<td>6.86 (0.32)</td>
<td></td>
</tr>
<tr>
<td><em>P. linearis</em></td>
<td>1/13/2006</td>
<td>0.12 (0.02)</td>
<td>6.98 (0.14)</td>
<td>8.16 (0.12)</td>
<td>7.81 (0.16)</td>
<td>10.70 (0.11)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>P. linearis</em></td>
<td>2/14/2006</td>
<td>1.27 (0.02)</td>
<td>9.53 (0.14)</td>
<td>11.99 (0.17)</td>
<td>12.01 (0.15)</td>
<td>25.52 (1.12)</td>
<td>21.06 (0.55)</td>
<td></td>
</tr>
<tr>
<td><em>P. linearis</em></td>
<td>2/28/2006</td>
<td>0.33 (0.04)</td>
<td>10.02 (0.08)</td>
<td>11.20 (0.05)</td>
<td>13.48 (0.07)</td>
<td>11.01 (0.77)</td>
<td>14.28 (0.08)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.2: Michaelis-Menten type relationship ammonium uptake rate of *Porphyra umbilicalis* and ammonium concentration. $R^2 = 0.956$, Error bars are ±SE. Maximum rate of ammonium uptake ($V_{\text{max}}$) = 12.72 μmol gFW$^{-1}$ hr$^{-1}$ and half saturation constant ($K_s$) = 61.33 μM.
Figure 3.3: Michaelis-Menten type relationship ammonium uptake rate of *Porphyra linearis* and ammonium concentration. $R^2 = 0.932$, Error bars are ±SE. Maximum rate of ammonium uptake ($V_{\text{max}}$) = 9.68 $\mu$mol gFW$^{-1}$ hr$^{-1}$ and half saturation constant ($K_s$) = 8.52 $\mu$M.
CHAPTER IV. AMMONIUM DYNAMIC MODEL FOR THE OPTIMIZATION OF A MODULAR INTEGRATED AQUACULTURE SYSTEM (MIRAS)

INTRODUCTION

Fish are an important nutrient source for people throughout the world, and as food demand increases so does the need for sustainable sources of fish. Aquaculture is a rapidly growing economic sector and has the potential to produce adequate fish to meet global demand. Throughout the world research efforts are turning towards the development of environmentally sustainable aquaculture. Recirculating aquaculture systems (RAS) have been used primarily in hatcheries to produce fish for grown out in coastal or open ocean net pens. Such systems are not commonly used to raise fish to market size, since they are costly to set up and operate (Foss et al. 2004). However, RAS are used to raise fish in areas where access to water resources is limited (Bolton 2006, Dey et al. 2006, FAO 2006, Metaxa 2006). Moreover, recirculating tank and pond systems offer an excellent opportunity for effective remediation of aquaculture effluent and may become increasingly attractive with growing environmental concerns (Behrends et al. 2002, Clonts and Cerezo 1996, Muir 1998, Phillips and Love 1998, van Gorder and Jug-Dujakovic 2005).

Fish effluent contains metabolites including carbon dioxide (CO₂), ammonia (as NH₃/NH₄⁺) and phosphate (PO₄) (Jobling 1996, Ip et al. 2001). Recirculating Aquaculture Systems contend with accumulating waste products by
using physical and biological filtration (Atwood et al. 2005, Gelfand et al. 2003, Philips and Love 1998). Ammonia (NH$_3$/NH$_4^+$) is a major metabolite and its production is second only to CO$_2$ production (Ip et al. 2001). Since NH$_3$/NH$_4^+$ is toxic to fish bacterial biofilters are used to reduce the accumulation of ammonical N in recirculating water (Atwood et al. 2005, Van Gorder and Jug-Dujakovic 2005). However, bacterial biofilters convert NH$_3$/NH$_4^+$ to nitrate (NO$_3$) via nitrite (NO$_2$) which become part of the effluent and there is no reduction in environmental nitrogen (N) loading. Therefore NH$_3$/NH$_4^+$ production is a key process both in terms of fish production and environmental sustainability. The most important factors that determine the rate of ammonium excretion are feed consumption, feed protein content, body weight, water temperature, and pH (Beamish and Thomas 1984, Ip et al 2001, Gallagher and Mathews 1987, Handy and Poxton 1993, Jobling 1981, Lin and Yi 2003, Paulson 1980, Ramnarine et al. 1987, Rychly and Marina 1977, Wood 2001).

Computer simulation models can be used as a tool for the management of recirculating aquaculture systems. Models can be used to understand the effect of known biological processes and system parameters on various aspects of the system such as productivity and nutrient dynamics (Clark 1985, Ellner et al. 1996). A model can also be used to simulate experiments and predict optimum operating conditions to meet specific objectives. Complex models have been developed to study the interactions of aquaculture operations on the surrounding ecosystem and predict the maximum sustainable stocking density of fish or shellfish (Buonomo et al. 2005, Cranford et al. 2007, Sato et al. 2007, Wu et al. 2005).
A few models have characterized the nutrient dynamics in recirculating fish aquaculture systems (Halachmi et al. 2005, Pagand et al. 2000) and integrate fish and plant systems (Chapelle et al. 2000, Ellner et al. 1996, Jamu and Piedrahita 2002).

Integrated multitropic aquaculture (IMTA) uses desirable opportunistic plants as biofilters to remove the metabolites from aquaculture effluent. The principal advantage of IMTA systems is that the metabolic wastes of fish become fertilizers for the plants and enhance plant growth and biosynthesis (Chopin and Yarish 1999, Chopin et al. 2008, Neori et al. 2004). Ellner et al. (1996) developed a nutrient dynamics model of a RAS that integrated Gulthead sea bream (*Sparus aurata* L.) with the green seaweed *Ulva* sp. and determined that fish ammonium production rate and seaweed uptake kinetics were the governing factors determining ammonium concentration of the system compartments and effluent. Since the model was reported, *Ulva* has been successfully used as a seaweed biofilter in an RAS to treat sea bream effluent; decreasing the amount of fresh seawater needed and reducing environmental nutrient loading (Buschmann et al. 2001, Neori et al. 2000, Schuenhoff et al. 2003).

If nutrient removal is considered to be the primary function of a seaweed biofilter, then the goal is to maximize nutrient uptake efficiency by maintaining high seaweed biomass and low nutrient concentrations. Here, the most important characteristic of the seaweed biofilter is rapid, sustained nutrient uptake. However, it has been proposed that the seaweed biomass produced could provide a valuable product that could offset biofiltration costs (Chopin and Yarish 1999).
If this secondary goal is added, nutrient concentrations must be kept high enough to optimize seaweed biomass production or biochemical synthesis while simultaneously maintaining safe water quality for the fish and reducing nutrients in the effluent. In a RAS with very low water replacement, it may be possible to have a fairly high nutrient removal efficiency and biomass production. Therefore, the efficiency of the filtration component (Neori et al. 2003, Troell et al. 2006) hinges on balancing nutrient production from fish metabolism and nutrient uptake by the seaweed biofilter.

