SUMMER GROWTH NEAR SALMON SEA CAGES AND NITROGEN UPTAKE OF KELP

By

OK-HYUN AHN

F.Sc., Pukyong National University, Korea, 1991

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE in THE FACULTY OF GRADUATE STUDIES (CHEMICAL & BIO-RESOURCE ENGINEERING DEPARTMENT)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
October 1997

© Ok-hyun Ahn, 1997
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.
ABSTRACT

The purpose of this study was to test the hypothesis: a fish farm can fertilize kelp during summer when only nitrogen is a limiting factor for the growth of kelp.

Temperature (10.0-14.7°C), salinity (22.3-30.0‰), water visibility (3.5-9.5 m) and daytime-irradiance data at 2 m depth in summers of 1995 and 1996 indicated suitable levels for the growth of kelp.

Three kelp species, Laminaria saccharina, L. groenlandica and Nereocystis luetkeana, were collected from salmon fish farm nets and cultivated adjacent to and far from a salmon farm. Distal sites as control sites did not receive effluent from salmon farms. During the summer of 1995, the blade growth rates in length at the sites ranged from 2.50-5.4% d⁻¹ for L. saccharina and 5.15-6.57% d⁻¹ for N. luetkeana. Significant differences in the blade growth between L. saccharina control and experimental plants were observed (p > 0.05), and C/N ratio in the plant tissues increased with distance from the salmon farm. At both the farm and control sites, ambient NO₃⁻ concentrations were similar (above 12 μM). Ammonium concentration adjacent to the farm varied from 1.7-13.3 μM, while at the control site it was < 2 μM.

For two consecutive 4-weeks experiments from May-July in 1996, in general, significant differences in growth rates were recorded between three species of control and experimental plants ranging from 2.70-3.79% d⁻¹ from May-June and 2.16-4.79% d⁻¹ from June-July. The ambient concentrations of NO₃⁻ near the fish farm and control sites were low (< 5μM). The concentrations of NH₄⁺ next to sea cages varied over time (1-34 μM) and were associated with fish feeding events.

In the nitrogen uptake experiments involving various combinations of NH₄⁺ and NO₃⁻ concentrations, the uptakes of NO₃⁻ by both L. saccharina and Nereocystis increased linearly
with dissolved inorganic nitrogen (up to 70 μM). In contrast, NH$_4^+$ uptake by the kelps was saturated at approximately 11-13 μM. Only *Nereocystis* exhibited preference of NO$_3^-$ over NH$_4^+$ when they were present equally in medium of 20 μM NO$_3^-$ plus 20 μM NH$_4^+$ (p > 0.05).

Any site where ambient concentration of ammonium is high (near 15 μM) during summer may be suitable for both *L. saccharina* and *Nereocystis*. However, it seems more efficient to fertilize kelp by nitrate than ammonium since nitrate uptake by both species increased linearly without the effect of external concentration of ammonium in the medium.
# TABLE OF CONTENTS

ABSTRACT ...................................................................................................................... ii
TABLE OF CONTENTS .................................................................................................... iv
LIST OF FIGURES ........................................................................................................ vi
LIST OF TABLES ........................................................................................................... viii
ACKNOWLEDGEMENTS ................................................................................................. ix

GENERAL INTRODUCTION .......................................................................................... 1
  a. General Biology of Kelp ......................................................................................... 4
  b. General Kelp Cultivation Techniques ................................................................. 5
  c. Nutrient Loading from Fish Farms .................................................................... 6
  d. Integrated Culture of Seaweed/Animal ............................................................. 7

CHAPTER 1. SUMMER GROWTH OF KELP NEAR BRITISH COLUMBIAN SALMON SEA CAGES

  1.1 Introduction ........................................................................................................ 9
  1.2 Objectives ........................................................................................................ 12

  1.3 Materials and Methods
    1.3.1 Study Sites .................................................................................................... 13
    1.3.2 Plant Preparation ......................................................................................... 13
    1.3.3 Plant Measurement ...................................................................................... 15
    1.3.4 Kelp Line Installation .................................................................................... 15
    1.3.5 Environmental Parameters ......................................................................... 16
    1.3.6 Nitrate and Ammonium Analysis ............................................................... 17
    1.3.7 Theoretical Growth Model .......................................................................... 17
    1.3.8 Tissue Nitrogen Analysis ............................................................................ 20
    1.3.9 Statistical Analysis ....................................................................................... 20

  1.4 Results
    1.4.1 Environmental Parameters ........................................................................ 22
    1.4.2 Nitrate and Ammonium Analysis ............................................................... 22
    1.4.3 C/N ratio in Tissue ....................................................................................... 25
    1.4.4 Mortality and Growth Rate ......................................................................... 29
    1.4.5 Comparison of Actual Growth to Theoretical Growth ............................... 34
    1.4.6 Estimation of Benefit of Fish Culture Effluent for Kelp Growth ................. 39

  1.5 Discussion ........................................................................................................... 41
CHAPTER 2. AMMONIUM AND NITRATE UPTAKE BY Laminaria saccharina AND Nereocystis luetkeana ORIGINATING FROM A BRITISH COLUMBIAN SALMON SEA CAGE FARM

2.1 Introduction ........................................................................................................ 48
2.2 Objectives ............................................................................................................ 51
2.3 Materials and Methods
   2.3.1 Plant Materials .......................................................................................... 52
   2.3.2 C/N ratio Analysis ...................................................................................... 53
   2.3.3 Analysis of Ammonium and Nitrate Next to Sea Cages ......................... 54
   2.3.4 Statistical Analysis .................................................................................... 54
2.4 Results ................................................................................................................. 55
2.5 Discussion ............................................................................................................ 61

OVERALL DISCUSSION .......................................................................................... 65
CONCLUSION ........................................................................................................... 67
SUGGESTIONS FOR FUTHER WORK ...................................................................... 69
REFERENCES ............................................................................................................ 71
LIST OF FIGURES

Figure 1. The locations of the study sites. ................................................................. 14

Figure 2. Current speed (cm s\(^{-1}\)) and direction (0=North, 90=East, 180=South and 270=West) next to sea cages at 2 m depth at Simond’s Bay from July-August in 1995 and at Cliff Bay from May-August in 1996. Rectangles indicate net pen system locations. Gray lines next to the systems indicate kelp rope locations. ................................................................. 24

Figure 3. The ambient concentration of ammonium next to sea cages at 2 m depth on May 18, June 18 and July 18-19, 1996. Error bar denotes standard error of hourly samples from triplicates and vertical lines indicate fish feeding times on certain days. ................................................................. 26

Figure 4. The ambient concentration of nitrate next to sea cages at 2 m depth on May 18, June 18 and July 18-19, 1996. Error bar denotes standard error of hourly samples from triplicates and vertical lines indicate fish feeding times on certain days. ................................................................. 27

Figure 5. Theoretical changes in ammonium concentration next to sea cages at Simond’s Bay in 1995 depending on current speed and direction. Current A flows through the side of largest numbers of cages whereas Current B flows through the side of smallest number of cages. A vertical line in Graph B denotes average current speed during the time. ...... 36

Figure 6. Theoretical changes in ammonium concentration next to sea cages at Cliff Bay in 1996 depending on current speed and direction. Current A flows through the side of largest numbers of cages whereas Current B flows through the side of smallest number of cages. A vertical line in Graph B denotes average current speed during the time. ...... 37

Figure 7. Currents flowing within the above angles were assumed to bring fish culture effluent to kelp. Data within the angles were counted to estimate the benefit of fish culture effluent as fertilizer during the summer of 1995 and 1996. Gray lines next to sea cages indicate kelp ropes and arrows denote current directions. ................................................................. 38

Figure 8. C and N contents in 2x2 cm tissue samples at the beginning and the end of the growth periods. ................................................................. 43

Figure 9. Time course of decrease in nitrate and ammonium in four combinations of culture medium for 7x7 cm tissue segments from first-year plants of
*Laminaria saccharina* and *Nereocystis luetkeana* at 12°C under 125rpm agitation and 135 μmol photon m⁻² s⁻¹ of irradiance.

Figure 10. Ammonium and nitrate uptake rates for *Laminaria saccharina* and *Nereocystis luetkeana* in four culture media containing different concentrations of NH₄⁺ and NO₃⁻ for the first hour of 3 h incubation.
LIST OF TABLES

Table 1. Various environmental parameters measured next to sea cages during the summers of 1995 and 1996. ............................................................... 23

Table 2. Total C and N contents of three 2x2 cm tissue segments for Laminaria saccharina, L. groenlandica and Nereocystis between two consecutive measurements during the experimental period (C and N expressed as % dry weight and as C/N ratio by weight). ...................................................... 28

Table 3. Differences in growth of kelp (Laminaria saccharina and Nereocystis luetkeana) during July-June in 1995. .................................................. 30

Table 4. Differences in growth of kelp (Laminaria saccharina, L. groenlandica and Nereocystis luetkeana) during May-June in 1996. .............................. 31

Table 5. Differences in growth of kelp (Laminaria saccharina, L. groenlandica and Nereocystis luetkeana) during June-July in 1996. ............................. 32

Table 6. The estimation of the benefit of fish culture effluent as fertilizer for growth of kelp at the sites during the time, based on current meter readings (current direction and speed). Data were sampled every 30 min during the summer of 1995 and 1996. ................................................................. 40

Table 7. The wet and dry weights (g) and total C and N contents of 7x7 cm tissue segments for Laminaria saccharina and Nereocystis luetkeana (C, N expressed as % dry weight and as C:N ratios by weight; Mean values ± SE for Wet and Dry weights, and W/D; n = 6 for wet and dry weights and n = 3 for C:N ratios by weight). ......................................................... 57

Table 8. Ammonium and nitrate uptake rates in pmol g-1 dry wt h-1 (± SE where n = 3) for L. saccharina and Nereocystis luetkeana in four combinations of NH₄⁺ and NO₃⁻ during a 3 h incubation. .................................................. 59
ACKNOWLEDGEMENTS

Many people have made contributions to this research. Although I cannot begin to mention them all, there are those among them that deserve special thanks.