In the present study we describe a simple ammonium dynamic model of a demonstration-scale modular integrated recirculating aquaculture system (MIRAS) that was constructed at Great Bay Aquaculture, LLC (GBA) in Newington, New Hampshire, USA. The MIRAS uses Porphyra (nori) to treat the effluent from Atlantic cod (Gadus morhua L.) production. Porphyra was selected because of its fast growth, nutrient uptake characteristics and economic value (FAO 2006). Porphyra is used as human food and as a commercial source of phycobilin pigments in biotechnology. Porphyra requires nitrogen, as nitrate or ammonium, to support growth and pigment-protein production (Carmona et al. 2006, Day 2003, Kim 2003, Pereira et al. 2006). The present study focused on ammonium because it is the primary nitrogenous metabolite of fish effluent and because at elevated concentrations it is toxic to fish (Ashe et al. 1996, Foss et al. 2004, King and Berlinsky 2006). Nutrients such as CO₂ and PO₄ were assumed to be present in sufficient quantities to support Porphyra growth as they are

Objectives

The modular integrated recirculating aquaculture system (MIRAS) at GBA was designed as an experimental-scale system to study the use of seaweed as a biofilter. The primary purpose of the seaweed biofilter was to reduce the levels of ammonium and other nutrients and reduce the total discharge of nutrients in from its effluent. A secondary goal was to produce harvestable quantities of Porphyra.

Chapter 3 of this dissertation quantified the ammonium production and uptake rates of the fish and seaweed in the system. The ammonium production and uptake rates and system parameters (system volume, water replacement rate, fish feed rate and seaweed biomass) were used in the present chapter to generate two computer models to describe the nutrient dynamics of the MIRAS. The first model was developed to predict the temporal dynamics of recirculation and water replacement rates, fish feed rate or fish biomass, and seaweed biomass on system ammonium concentration. A second model was developed as a tool to optimize seaweed biomass in relation to biomass or fish feeding rate while maintaining system ammonium concentration safely below stress levels for the fish.

During the study, a cohort of Atlantic cod (Gadus morhua L.) was grown in the system. The native seaweed species Porphyra umbilicalis (L.) Kützing and P.
*linearis* Greville were used in a series of trials to validate the models and to test their effectiveness as biofilters.

The five major objectives of the study were to:

Objective 1: develop a model that describes temporal changes in ammonium concentration of the system as a function of system water recirculation and replacement rates.

Objective 2: generate a model that could predict the ammonium concentration of the system in relation to seaweed and fish biomass or fish feeding rates.

Objective 3: validate the predictive computer model of the MIRAS using a series of trial runs with different seaweed biomasses and fish feeding rates.

Objective 4: evaluate the growth of cod and *Porphyra* in the MIRAS during integrated trials.

Objective 5: demonstrate continuous long term operation of the MIRAS.

The models, which are based on fish ammonium production rates, seaweed uptake rates, and system operating parameters, can be used to determine optimum parameters and seaweed/fish biomass ratios for the
reduction of ammonium in the MIRAS effluent and production of fish and seaweed. Trial runs using *Porphyra umbilicalis* and *P. linearis* were run to evaluate the effectiveness of the model in predicting effects of parameter changes in ammonium concentration of the system or individual tanks.

**METHODS**

*System Description*

The models were developed and validated based on a demonstration scale modular integrated recirculating aquaculture system (MIRAS) constructed in a 20 x 36 foot greenhouse (TekSupply, South Windsor, CT). The MIRAS was comprised of two independent systems, each with four 4m$^3$, 3600 L white fiberglass tanks (Marine Biotech, Beverly, MA). Seawater in the system came from the Piscatiqua River and was stored in a tank at GBA. The water flow system was designed such that the MIRAS could be run in three modes: static (no flow), flow-through, or recirculating. The fish and seaweed were grown in separate tanks and the water overflowed stand pipes, and was collected in a 174 L sump tank, pumped through a series of filters and a chiller (when necessary), and then pumped back to the fish and seaweed tanks. Large particles were filtered by a parabolic filter (FIAP Aquaculture, AN2875) and smaller particles by two 10-25 micron polymicro cartridge filters. A 330 Watt Ultraviolet sterilizer (Tropical Marine Centre Ltd., UV 180) was used to sanitize water prior to circulation back into the tanks. A more detailed description of the MIRAS can be found in Chapter 2 of this dissertation.
Model Development

The models were developed in Microsoft Excel using the ammonium dynamics of the fish and seaweed components and system parameters such as water replacement rate and system recirculation rate. The seaweed ammonium uptake kinetics were determined using flask experiments within the greenhouse so the plants were exposed to the same temperature and light levels (See chapter 3 for details). Fish ammonium production was determined for specific feeding rates by independent studies using the MIRAS where one tank was used to grow the fish. The fish tank was initially stocked at a density of 3 Kg per cubic meter with 205 fingerling cod with a total biomass of 9 Kg. The fish were weighed towards the end of the integrated trials and weighed an average of 456 g per fish for a total biomass of 18 Kg. The cod were fed Zeigler Marine Grower 55-15 Slow Sinking 8.0 mm pellets (55% protein, 15% fat). In order to ensure that most of the food was consumed, the fish were feed at a rate slightly low than recommend by the feed manufacturer for the size of the fish and water temperature. A constant rate of 100 g fed per day was used to minimized variability in the in the cod fish N production rate. The models assume that all of the feed offered was consumed and trials were suspended when the fish did not appear to be actively eating the feed. Any uneaten feed was removed from the system daily via the tank drain pipes and parabolic filter. An average ammonium production rate was calculated from studies run periodically throughout the time course of the integrated trials. The N production rate assumed to be representative as feed composition,
feeding frequency and environmental parameters (e.g. temperature and pH) were constants.

The main processes determining system ammonium concentration were the ammonium input by the fish, its removal by the seaweed, and seawater replacement rate. It was assumed that the seawater replacement rate also accounted for minimal losses due to evaporation or small leaks. The model assumes that nitrification is minimal since there was no bacterial biofilter or sedimentation tank and the system was cleaned regularly to reduce the build up of bacteria and fouling algae. The two models developed are simple models that consider the major parameters that influence the ammonium concentration of the water in the MIRAS. Both models were used to understand the effect of changing a fixed set of parameters such as water exchange rate, recirculation rate, fish feed rate or biomass, and seaweed biomass.

Model I: Ammonium concentration versus time

The temporal model of the system's ammonium level included the following system parameters: fish feed consumption rate or fish biomass, seaweed biomass, and water replacement rate. The simplest form of model I (Figure 4.1) predicts the ammonium concentration when the system has fish only and no water replacement. The system ammonium concentration at time interval \(S_t\) can be calculated as

\[ S_t = \frac{P_B t}{Vol_s} \]
where, $P$ is the ammonium production rate ($\mu$mol h$^{-1}$ g feed$^{-1}$), $B_f$ is the fish feed rate (g d$^{-1}$), $t$ is the magnitude of the time interval, and the system volume is $V_{ol_s}$.