I would like to express my sincere appreciation to my supervisor Dr. Royann Petrell for her suggestion, guidance and financial support throughout my graduate studies, and for constructive criticisms in reviewing my thesis. I wish to express my deep gratitude to Dr. Paul Harrison for reviewing and revising my thesis work at various stages of the research. Also, special appreciation is extended to Dr. Anthony Lau for his review, comments and interest. I am also thankful to Dr. Louis Druehl at Simon Fraser University for his guidance and knowledgeable advice during a course work at Bamfield Marine Station, Vancouver Island, B.C., to Dr. Steve Pond at U.B.C. for providing a current meter for the research that is very delicate and expensive and to Dr. Jeong-Ha Kim for providing research facilities.

Thanks are also extended to my colleague, Mr. Eric Boucher, for helping and making field trips joyful. The technical advice of the other colleagues, Mr. Chang-Six Ra, Ward Prystay and Keng-Pee Ang is also appreciated. I would also like to thank the people in the Department of Oceanography for their technical assistance for water and C/N analysis.

I gratefully acknowledge Mr. Sylvain Alie and the fish farmers of Heritage Salmon Ltd. for their assistance and interests during the research. I also acknowledge the Natural Science and Engineering Research Council and Heritage Salmon Ltd. for their financial support and the Department of Chemical and Bio-Resource Engineering for laboratory facilities.

Special thanks must also be given to all of those who made indirect contributions: to Dr. Chul-Hyun Sohn, Dr. Sung-Bum Huh and Dr. Ki-Wan Nam at Pukyong National University, Korea for their encouraging for graduate studies and writing the references for my graduate studies at U.B.C. and the members of Vancouver Lutheran Church and friends for their encouragement throughout all my studies.

My most heartfelt appreciation, however, goes to my parents and sisters for their patience, understanding, advice and encouragement. Their support has made, and will forever make my life’s challenges seem so much easier.
1. GENERAL INTRODUCTION

During the last decade aquaculture has become a well-established industry in the world. In 1994, the production of finfish, shellfish and aquatic plants reached 25.5 million tonnes with a value of US$ 39.83 billion, in which the aquaculture production of aquatic plants reached 6.89 million tonnes with a value of US$ 6.05 billion (SOFIA, 1996). The demand of seaweed has expanded along with the increase of the world population and the interest in health foods (Csavas, 1990). The global demand of fish has also dramatically increased and was projected by FAO in 1995 as 140-150 million tonnes for the year of 2010. Among fish production, total salmon production in the world now exceeds 1.4 million tonnes per annum, of which farmed salmon contributes about 440,000 tonnes (SOFIA, 1996).

However, concerns has risen due to rapid expansion of finfish farming that fish farm may negatively impact the environment (Gowen et al., 1987; Folke and Kautsky, 1989; Ackerfors and Enell, 1990; Findlay et al., 1995; Dosdat et al., 1995; Kautsky et al., 1996). Intensive cultivation generates large amounts of organic and inorganic waste (uneaten food, faecal and excretory material) all of which are continuously produced and released into the environment (Gowen and Bradbury, 1987). Many methods intended to reduce the environmental degradation have been discussed, such as the rotation of cultivation areas, use of ecological feeds, increase of the food conversion index, installation of collecting or diffusion devices under the cages (Buschmann, 1996). These solutions may require an additional net cost to the producer. Thus, integrated farming techniques have been focused to utilize the waste products of high trophic-level cultivated fish by integrating invertebrates and seaweed. The advantages of integrated culture of salmon/kelp may be summarized as waste removals by biofilters, increasing seaweed yield, multiple crop, oxygen supplementation and the share of farm labor and facilities (Petrell et al., 1993; Petrell and Alie, 1995; Buschmann, 1996; Kautsky et al., 1996).
In temperate coastal waters, the availability of inorganic nitrogen is a critical limiting factor for the production of seaweed during late spring and throughout summer, even though temperature and irradiance are optimal (Chapman and Craigie, 1977; Chapman et al., 1978; Hanisak, 1983). However, unlike nitrogen, phosphorus is generally not considered to be a limiting nutrient in the sea (Lobban and Wynne, 1981). During times of nitrogen shortage, some large scale Laminaria farms in China routinely use fertilizer to enhance the growth of plants (Tseng 1981). Chapman and Craigie (1977) observed significant growth improvement of Laminaria longicruris by NaNCh supply during a dissolved nitrogen deficient period. Topinka and Robbins (1976) obtained better growth for Fucus spiralis by enrichment with NH\textsubscript{4}+ and NO\textsubscript{3}\textsuperscript{-} than plants maintained in ambient summer seawater. The use of recycling animal effluent seawater as fertilizer has been proposed to remove dissolved ammonium and increase the yields of seaweed (Ryther et al., 1975; Harlin et al., 1979; Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Subandar et al., 1993; Buschmann et al., 1994). In a case study that integrated salmon and the red alga, Gracilaria in an tank cultivation system, fish culture effluent as a nitrogen source proved to be effective for the high yields of the plants (Buschmann et al., 1994).