The exchange rate is the rate at which effluent (and other losses) from the system is replaced with new incoming water. At each time interval the system's concentration changed by a quantity determined by the length of the time interval ($t$), the volume of water exchanged ($V_{ol_x}$) compared to the total system volume ($V_{ol_s}$), and the difference between the concentration of the system during the previous time interval ($S_{t-1}$) and new water ($S_{new}$):

$$S_{x} = t\cdot V_{ol_p}/V_{ol_s} \cdot (S_{t-1} - S_{new})$$

When the model includes a fish component and the water exchange rate the system ammonium concentration at time interval ($S_t$) can be calculated over a series of time segments (Figure 4.2) as follows:

$$S_t = S_{t-1} + (t / V_{ol_s}) \cdot [P \cdot B_f - V_{ol_x} (S_{t-1} - S_{new})]$$

Further development of the model to account for the seaweed component included the ammonium uptake kinetics of the _Porphyra_ species used in the MIRAS. The ammonium uptake characteristics were measured in the MIRAS and described by a Michaelis-Menten type equation. The temporal change in MIRAS ammonium concentration when the fish and seaweed components are included can be describe by the following equation.

$$S_t = S_{t-1} + (t / V_{ol_s})\cdot [P \cdot B_f - (B_s \cdot V_{max} \cdot S_{t-1})/(K_s + S_{t-1})]$$

where $B_s$ is the seaweed biomass (g FW), $V_{max}$ is the seaweed's maximum ammonium uptake rate, and $K_s$ is the half saturation constant for the seaweed.
uptake kinetics curve. The temporal equation for system concentration was corrected for exchange rate (Figure 4.3) was derived as follows:

$$S_t = S_{t-1} + (t / Vol_s) *[P*B_f - (B_s * V_{max} * S_{t-1}) / (K_s + S_{t-1}) - Vol_x * (S_{t-1} - S_{new})]$$

The temporal model was modified to predict the effect of recirculation rate on the ammonium concentrations of the water in individual tanks (Figure 4.4). The ammonium concentration of the fish tank ($S_f$) was calculated as follows:

$$S_f = S_{t-1} + (t / Vol_f) *[P*B_f + R*(S_{sys} - S_{t-1})]$$

where $S_{t-1}$ is the concentration of the fish tank during the previous time interval, $R$ is the recirculation rate (L h$^{-1}$), and $S_{sys}$ is the concentration of the whole system. Likewise, the ammonium concentration of the seaweed tanks ($S_{SW}$) was calculated as follows:

$$S_{SW} = S_{t-1} + (t / Vol_{SW}) *[B_s * V_{max} * S_{t-1}] / (K_s + S_{t-1}) + R*(S_{sys} - S_{t-1})]$$

where Vol$_{SW}$ is the volume of the seaweed tanks (L). For simplicity water exchange is not included in this version of the temporal model.

**Model II: Ammonium concentration of the MIRAS at Equilibrium**

The temporal model indicates that starting from the ammonium concentration of incoming seawater, the system ammonium concentration increases for a time and then reaches a steady state. When the system reaches this steady state or equilibrium, seaweed ammonium uptake occurs at the same rate as ammonium production by the fish. Therefore,

$$S_{eq} = P*B_f*K_s / (B_s * V_{max} - 1)$$
where $S_{eq}$ = the ammonium concentration of the system at time of equilibrium, $P_f$ = the ammonium production at a feed rate, $B_f$ is the fish feed rate ($g \text{ d}^{-1}$), $K_s$ = the half saturation constant, $B_s$ is seaweed biomass ($g \text{ FW}$) and $V_{max}$ is the maximum uptake rate. The correction for water exchange was calculated based on the volume of water exchanged ($Vol_x$) compared to the total system volume ($V_s$) and the difference between the concentration of the system at equilibrium ($S_{eq}$) and new water ($S_{new}$):

$$S_x = (Vol_x/Vol_s) \cdot (S_{eq} - S_{new})$$

The predictive model corrected for water exchange (Figure 4.5) was:

$$S_{eq} = \frac{(P_f \cdot B_f \cdot K_s)}{(B_s \cdot V_{max} - 1)} - S_x$$

where $S_x$ = the ammonium concentration released from the system via water exchange. The concentration of the system at equilibrium was calculated for a feed rate of 100 g feed d$^{-1}$ and expressed in terms of seaweed biomass to fish feed ratio. The equilibrium model was also expressed in terms of seaweed biomass and fish feed rate (Figure 4.6), rather than seaweed to fish feed ratio. The concentration of the system at equilibrium was calculated for feed rates of 50, 100, 200, 300, and 400 g feed d$^{-1}$ and seaweed biomass range of 0 to 6000 g FW seaweed.

**Model Validation:**

A series of integrated trials was run in the MIRAS at GBA to validate the predictive model (model II). When the integrated seaweed/fish trials began the fish tank (3150 L filled volume) was stocked with 205 fingerling Atlantic cod
(Gadus morhua) with a mean weight of 44.01 g per fish. Near the end of the integrated trials the fish had a mean biomass of 456 g and the total biomass was 18 Kg. Cod stocking density was kept low (2-4 Kg m\(^{-3}\)) to avoid stress factors that might affect feeding behavior and ammonium production. Two native Porphyra species were used as the biofilter component. When not available from cultures at the University of Connecticut, Porphyra umbilicalis was collected from Wallis Sands, Rye NH (43°01'40.68N",70°43'29.86"W) and P. linearis from Popple Cove, Gloucester MA (42°36'13.18N",70°30'02.09"W). Each seaweed species was investigated in separate experiments and the MIRAS was run in recirculating mode with a minimal water replacement of 39.4 L hr\(^{-1}\) (±23.5 SD) to replace water lost to evaporation and small leaks. The water replacement rate was controlled by a one way valve and measured by a flow meter. The system was set to recirculate at a turnover rate equal to one system volume every three hours. Dissolved oxygen was monitored periodically, and the water temperature and pH were monitored daily.

Prior to each trial the tanks were refilled with new seawater and then set to static mode and 1 L of chlorine was added. The chlorine was neutralized after 12-24 hours with 300 ml sodium thiosulfate. We began by using seaweed:fish biomass ratios of 0.2, 0.33, and 0.5 to test the steady state predictive model, but later we simply adjusted the seaweed biomass and used a feed rate of 100 g day\(^{-1}\). The environmental factors effecting N production (e.g. temperature and pH) were stable and so the shift to a constant fish feed ration led to a more consistent ammonium production rate. This allowed seaweed biomass was the
single variable. Since we were not concerned with maximizing fish growth rate, the fish were fed at a level slightly lower than the maximum recommended rate to ensure that they would consume the feed provided. Any unconsumed feed was removed from the tank through the drain at the bottom of the fish tank and filtered out via the parabolic filter. The fish usually consumed most of the food provided during the trials. On the occasion that there was an apparent decrease in fish appetite or increase uneaten feed in the parabolic filter, trials were suspended until the fish recovered.