Canada is the fourth largest producer of farm salmon in the world (BCMAFF, 1991). Salmon farming in British Columbia is the most important aquaculture activity. In 1995, B. C. produced 28,100 tonnes of product with the total farmgate value of $170.6 million (BCMAFF, 1995). In addition, B.C. is known to have a vast potential to produce large volumes of seaweed products (BCMAFFa, 1991). Marine plant farming technologies have been developed and successfully applied commercially on a small scale in B.C. for several local edible kelp species and Japanese Porphyra species (Druehl, 1987; BCMAFF, 1991). Druehl (1987) reported that the coastal environment of Northwest Pacific is well suited to marine plant production and supports 15 genera and 50 species of kelp, which may provide many options for different products from different species of kelp.
In Canada, especially in B.C., the integrated culture of kelp/salmon may be ideal to enhance the production of kelp since the application of artificial fertilizer is not allowed in the Federal Fisheries Act to protect fish habitats and the Canadian Environmental Protection Act prohibits ocean dumping of almost all substances without a permit. The integrated culture of kelp/salmon was evaluated by a mathematical production model and proved to be technically and economically feasible in B.C. (Petrell et al., 1993; Petrell and Alie, 1995). Buschmann (1996) also suggested that the seaweed could result in higher production if the cultivation is installed near the salmon cages, as compared to a monoculture. However, the technologies for integrated culture of kelp/salmon have not generally been implemented on a commercial scale. Further research is needed to determine which species is the best suited for integrated culture of salmon/kelp, if a salmon farm can replace the need of artificial fertilizer and if final products are valuable for commercial usage.

This lack of information on the integrated culture of salmon/kelp provided motivation for the present study. Kelp growth studies were conducted at variable distances from salmon sea cages for two consecutive summers in 1995 and 1996, and an ammonium and nitrate uptake experiment was also carried out in a growth chamber room to examine nutrient uptake by the two species of kelp originating from salmon sea cages where ammonium concentration is richer than background levels.
a. GENERAL BIOLOGY OF KELP

Kelp are species of brown algae belonging to the order Laminariales. They are distributed worldwide along rocky coasts in both temperate and cold waters (Lüning, 1985). In Laminariales, there are three large families, Laminariaceae, Lessoniaceae and Alariaceae. The largest genera is *Laminaria* of approximately 30 species (Guiry and Blunden, 1991). Generally, most of *Laminaria* species are perennial, maturing in 2 to 3 years, and grow best at 8-16°C (Lobban and Harrison, 1994) and from the lowest part of the intertidal zone to about 28 m, where they become light limited (McConnaughey and Zottoli, 1983). Their sporophyte consists of a root-like holdfast, a strong flexible stipe, or stem, and a large flat blade, which in some species is dissected. Growth occurs in the basal part of the blade, and fragmentation and loss occur near the tips (McConnaughey and Zottoli, 1983). The harvestable sporophyte alternates with microscopic gametophytes (Lobban and Harrison, 1994).

*Laminaria saccharina* (L.) Lamouroux is widely distributed in north-west Europe and Northwest Pacific and occurs on the lower shore and in the sublittoral while small, isolated plants are found in rock pools on the middle shore (Fish and Fish, 1996). It has a rich brown colour with a conspicuous holdfast, blade and stipe. The stipe varies in length from 5 to 50 cm and from 6 to 9 mm in diameter. It is terete, flattening above the base of blade. The blade is 12-18 cm wide and 2.5-3.5 m long (Scagel, 1967).

*Laminaria groenlandica* Rosenvinge is a perennial and each subsequent year class has a broader blade. It grows on rocks in the lower intertidal or upper subtidal zone, especially in areas with substantial water motion. This species occurs from Alaska to southern Oregon (Waaland, 1977). Generally, a blade is up to 2 m long and 0.5 m wide, with or without bullae, often torn. A stipe length is usually 10-30 cm, slightly flattened or terete and a holdfast has many branched haptera (Druehl, 1980a).
Nereocystis luetkeana (Mertens) Postels and Ruprecht belonging to the family Lessoniaceae, is one of the largest and most common kelps in the Northeast Pacific Ocean. It is distributed from Northern California to Alaska (Abbott and Hollenberg, 1976). The plant body is composed of root-like haptera at the base, a long tubular stipe, bladder and a large number of (16-66) of ribbon-like blades attached to bladder (Merrill and Gillingham, 1991). The blades are linear, 6-15 cm wide and up to 4.5 m long (Scagel, 1967).

b. GENERAL KELP CULTIVATION TECHNIQUES

Edible Laminaria was collected by the Ainu in northern Japan as early as the eighth century A.D (Druehl, 1988), and its cultivation was initiated in China and Japan in the early 1950s (Tseng, 1981).

The cultivation technique consists of two major components: (a) Seedstock production, which involves establishment of small sporophytes on string or other easily transported substrate. (b) Raft/rope cultivation in the sea, which involves commercial systems for rearing seedstock maturity in the sea and usually consist of an anchored horizontal or vertical grid of ropes buoyed 2-7 m below the surface (Druehl, 1988).

Many edible Laminaria species require two years to obtain properties desirable to Asian cuisine (Druehl, 1988). However, with the development of forced-cultivation method of seedstock by Hasegawa (1971), total cultivation period of Laminaria can be shortened. Sporophytes, which are produced from seedstock in the summer and spend 3 months in the autumn in the field prior to their second growth season, behave as second-year plants (Druehl, 1988; Lobban and Harison, 1994). The technique of Laminaria cultivation varies slightly due to dependency on the Laminaria species, environmental factors such as temperature, nitrogen availability and salinity, and cultivation strategy for final products. For example, the cultivation
techniques in Japan differs somewhat from that used in China due to the different latitude (Guiry and Blunden, 1991).

In Europe, the species cultivated are *Laminaria saccharina*, *Alaria esculenta* and *Saccorhiza polyschides* using Asian techniques (Guiry and Blunden, 1991). In contrast, in British Columbia, Canada, a series of experimental farms were set up, and *Laminaria groenlandica*, *L. saccharina* and *Cymathere triplicata* were examined in pilot project (Druehl et al., 1988b). The production technique of seedlings were adapted from Japanese protocol and evolved by other researchers and farmers. String seeded with spores was maintained in a greenhouse and outplanted in January and February by inserting short lengths into the rope at 30 cm intervals along a 60 m kelp rope, which was held horizontally at 2 m below the sea surface at several different sites. When cultivated in the sea, these seedlings of three species produced up to 8 wet kg m\(^{-1}\) of cultivation rope for *L. saccharina*, 20 wet kg m\(^{-1}\) for *L. groenlandica* and 2.7 wet kg m\(^{-1}\) for *C. triplicata*. These values are similar to those reported from other parts of the world (Druehl et al., 1988b).

c. NUTRIENT LOADING FROM FISH FARM CAGES

Modern technologies for intensive fish farming involving higher stocking densities and inputs of feed, increase the nutrient load discharged from a fish farm (Gowen and Bradbury, 1985; Ackerfors and Enell, 1990; Findlay et al., 1995). The investigations of environmental impacts caused by net-pen aquaculture on marine benthic communities have been conducted with the measurement of material fluxes in sea cage farms including sedimentary processes (Hall et al., 1992; Findlay et al., 1995). Fish farm waste generally includes organic carbon and organic nitrogen (carbohydrate, lipid and protein), ammonium, urea, bicarbonate, phosphate, vitamins, therapeutants and pigments (Gowen and Bradbury, 1985). The input of soluble nitrogenous compounds generally cause hyper-nutrification of coastal waters (Ryther and Dunstan, 1971) and
soluble nitrogenous waste (ammonium and urea) from fish farms may create similar effects (Gowen and Bradbury, 1985).

The total load of N and P from a typical cage-fish farm is estimated to be 78 kg and 9.5 kg, respectively, per ton of fish produced per year with a feed coefficient (wet weight of feed used per weight fish produced) of 1.5. (Ackerfors and Enell, 1990). However, there may be enormous variability in the rate of waste build up due to local site conditions, species, feed type and management (Beveridge, 1987). With 0.9% phosphorus and a feed coefficient of 1.5, 2.2 kg of phosphorus are dissolved and 7.3 kg of particulate are produced per tonne of fish (Ackerfors and Enell, 1990). In contrast to the P load, the main part of the total nitrogen load is in the dissolved form, mainly as urea and ammonium from the fish excreta (Fivelstad et al., 1990). A salmonid fish-cage farm produced approximately 32 kg of ammonium per tonne of food fed, which was similar with the estimates of ammonium production by salmonids in land-base farms: 45 kg/tonne of fish produced (Warrer-Hansen, 1982); 55 kg/tonne of fish produced (Solbe, 1982). Buschmann (1996) observed that ammonium in salmonid effluents from intensive tank systems reached concentrations as high as 500 µg L⁻¹ in spring and summer. Gowen and Bradbury (1987) estimated 4.0 tonnes of soluble nitrogenous waste (3.2 tonnes of ammonium-N, 0.8 tonne of Urea-N) to be produced from a salmon farm with an annual production of 50 tonnes. Hall et al. (1992) found that of the total nitrogen input to a trout sea cage farm, 27 to 28% was recovered in harvest, while fish loss constituted 2 to 5%, and 67 to 71% (or 95 to 102 kg N per tonne produced fish) was lost to the aquatic environment.

d. INTEGRATED CULTURE OF ANIMAL/SEAWEED

Integrated culture, or Polyculture – the cultivation of different species within the same cultivation or similar environment – is strongly recommended as a very practical, productive and cost-efficient way of growing organisms (Indergaard and Jensen, 1983; Petrell et al., 1993;
Petrell and Alie, 1995; Kautsky et al., 1996). Integrating farming is an alternative way to achieve sustainable production in coastal areas and also produce income for the producers (Buschmann, 1996). Integrated pond cultivation is well developed in China, where four or more fish species are commonly grown together (Stickney, 1994). Mussel production can increase next to a fish farm since they utilize particulate feed fragments from pellets (Wallace, 1980).

In the past time, however, traditional aquaculture technique in China and Europe has relied almost exclusively on the resources shared and recycled locally with animal and crop production.