The ammonium levels in seaweed and fish tanks were monitored daily and seaweed tissue samples were taken daily for pigment analysis. The seaweed was removed from the tanks twice weekly, squeezed dry and weighed. An amount equal to the original seaweed biomass was returned to the tanks; the excess was rinsed with freshwater and dried on racks in the greenhouse (Figure 4.8).

Ammonium recovered

Recovered NH₄-N was estimated from the concentrations of the system, the new replacement water and the replacement rate as follows:

\[ N_{\text{rec}} = 100 \times \frac{(N_{\text{exc}} - N_{\text{lost}})}{N_{\text{exc}}} \]

where \( N_{\text{rec}} \) = the percent of the ammonium excreted by the cod that was taken up by the seaweed biofilter, \( N_{\text{exc}} = \) ammonium excreted (mmol day\(^{-1}\)), and \( N_{\text{lost}} = \) ammonium lost from the system via water exchange.
The ammonium lost from the system is calculated as follows:

\[ N_{\text{lost}} = E \times S_x \cdot S_w \]

Where \( E \) = the water exchange rate \((L \text{ d}^{-1})\) and Where \( S_x \) = the ammonium concentration released from the system via water exchange \((\text{mM})\).

**Ammonium analysis**

All water samples were frozen and stored at \(-20^\circ\text{C}\) until analyzed. Ammonium concentration was measured via the salicylate method and the reagents used were from a salt water ammonium-N test kit, code 3304 (LaMotte Co, Chestertown, MD, USA). The method was modified for 1 ml water samples and concentrations were determined using a Helios Alpha UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

**RESULTS**

*Model I*

The temporal model indicated a linear increase in system ammonium concentration when the MIRAS is stocked with fish and set to recirculating mode with no water replacement (Figure 4.1). Further, the increase in ammonium concentration occurs more rapidly given a greater biomass of fish fed at 1% of their biomass per day. When a water exchange rate of 30 L h\(^{-1}\) is included in the model (Figure 4.2) the ammonium concentration increases linearly until approximately day 5 when the rate of increase becomes less rapid.
When 1500 g of *Porphyra umbilicalis* are added to the temporal model, system ammonium concentrations are predicted to be markedly lower than when the system has fish only. When starting from a low ammonium concentration, the temporal model (Figure 4.3) indicated that the ammonium concentration in the MIRAS would increase rapidly for an initial period of time and then level off. The model indicated that with the seaweed biofilter the MIRAS reached a point of equilibrium where the concentration was at a steady state and ammonium uptake and production were equal. The ammonium concentration at equilibrium is determined primarily by the linear rate of ammonium production, the biomass of the seaweed and the dynamic ammonium uptake characteristics of the seaweed. The predicted initial rate of increase of the ammonium concentration, the ammonium concentration at equilibrium, and the length of time the system takes to reach equilibrium decreased as the seaweed biomass to fish feed ratio increases, or as more seaweed biomass was added relative to daily feed rate.

The temporal model was also used to predict the effect of recirculation rate (L h$^{-1}$) into individual tanks (Figure 4.4). The model showed that high recirculation rates (e.g. 2520 L h$^{-1}$) decreased the difference between the equilibrium concentrations of the fish and seaweed tanks. While the average system ammonium concentration did not change, decreasing the flow rate into the tanks (e.g. 360 L h$^{-1}$) increased the ammonium concentration in the fish tank concentration and decreased the concentration in the seaweed tanks.
Model II

The ammonium vs biomass model produced the curves shown in Figure 4.5 and indicate that the steady state system ammonium concentration was an inverse function of the seaweed biomass to fish feed ratio. In other words, as seaweed biomass increased relative to the fish feed rate, the ammonium concentration started out very high and dropped rapidly until 50-100 μM. Below approximately 50 μM, the concentration decreased less rapidly with successive increases in seaweed biomass. The model indicated that there was a minimum seaweed biomass to fish feed ratio below which ammonium levels could rapidly increase to concentrations toxic for the fish. Conversely, there was a practical maximum seaweed to fish feed ratio above which additional seaweed would not significantly increase the biofiltering capacity. Additionally, the model for *P. umbilicalis* predicted higher ammonium concentrations than the model for *P. linearis* (Figure 4.5).

Modell II Validation

Each model validation trial was run for two to three weeks during which ammonium concentrations were consistently maintained at safe levels and the fish remained healthy. The details of individual trials are listed by *Porphyra* species and trial date (Table 4.1). Periodic measurements showed stable dissolved oxygen (DO) with a mean DO of 7.28 (SD ± 0.17) mg L$^{-1}$. The mean pH throughout the trials was 7.8 (SD ± 0.24) and temperature was maintained at 14.9 °C (SD ± 8.7) and 11.0 °C (SD ± 1.7) for the *P. umbilicalis* and *P. linearis*...
trials, respectively. All of the *Porphyra umbilicalis* trials resulted in system ammonium levels below those predicted by the model (Figure 4.7). Most of the *P. linearis* trials resulted in ammonium concentrations above what was predicted for *P. linearis* but below predicted values for *P. umbilicalis* (Figure 4.7). The efficiency of the seaweed biofilter, expressed as percent ammonium recovered, was calculated from system ammonium concentrations and the exchange rate for each trial. On average *Porphyra umbilicalis* recovered 95.26% (±0.82 SE) and *P. linearis* 85.54% (±3.40 SE) of the ammonium excreted by the fish.

The highest relative growth rate (RGR) of the seaweed was 15.14% (SE ± 1.71) for *Porphyra umbilicalis* and 11.89 (SE ± 1.38) for *P. linearis*. The growth of *P. umbilicalis* and *P. linearis* was not affected by seaweed to feed ratio (*P. umbilicalis*: R²=0.101, p=0.124 and *P. linearis*: R²=0.000, p=0.383) or system ammonium concentration (*P. umbilicalis*: R²=0.000, p=0.791 and *P. linearis*: R²=0.119, p=0.236). Stocking density ranged from 0.0688– 0.313 Kg m⁻³ for *P. umbilicalis* and 0.0688 – 0.25 Kg m⁻³ for *P. linearis*. While stocking density had no effect on the growth of *P. umbilicalis* (R²=0.0354), the relative growth rate of *P. linearis* was inversely related to stocking density (R²=0.693). Further, the *P. linearis* blades were collected during the winter, often from iced over rocks, and appeared to quickly become reproductive once in the MIRAS tanks.