Integrated fish/seaweed cultivation experiments have used both open and semi-closed culturing techniques. Buschmann et al. (1996b) found that an intensive cultivation system for *Onchorhynchus kisutch, O. mykiss* and *Gracilaria chilensis* is feasible, resulting in 30 kg m$^{-3}$ of fish production with a food conversion of 1.4 g food g fish$^{-1}$ production and 48.9 kg m$^{-2}$ year$^{-1}$ for *Gracilaria* production. *Laminaria* cultivation near salmon sea cages is economically feasible due to higher production produced as compared to a monoculture (Petrell et al., 1993; Petrell and Alie, 1995). The rapid growth in length for *Fucus esiculosus* was obtained in the vicinity of a fish farm due to a constantly higher nutrient availability (Rönnberg et al., 1992). Mathematical models have also been developed for the integrated culture of kelp/salmon (Petrell et al., 1993; Petrell and Alie, 1995) and of salmon, mussels and seaweed in floating system (Bodvin et al., 1996) to access the feasibility of the integrated culture of animal/seaweed. Integrated aquaculture of fish, mussels and seaweed based on ecological engineering has been reported to reduce energy subsidies, costs and environmental impacts per unit of production (Kautsky et al., 1996).
1. Summer Growth of Kelp

Near British Columbian Salmon sea cages

1.1 INTRODUCTION

Growth and productivity of seaweeds are controlled by environmental factors such as irradiance, temperature, nutrient availability and water movement (Lobban et al., 1985). As stated previously, vital nutrients are available adjacent to a salmon farm. The question arises: Can seaweeds benefit from such nutrients?

Nitrogen availability plays an important role in the seasonal growth of kelp since its availability exhibits seasonal variability: the highest concentration is in winter and the lowest concentration is in summer (Chapman and Craigie, 1977; Chapman and Lindley, 1980; Wheeler and North, 1980; Gagne et al., 1982; Sjøtun, 1993). This pattern was experimentally observed in the field (Chapman and Craigie, 1977; Druehl, 1980a, 1980b; Gagne et al., 1982), and it has been proposed that the summer decline of growth due to low external N-supply could be enhanced by inorganic fertilization (Chapman and Craigie, 1977; Neushul et al., 1992). Some kelps are known to build up internal reserves of nitrogen in winter to maintain a high growth rate through most of the summer (Chapman and Craigie, 1977; Gerard and Mann, 1979; Chapman and Lindley, 1980). In contrast, Macrocystis pyrifera was revealed not to build a large internal pools of NO$_3^-$ as do some Laminaria spp. (Chapman and Craigie, 1977; Gerard and Mann, 1979; Gerard, 1982). During the winter, L. longicuris accumulated NO$_3^-$ as a N reserve up to 150 μmoles per g fresh weight, and rapid growth for L. longicuris was maintained for about 3 months after depletion of the ambient NO$_3^-$ and for approximately 1 month after the internal reserves of NO$_3^-$ were exhausted (Chapman and Craigie, 1977). However, starting with high
internal nitrogen reserves, *Macrocystis pyrifera* can sustain relatively rapid growth for at least 2 to 3 wk in the absence of a significant external N supply (Gerard, 1982).

Unlike nitrogen, phosphorus, which is one of element required by kelp, is generally not considered to be a limiting nutrient in the marine environment (Lobban and Wynne, 1981). The major form in which algae required phosphorus is as orthophosphate ions (Sze, 1991).

Water movement stimulates algal metabolism and nutrient uptake since turbulence enhances gas exchange and nutrient diffusion through the nutrient depleted boundary layer surrounding algal cells (Gerard and Mann, 1979; Wheeler, 1980; Lobban *et al.*, 1985). In stagnant water, photosynthesis, respiration and growth of seaweed is greatly restricted (Lobban *et al.*, 1985), and seaweeds may become less resistant to diseases (Merrill and Gillingham, 1991). Water motion, which is too violent, however, can limit production of kelp (Gerard and Mann, 1979). A good current or tidal mixing is considered to be an important criterion for a kelp farm site (Druehl, 1980a, 1987; Merrill and Gillingham, 1991).

Light provides the initial energy for photosynthesis, and ultimately for all biological processes and acts as a signal to control the reproduction, growth and distribution of seaweed (Lobban *et al.*, 1985). In general, in coastal areas, kelp become light-limited at depths of 30 m or less (McConnaughey and Zottoli, 1983). Gerard (1988) reported that the photosynthetic capacity and efficiency is generally higher for plants from the turbid habitat than plants from deep and shallow habitats since ecotypic differentiation exists. Three of European *Laminaria, L. digitata, L. saccharina* and *L. hyperborea* showed saturation of photosynthesis at about 150 μmol photon m$^{-2}$ s$^{-1}$. In general, irradiance saturation level of Laminariales and other macrophytes is between 30-100 μmol photons m$^{-2}$ s$^{-1}$ (Harrison and Druehl, 1982). Some *Laminaria* species near polar regions are exposed to lower annual inputs of solar radiation than their more equatorial counterparts. In Canadian Arctic, an annual photon-flux fluence rate (PFFR) for growth in *L. solidungula* was about 49 annual mol photons m$^{-2}$ for the lower depth limit of 20 m (Chapman
and Lindly, 1980). Similarly, 70 mol photon m$^{-2}$ y$^{-1}$ for *Laminaria digitata* was obtained for the lower limit of 8 m (Lüning and Dring, 1979).

Growth rate of seaweed is certainly affected by temperature and, as for individual enzyme reaction, there is generally a peak or high plateau above and below which growth rate falls off (Lobban *et al.*, 1985). However, in situations where light or nutrients are limiting, temperature may have little effect on growth (Lobban *et al.*, 1985). Atlantic *Laminaria saccharina* exhibited the optimal growth in length (15-18% d$^{-1}$) at 10-15°C in a culture (Bolton and Lüning, 1982).
1.2 OBJECTIVES

This study is the first to cultivate kelps adjacent to salmon farms.

The hypothesis is:

Kelps cultivated adjacent to salmon sea cages may receive the significant benefit of nitrogen release produced by fish for better growth, quality, and production during summer when nitrogen availability is the only growth limiting factor. The actual growth will be tested by a previously developed model (Petrell and Alie, 1995).

The objectives of kelp growth study are:

(1) To determine which species is more suitable for integrated culture of salmon/kelp.

(2) To validate an existing kelp production model with actual field growth data and refine it, if necessary.

(3) To measure ammonium dilution effects on kelp growth and N content in kelp tissue as a function of distance from a salmon farm.

(4) To determine a suitable depth of kelp lines when installed near a fish farm.

(5) To test the suitability of salmon/kelp integration in field.

(6) To apply the results to husbandry of kelp cultivation.
1.3 MATERIALS AND METHODS

1.3.1 STUDY SITES

Kelp growth studies were conducted during the summers in 1995 and 1996 at salmon sea cage farms operated by B.C. Packers LTD and located in northwestern B.C., Canada. The first summer's growth study was conducted between July 7 to August 17 in 1995 at Wehlis Bay and Simond’s Bay. The second summer’s growth study was performed at Cliff Bay from May 16 to July 15 in 1996 (Fig. 1). Fish farms selected for the studies had the largest fish biomass for those periods. Sea cages were 15 m x 15 m x 15 m deep. At Simond’s Bay, two sets of sea cage systems were positioned approximately 200 m apart. Each set were arranged 2 by 4 and 2 by 6. In contrast, the cages at Cliff Bay were arranged 2 by 10. Both fish farms generally produced approximately 660,000 kg of total fish biomass/2 y.

The oceanographic information near Wells Passage, including Wehlis Bay and Simond’s Bay, reveal temperatures of 9 to 12°C and salinity of less than 24 ppt for the 2 to 5 m depths during the summer season. The temperature near Cliff Bay was record to be 7.6 to 12.5°C for the late spring and early summer period (PBCMAF, 1987). Cliff Bay is located near Tribune Channel. This site was known to have an environment of relatively low wave stress and slow current, fairly constant temperature at 12.7°C and a salinity near 26.0%o at 2 m depth in the summer (PBCMAF, 1987).

1.3.2 PLANT PREPARATION

Three species of kelp, Laminaria saccharina, Laminaria groenlandica and Nereocystis luetkeana, were used for the studies not only because they grow naturally on fish farm nets, but because the kelps are already known to be commercially valuable. The success of these kelps on fish farm nets suggested that they are tolerant of the ammonium released from the fish farms and
Fig. 1. The location of the study sites.
utilize ammonium as a nitrogen source for growth. All plants (thali stage) were collected on fish farm nets. On the same day, they were kept in ice coolers filled with seawater and ice-packs, and immediately transported to an experimental site. The plants were maintained inside of a sea cage at the site until they were installed on each of 15m long kelp lines. During the summer in 1995, only two species of kelp, *L. saccharina* and *N. luetkeana* were used since insufficient numbers of *L. groenlandica* were available. In the summer of 1996, three species of kelp (including *L. groenlandica*) originating from the experimental site, Cliff Bay, were used for the growth experiment.

1.3.3 PLANT MEASUREMENT

The plants of each species were randomly selected, tagged and measured in total wet weight, the length and width of laminae, and the length of a stipe. For *N. luetkeana*, a diameter of pneumatocyst perpendicular to the stipe direction and the number of laminae were also measured. The laminae of *Nereocystis* was trimmed at around 5–30 cm above a pneumatocyst, but the laminae of *Laminaria* about 10–20 cm above the junction between a laminae and a stipe, respectively. After the measurements of growth parameters, the holdfast of each plant was inserted into a 15 cm long kelp at 30 cm intervals to avoid light competition between plants. Total experimental periods were approximately 7 weeks in 1995 and 9 weeks in 1996. The growth measurements were carried out on the initial day and 3-4 week intervals during the experiment periods. The appearance of the first sorus and epiphytes were also recorded at each measurement periods.

1.3.4 KELP LINE INSTALLATION

At the beginning of kelp growth study in 1995, 40 plants, 20 plants of each species, were used for a kelp line. Eight kelp lines were used for four treatments. Six kelp lines out of eight
were installed at three varied distances from net pen system: two kelp lines were installed far away from sea cages as a control site (Wehlis Bay) where the site had no fish culture effluent effect; to investigate the effects of ammonium dilution on kelp growth, 4 kelp lines were installed at two varied distances from net pen system (adjacent to and about 8 m away from sea cages) at the experimental site (Simond’s Bay). Two kelp lines for *Nereocystis* were also installed adjacent to sea cages at 1 m depth to examine the effect of depth of kelp lines on the growth.