Phycobilin content increased visibly in the first 24 hrs, regardless of system ammonium concentrations or seaweed to feed ratio. The maximum phycoerythrin (PE) content of *Porphyra umbilicalis* and *P. linearis* was 3.644 mg PE g FW⁻¹ (SE± 0.365) and 6.859 mg PE g FW⁻¹ (SE±0.228), respectively. The
maximum phycocyanin content (PC) of *P. umbilicalis* and *P. linearis* was 1.366 (SE± 0.146) and 1.840 (SE± 0.106), respectively. The phycobilin content was weakly related to the seaweed to feed ratio. The PE content of *P. umbilicalis* and *P. linearis* was inversely related to the seaweed to feed ratio (*P. umbilicalis*; R²=0.691, p=0.000 and *P. linearis*; R²=0.709, p=0.0098) as was the PC content (*P. umbilicalis*; R²=0.401, p=0.006 and *P. linearis*; R²=0.597, p=0.040).

**DISCUSSION**

The models described here are relatively simple compared to those of described for other recirculating systems and require only a few system parameters, including ammonium production rate (based on feed rate), system volume, water exchange, and seaweed nutrient uptake rate. The predictive models are tools that culturists can use to understand the nutrient dynamics of the MIRAS and determine optimal operational parameters such as seaweed biomass to fish feed ratio and water exchange rates.

The model reported by Buonomo and others (2005) predicts daily and seasonal effects on many system processes (including nutrients, detritus, and fish growth). Another model described by Pagand and others (2000) predicts the dissolved nitrogen content in seabass effluent using the variables of fish biomass (determined by initial weight and number of fish) and water replacement rate. The simulation model developed for an integrated seabream/ *Ulva* recirculating mariculture system (Ellner et al. 1996) predicts concentrations of dissolve
nitrogen species in various system components. The three models mentioned above accurately predicted nitrogen concentrations over the course of a year and therefore took into account seasonal effects. Additionally, while each of the previous models above have a biofilter component, which has a fixed capacity for nutrient removal. Our objective was to develop a simple model that could be used to determine optimal seaweed biomass needed to meet production goals and reduce the amount of nitrogen lost in the effluent.

In an integrated fish/seaweed recirculating system the operator must balance the potentially conflicting needs of the fish and seaweed, and address the issue of environmental nutrient loading. It is essential to maintain nutrient levels within the tolerance levels for the fish; for Atlantic cod the goal is to maintain ammonium concentrations under 1-2 ppm (56-112 μM NH₄⁺) to promote healthy growth of the fish (George Nardi and Chris Duffy pers comm., Foss et al. 2004). By contrast, seaweed growth and pigment production depend on having an adequate supply of nutrients (Lobban and Harrison 1994). If the seaweed component is serving solely as a biofilter, then the goal would be to maximize the biofilter nutrient removal and run the system at very low ammonium levels. However, if the seaweed is considered a secondary product then the culturist must maintain system nutrient levels that promote seaweed productivity. Additionally, higher system ammonium levels result in more ammonium loss in effluent that is exchanged for fresh seawater. If minimum nutrient discharge is required, then system nutrient levels should be kept lower than if the goal is simply to reduce environmental nutrient loading.
The first model (Figure 4.3) predicted system ammonium concentration as a function of time and showed that with adequate seaweed biomass, the ammonium concentration of the MIRAS came to equilibrium. Given set ammonium production and uptake rates, the predicted concentration at equilibrium was determined by seaweed biomass, or total nutrient uptake capacity. The model indicated that increasing the seaweed biomass, relative to fish biomass or feed rate will decrease both the ammonium concentration at equilibrium and time to equilibrium. The operator may also predict the effect of adjusting water flow rates, or seaweed biomass, on the ammonium level of individual tanks (Figure 4.4). Low recirculation rates result in high ammonium concentrations in the fish tanks and low concentrations in the seaweed tanks which is contrary to the objectives of fish health and seaweed productivity. Consequently, high recirculation rates are desirable.

The second model predicted the system ammonium concentration at equilibrium in relation to seaweed biomass and ammonium production rate (Figure 4.5). The model presented here was developed using feed ration rather than fish biomass, since this parameter could be held constant over the course of the study. The model can be easily reconfigured in terms of fish biomass and/or seaweed biomass (Figure 4.6). The culturist can use this model as a tool to determine the seaweed biomass needed for system specific feed consumption rates, ammonium production rates or fish biomass. Not surprisingly, the simulations run using this model indicate that choosing a very low seaweed biomass will result in extremely high system ammonium concentrations, which
would endanger the fish. Alternatively, very high seaweed biomass would remove almost all of the ammonium from the system. While low ammonium concentrations are ideal for promoting fish health, they do not support optimum seaweed growth. Therefore, if the seaweed is considered a secondary product, then it will be necessary to find an ammonium concentration that supports seaweed productivity while maintaining water quality that is safe for the fish. In the case of a system raising cod (Gadus morhua) and Porphyra umbilicalis, a target of 50-60 µM ammonium would meet both of these goals. The targeted ammonium level corresponds to the bend of the predictive model curve (Figure 4.5).

Both predictive models may be used to explore the effect of key parameters on the ammonium concentration of the system, or individual tanks. In an aquaculture operation, nitrogen production is likely to be of most interest. The ammonia/ammonium input into the system is influence by the amount of protein consumed, environmental factors and the fish species, age, and biomass (Beamish and Thomas 1984, Gallagher and Mathews 1987, Handy and Poxton 1993, Ip et al 2001, Jobling 1981, Lin and Yi 2003, Paulson 1978, Ramnarine et al. 1987, Rychly and Marina 1977, Wood 2001). For example, feed conversion ratios (FCRs) vary as fish age and between fish species (Copeland et al. 2003, Refstie et al. 2006, Rosenlund et al. 2004, Schuenhoff et al. 2003). The lower the FCR for a given fish, assuming the consumption rate is equal to the feed rate, the more efficiently the fish converts protein to muscle mass. It follows that a low the FCR will correspond to a relatively low N excretion rate, since a greater fraction
of feed nitrogen is tied up in muscle protein. Model I indicates that as the N production rate decreases relative to seaweed biomass, the system will equilibrate at lower ammonium concentrations in a decreases amount of time. Model II indicates that as N production decreases (e.g. lower FCR, fish biomass or fish feed consumption), the system will require less seaweed biomass to maintain a given system ammonium concentration and the shape the predictive curve changes such that the bend becomes sharper (Figure 4.6).

The most important aspects of biofilters, in the context of fish aquaculture, are their abilities to continuously take up ammonium at high rates and maintain safe system ammonium concentrations. Stable water quality is an essential characteristic of a recirculating system, as the lack of stability negatively affects fish health and environmental nutrient loading (Gal et al. 2007, Gelfand et al. 2003, Neori et al. 1996). Nutrient uptake rates based on short term studies or nutrient starved specimens may overestimate the capacity of seaweeds for long term nutrient removal. The study by Carmona and others (2006) reported long term (4 week) uptake rates for *P. umbilicalis* of that were much lower than in the present study. Although the predictive model developed for the MIRAS was based on short term (24 hr) studies, it used plants that were not nutrient starved because they were acclimated to existing MIRAS conditions.

While the nutrient uptake experiments were conducted using parameters that closely approximated the conditions in the integrated trials, there may have been some differences incurred by the experimental setup. Earlier studies indicated that ammonium uptake is affected by light and age of the seaweed
thalli (Caromona et al. 2006, Day 2003, Kraemer et al. 2004, Pereira et al. 2006). The uptake experiments were conducted in flask set on top of a MIRAS tank where the light intensity was likely to be more intense than in the large tanks. Further, the model was based on the ammonium uptake parameters of blades from the same collection as used to stock the tanks for the integrated trials. However, the uptake experiments were conducted soon after the seaweed was collected and therefore was likely to be in the best condition. The *P. linearis* appeared to quickly become reproductive, which may have affected uptake rates over time.

The high nitrogen uptake and growth rates of *Porphyra umbilicalis* and *P. linearis* suggested that these species would efficiently remove nutrients from aquacultural effluent (Kim et al. 2007). Our results demonstrated that these *Porphyra* biofilters consistently removed most of the ammonium generated by the fish and provided stable pH and ammonium levels within the system. The general agreement with results predicted by the model confirmed the consistency of the biofilter's capacity to treat fish effluent. While *P. linearis* performed well as a biofilter, system ammonium concentrations for all trials were slightly higher than predicted indicating that the short term uptake kinetics may have overestimated the long term nutrient uptake capacity of this species. Kim and others (2007) found that while the growth, phycobilin content and nitrogen uptake of *P. linearis* were comparable to *P. umbilicalis* at lower temperature levels, *P. umbilicalis* performed better at 20°C. High short term nutrient uptake at low temperatures may allow the high intertidal, winter-spring, seaweed *P. linearis* to take
advantage of the nutrients available on the occasion that it is exposed to
unfrozen seawater. By contrast, ammonium levels were lower than predicted for
*P. umbilicalis* which indicates that this species has a higher capacity for long term
ammonium uptake.

The increase in phycobilin content with increased nitrogen availability
(decreased seaweed to feed ratio) indicates that the available ammonium is
utilized for pigment production rather than growth. Further, the greater phycobilin
content and lower growth rates of *P. linearis* may be affected by light availability
since the trials of this winter-spring species, were run in December through
March when daily light availability is low.

The effect of stocking density on the growth rate of *P. linearis* may also
indicate a seasonal light limitation. The growth rates of *P. umbilicalis* and *P.
linearis* used in the MIRAS were comparable to those reported by Kim et al.
(2007) for the same species and *P. dioica* (Pereira et al. 2008) grown at 15°C
and 25 μM ammonium. However, growth rates were lower than those reported
for *Porphyra* species grown at higher ammonium levels of 150 to 300 μM
(Carmona et al. 2006, Day 2003, Kim et al. 2007, Pereira et al. 2008). Therefore,
if *Porphyra* biomass is considered a secondary product, the MIRAS should be
operated at the maximum ammonium concentration that is safe for the fish.

The *Porphyra* biofilter used in the MIRAS removed as much or more
nitrogen than other algal biofilters such as *Palmaria palmata* (L.) Kuntze,
*Chondrus crispus* (Stackhouse), and *Ulva lactuca* (L.) (Kebede-Westhead et al.
2006, Matos et al. 2006, Neori et al. 2003). Trial results indicated that *Porphyra*
*umbilicalis* was a slightly more efficient biofilter than *P. linearis* in the MIRAS. These results contrast with those reported by Kim et al. (2007) who found similar uptake capacities for *P. umbilicalis* and *P. linearis*. However, the majority of the *P. linearis* trials were run at lower seaweed to feed ratios, and efficiency may be reduced simply because the system ammonium concentrations were high and therefore more N is lost with each unit of water replaced.

The nutrient removal efficiency of the *Porphyra* in the present study were consistent with those reported by Carmona et al. (2006) which were 70-100%. Maximum nutrient removal (95-100%) by the *Porphyra umbilicalis* biofilter occurred when the system ammonium concentration was below 20 μM and this corresponded to seaweed to feed ratios of 15 and higher. The biofilter efficiency was still very high (above 90%) when system ammonium concentrations were 35 μM, or when the seaweed to feed ratios were greater than 7.5. The present results are consistent with previous studies of *Ulva* which found that effective buffering occurs at system nutrient concentrations corresponding to the middle of the ammonium uptake curve (Neori et al. 1996).

Cascade systems and polishing tanks (e.g. a series of seaweed tanks with increasing biomass) have been suggested to maximize ammonium removal (Matos et al. 2006, Neori et al. 2003). The *Porphyra* species used in the MIRAS efficiently reduced the nutrients released from the system without the need for a cascade system or polishing tanks. The efficiency of the biofilter was enhanced by the low water replacement rate which essentially held the nutrients in the system. Additionally the water in the seaweed tanks was constantly agitated via
aeration, presumably breaking the seaweed boundary layer. However, a polishing tank could be added to capture residual nutrients prior to release, depending on the goals of the system. If minimal nutrient release is desired, a polishing tank may be indicated, particularly if the MIRAS is run at high ammonium concentrations (low seaweed to feed ratio). Alternatively, the water replacement rate could be minimized.

While one of the most crucial aspects of a seaweed biofilter is its nutrient uptake efficiency, other factors must be considered depending on the goals of the aquaculture operation. Nitrogen limitation decreases *Porphyra* growth rate and pigment production, and so the choice of a low target ammonium level in the MIRAS may reduce seaweed productivity. If the seaweed is thought of as a secondary product, then it is important to choose a target ammonium concentration that results in high biofilter efficiency and supports seaweed growth and biochemical synthesis. The results of the integrated trials using the MIRAS suggested that the growth and pigment production of *P. umbilicalis* and *P. linearis* were not limited by nitrogen availability at any of the seaweed to feed ratios. Therefore choice of target ammonium concentration may be based on nutrient uptake efficiency and the fish's well being.

In summary, a predictive model was developed for a demonstration-scale modular recirculating aquaculture system using a seaweed biofilter. The MIRAS at GBA was successfully run continuously for one and a half years to test the system and evaluate the predictive model. The results indicate that *Porphyra umbilicalis* and *P. linearis* may be used as efficient biofilters to treat Atlantic cod
effluent in recirculating aquaculture systems. The use of these seaweed biofilters counter balance the metabolism of the fish and effectively capture dissolved nitrogen in harvestable seaweed biomass that can be used for a number of applications including, human food, pigment production, or as a protein source in finfish feed pellets. The trials showed that the model may be used to predict the effect of various parameters on systems nutrient levels. Therefore, an aquaculture operation may use the model to maintain desired system nutrient levels that will meet the needs of both the fish and seaweed and meet production goals. The MIRAS is a fully scalable system that may be used to expand the aquaculture industry while reducing the detrimental environmental effects of nutrient loading. The use of native or locally available Porphyra species to treat aquaculture effluent in a large scale recirculating system requires a consistent supply of Porphyra sporelings to stock tanks.
Model I: Predicted Ammonium Concentrations - MIRAS with Fish

\[ S_t = P \times B_f \times t / \text{Vol}_s \]

**Parameters:**

- Feed = 55% protein
- \( \text{NH}_4^+ \) excr = 4.72% feed protein
- \( P = 109.17 \ \mu\text{mol hr}^{-1} \ \text{g feed}^{-1} \)
- Feed rate = 1% Fish biomass d\(^{-1}\)
- \( B_f (\text{g feed d}^{-1}) = \text{variable} \)
- \( \text{Vol}_s = 12881 \ \text{L} \)

Figure 4.1: Predicted ammonium concentration (\(\mu\text{M}\)) in the MIRAS versus time (hours) when the system is recirculating with no water exchange. Curves represent ammonium predicted for the system with 10, 7.5 and 5 Kg cod (*Gadus morhua*) in the MIRAS fed at a rate of 1% fish biomass per day.
Model I: Predicted NH$_4^+$ Concentrations -MIRAS with Fish & Exchange Rate.

\[ S_t = S_{t-1} + \left( \frac{t}{V_{ol_x}} \right) \ast [P \ast B_f - V_{ol_x} (S_{t-1} - S_{\text{new}})] \]

**Parameters:**
- Feed = 55% protein
- NH$_4^+$ excr = 4.72% feed protein
- Feed rate = 1% Fish biomass d$^{-1}$
- $P = 109.17$ μmol hr$^{-1}$ g feed$^{-1}$
- $B_f$ (g feed d$^{-1}$) = variable
- $V_{ol_x} = 30$ L h$^{-1}$
- $S_{\text{new}} = 4$ μM
- $t = 6$ h
- $V_{ol_x} = 12881$ L

Figure 4.2: Predicted ammonium concentration (μM) in the MIRAS versus time (hours) when the system is recirculating with a low (30 L h$^{-1}$) water exchange rate. Curves represent ammonium predicted for the system with 10, 7.5 and 5 Kg cod (*Gadus morhua*) in the MIRAS fed at a rate of 1% fish biomass per day.
Model I: Predicted Ammonium Concentrations - MIRAS with Cod, *Porphyra* Biofilter & Water Exchange Rate

\[ S_t = S_{t-1} + \left( \frac{t}{Vols} \right) \left[ P^{*}Br - \left( B_s * V_{max} * S_{t-1} \right) (K_m + S_{t-1}) - Vol_x (S_{t-1} - S_{new}) \right] \]

**Parameters:**
- Feed = 55% protein
- $NH_4^+$ excr = 4.72% feed protein
- Feed rate = 1% Fish biomass d$^{-1}$
- $t = 6$ h
- $Vols = 12881$ L
- $P = 109.17$ μmol hr$^{-1}$ g feed$^{-1}$
- $B_r (g$ feed $d^{-1}) = \text{variable}$
  - $B_s = 1.5$ Kg FW
  - $V_{max} = 12.72$ μmol g FW$^{-1}$ hr$^{-1}$
  - $K_s = 61.33$ L hr$^{-1}$
  - $Vol_x = 30$ L hr$^{-1}$
  - $S_{new} = 4$ μM

**Figure 4.3:** Predicted ammonium concentration (μM) in the MIRAS versus time (hours). Curves represent ammonium predicted for the system with seaweed to fish feed ratios of 15, 20 and 30 (g FW seaweed: g feed d$^{-1}$). For the demonstration scale MIRAS the seaweed biomass:feed ratios represent when the cod are fed 50, 75 or 100 g feed d$^{-1}$ and the biofilter has 1.5 Kg of *Porphyra umbilicalis*. 
Model I: Predicted Ammonium Concentrations – Effect of Recirculation rate on MIRAS Fish and Seaweed Components

\[S_f = S_{t-1} + \left( \frac{t}{V_{olf}} \right) \left[ P \cdot B_f + R \cdot (S_{sys} - S_{t-1}) \right]\]

\[S_{sw} = S_{t-1} + \left( \frac{t}{V_{olsw}} \right) \left[ \left( B_s \cdot V_{max} \cdot S_{t-1} \right) / (K_s + S_{t-1}) + R \cdot (S_{sys} - S_{t-1}) \right]\]

Parameters:
- Feed = 55% protein
- NH₄⁺ excr = 4.72% feed protein
- \(t = 1\) h
- \(P = 109.17\) μmol hr⁻¹ g feed⁻¹
- \(B_f = g\) feed d⁻¹ = 100 g d⁻¹
- \(B_s = 1500\) g FW
- \(V_{max} = 12.72\) μmol g FW⁻¹ hr⁻¹
- \(K_s = 61.33\) L h⁻¹

Component Vol = variable
- \(V_{olf} = 3150\) L
- \(V_{olsw} = 9731\) L

Recirc. Rate = variable:
- A: \(R = 2520\) L hr⁻¹
- B: \(R = 360\) L hr⁻¹

Figure 4.4: Predicted ammonium concentration (μM) in the MIRAS versus time (days). Curves represent predicted ammonium concentration for the fish tank and seaweed tanks when the flow rate into the tanks is set at 2520 L h⁻¹ (A) or 360 L h⁻¹ (B). The ammonium dynamic parameters used in the model are for cod and \(P. umbilicalis\) in the MIRAS.
Model II: Predicted Ammonium Concentrations - MIRAS at Equilibrium

\[ S_{eq} = \left( P \cdot B_f \cdot K_s \left( B_s \cdot V_{max}^{-1} \right) \right) - \left( V_{ol} \cdot V_{ol_s} \right) \left( S_{eq} - S_{new} \right) \]

**Parameters:**

- \( P = 109.17 \mu \text{mol hr}^{-1} \text{ g feed}^{-1} \)
- Feed = 55% protein
- \( B_s = 1500 \text{ g FW} \)
- \( S_{new} = 4 \mu \text{M} \)
- \( \text{P. umbilicalis} \)
  - \( V_{max} = 12.72 \mu \text{mol g FW}^{-1} \text{ hr}^{-1} \)
  - \( K_s = 61.33 \text{ L h}^{-1} \)
- \( \text{P. linearis} \)
  - \( V_{max} = 9.68 \mu \text{mol g FW}^{-1} \text{ hr}^{-1} \)
  - \( K_s = 8.52 \text{ L h}^{-1} \)
  - \( \text{NH}_4^+ \text{ excr} = 4.72\% \text{ feed protein} \)
- \( B_f = \text{Feed rate} = 100 \text{g d}^{-1} \)
- \( \text{Vol}_s = 12881 \text{ L} \)
- \( \text{Vol}_x = 300 \text{ L} \)

Figure 4.5: Ammonium concentration at system equilibrium versus seaweed biomass to daily cod feed ratio. For the demonstration scale MIRAS the seaweed to feed ratios represent increasing seaweed biomass while the fish are fed 100g feed per day. The curves represent predicted ammonium levels when \textit{Porphyra umbilicalis} and \textit{P. linearis} are used as the biofilter and cod are used as the N source.
Model II: Predicted Ammonium Concentrations - MIRAS at Equilibrium

\[
S_{eq} = (P*B_f*K_s/(B_s*V_{max}^{-1})) - (Vol_s/Vol_s) * (S_{eq}-S_{new})
\]

**Parameters:**
- Feed = 55% protein
- \(NH_4^+\) excr = 4.72% feed protein
- \(P = 109.17\ \mu mol\ \text{hr}^{-1}\ \text{g\ feed}^{-1}\)
- Feed rate = 1% fish biomass \(d^{-1}\)
- \(B_f = \text{variable fish biomass}\)
- \(B_f = 5\ \text{Kg}\)
- \(B_f = 10\ \text{Kg}\)
- \(B_f = 20\ \text{Kg}\)
- \(B_f = 30\ \text{Kg}\)
- \(B_f = 40\ \text{Kg}\)
- \(S_{new} = 4\ \mu M\)
- \(Vol_s = 12881\ L\)
- \(Vol_s = 300\ L\)
- \(V_{max} = 12.72\ \mu mol\ \text{g\ FW}^{-1}\ \text{hr}^{-1}\)
- \(K_s = 61.33\ \text{L\ hr}^{-1}\)

**Figure 4.6:** Ammonium concentration at system equilibrium versus seaweed biomass for cod fish feed rates of 50, 100, 200, 300 and 400 grams per day. The curves represent predicted ammonium levels when *Porphyra umbilicalis* is used as the biofilter.
Model II: Validation via Integrated Porphyra/Cod Trials

\[ S_{eq} = \left( P \cdot B_f \cdot K_m / (B_s \cdot V_{max} - 1) \right) - (V_{oi}/V_{os}) \cdot (S_{eq} - S_{new}) \]

**Parameters:**
- Feed = 55% protein
- \( \text{NH}_4^+ \) excretion = 4.72% feed protein
- \( P = 109.17 \, \mu\text{mol g}^{-1} \text{hr}^{-1} \)
- \( B_f = \text{feed rate} = 100 \, \text{g d}^{-1} \)
- \( B_s = \text{variable} \)
- \( V_{oi} = 145.5 \, \text{L h}^{-1} \)
- \( V_{os} = 12881 \, \text{L} \)
- \( S_{new} = 4 \, \mu\text{M} \)

**P. linearis**
- \( V_{max} = 9.68 \, \mu\text{mol g FW}^{-1} \text{hr}^{-1} \)
- \( K_s = 8.52 \, \text{L h}^{-1} \)

**P. umbilicalis**
- \( V_{max} = 12.72 \, \mu\text{mol g FW}^{-1} \text{hr}^{-1} \)
- \( K_s = 61.33 \, \text{L h}^{-1} \)

Figure 4.7: Ammonium concentrations from integrated trials using Porphyra umbilicalis and P. linearis compared to ammonium levels predicted by the predictive model. Results are presented based on seaweed biomass to cod fish feed ratio.
Figure 4.8: *Porphyra umbilicalis* that was produced in the MIRAS spread out on a drying rack in the greenhouse (A) and a close up of the seaweed (B).
Table 4.1: Results of Integrated trials listed by *Porphyra* species and the date associated with the beginning of the system equilibrium period.

<table>
<thead>
<tr>
<th>Date</th>
<th>Seaweed Biomass (g FW)</th>
<th>Fish Biomass (g WW)</th>
<th>Feed Rate (g day⁻¹)</th>
<th>New Water NH₄ (µM) (SE)</th>
<th>Fish Tank NH₄ (µM) (SE)</th>
<th>Seaweed Tanks NH₄ (µM) (SE)</th>
<th>Ave Tank NH₄ (µM) (SE)</th>
<th>AGR (% day⁻¹) (SE)</th>
<th>RGR (% day⁻¹) (SE)</th>
<th>NH₄ recovered (%)</th>
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<tbody>
<tr>
<td>2/2/2005</td>
<td>1000</td>
<td>4090</td>
<td>15</td>
<td>2.78 (1.75)</td>
<td>7.11 (1.25)</td>
<td>4.51 (1.20)</td>
<td>5.81 (1.29)</td>
<td>11.94 (na)</td>
<td>10.22 (na)</td>
<td>98.07</td>
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<tr>
<td>12/11/2005</td>
<td>550</td>
<td>18263</td>
<td>100</td>
<td>7.29 (2.04)</td>
<td>74.75 (14.02)</td>
<td>65.91 (12.37)</td>
<td>68.85 (12.90)</td>
<td>5.70 (1.08)</td>
<td>5.11 (0.14)</td>
<td>77.19</td>
</tr>
<tr>
<td>12/23/2005</td>
<td>850</td>
<td>18263</td>
<td>100</td>
<td>10.54 (3.91)</td>
<td>40.88 (11.66)</td>
<td>38.14 (5.88)</td>
<td>39.05 (8.14)</td>
<td>6.45 (0.90)</td>
<td>5.91 (0.01)</td>
<td>87.94</td>
</tr>
<tr>
<td>1/11/2006</td>
<td>1500</td>
<td>18263</td>
<td>100</td>
<td>8.30 (2.10)</td>
<td>43.05 (8.51)</td>
<td>39.48 (2.95)</td>
<td>40.67 (3.19)</td>
<td>8.15 (0.50)</td>
<td>7.05 (0.36)</td>
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</tr>
<tr>
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<td>100</td>
<td>2.19 (0.98)</td>
<td>101.25 (6.11)</td>
<td>92.65 (7.45)</td>
<td>95.52 (7.06)</td>
<td>5.68 (0.25)</td>
<td>7.75 (0.56)</td>
<td>67.67</td>
</tr>
<tr>
<td>2/7/2006</td>
<td>1500</td>
<td>18263</td>
<td>100</td>
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<td>43.89 (2.77)</td>
<td>30.70 (2.38)</td>
<td>35.03 (3.97)</td>
<td>7.55 (2.60)</td>
<td>7.02 (0.88)</td>
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<tr>
<td>2/26/2006</td>
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<td>100</td>
<td>-1.41 (1.44)</td>
<td>56.89 (2.87)</td>
<td>47.57 (3.23)</td>
<td>50.45 (3.81)</td>
<td>15.31 (1.07)</td>
<td>11.89 (1.38)</td>
<td>83.77</td>
</tr>
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*Porphyra umbilicalis*
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93


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