In contrast, for the second growth study in the summer of 1996, 4 kelp lines (each line had a total of 60 plant-20 plants of each of the three species) were installed both at approximately 300 m away from net pen system (control site) and adjacent to sea cages (experimental site) at Cliff Bay.

Kelp lines were horizontally positioned at 2 m depth. During plant transportation to experimental sites, handling, and measurements, care was taken to avoid overheating, desiccation, and physical damage.

1.3.5 ENVIRONMENTAL PARAMETERS

In the summer of 1995, the water temperature, salinity, current directions and speeds were measured by S4 current meter (Ocean Inc.) at 2 m depth every 30 min from July 6\textsuperscript{th} to August 17\textsuperscript{th}. A lab top computer was used to transfer the accumulated environmental data from the S4 current meter. Light intensity was measured daily at 14:00 using a Lambda Instrument LI 192S quantum sensor. Water visibility was also recorded by a secchi disc daily at 14:30. During the summer of 1996, these environmental data were obtained monthly for 2-3 days from May to August.
1.3.6 NITRATE AND AMMONIUM ANALYSIS

The water samples to determine the ambient concentration of NO$_3^-$ and NH$_4^+$ near sea cages were taken into 500 ml plastic bottles at 2 m depth, both at the control and experimental sites at the beginning (July 13$^{\text{th}}$, 1995) and at the end (August 17$^{\text{th}}$, 1995) of the growth study period. After water sampling, they were immediately frozen in a refrigerator on the farm until they were transported to the lab. A different treatment for water samples was introduced in 1996. All water samples were filtered through a 1 μm G/FF syringe filter, poisoned with 0.01 ml chloroform /10 ml seawater to improve the preservation of these samples, and stored in 30 ml of polypropylene bottles. By using this method the samples are valid at ambient temperature for up to one week (Dosdat et al., 1995).

Water samples were taken in duplicate next to cages at 2 m depth and near the control site every month during the experimental period. Furthermore, in order to investigate the availability of ammonium and nitrate for the growth of kelp, water samples were taken hourly in triplicate next to cages at 2 m depth over a minute every month from May to July.

Samples were analyzed by a Technicon Autoanalyzer II using standard techniques (Harrison et al., 1986).

1.3.7 THEORETICAL GROWTH MODELS

The specific growth rate $G_k$, reported here as the percentage increase in length, width and weight per day, was calculated by the equation:

$$G_k = 100 \times (\ln X_2/X_1)/t$$  \hspace{1cm} (1)

where $X_1 =$ initial length, width, or weight, and $X_2 =$ length, width, or weight increase on day $t$. 

17
To compare actual growth data to theoretical growth, a mathematical model of integrated culture of salmon/kelp developed by Petrell and Alie (1995) was used. The basic assumptions in the model were:

1) Kelp growth is limited by inorganic dissolved nitrogen availability and seawater temperature.

2) The seasonal adjustments of kelp lines could meet the light requirement for the kelp growth.

3) Kelp grows only using ammonium excreted by fish (background nitrate and ammonium concentration was excluded).

4) Phosphorus is not a limiting nutrient

The theoretical ammonium excretion rate (kg d$^{-1}$ kg of fish mass$^{-1}$) based on feeding rate F (kg of feed kg$^{-1}$ d$^{-1}$) was calculated using the following equation of Liao and Mayo (1972):

$$\text{Ammonia excretion} = 0.0289F$$

(2)

The above equation was also valid for Atlantic salmon (Korman, 1989; Bergheim et al., 1991).

Ammonium concentration (μM) in the cage system was written as:

$$\text{Ammonium concentration (μM)} = \frac{\text{ammonia excretion}}{(\text{area} \times \text{velocity} \times 1000)}$$

(3)

where

\[\text{area} = \text{cage width} \times \text{cage depth} \ (m^2)\]

\[\text{velocity} = \text{current velocity} \ (m \ s^{-1})\]

The above equation was experimentally validated using salmon water from intensive fish culture up to a concentration of 9 μM dissolved inorganic nitrogen (Petrell et al., 1993).
The specific growth rates $G_k$ (% length increase d$^{-1}$) of *Laminaria saccharina*, *L. groenlandica* and *Nereocystis luetkeana* were estimated using the following equation (Chapman *et al.*, 1978) since the plants have similar nutrient uptake and temperature ranges for growth (Hurd *et al.*, 1994b). The saturated concentration of dissolved inorganic nitrogen (DIN) for kelp growth was assumed to be 10 µM (Chapman *et al.*, 1978):

\[
G_k = 1.5[DIN] (0.08T + 0.2) \text{ for } 5 < T < 10^\circ C \tag{4.1}
\]
\[
G_k = 1.5[DIN] \text{ for } 10 < T < 15^\circ C \tag{4.2}
\]

Petrell and Alie (1995) developed the logistic growth equation to predict change in size over time. The calculated maximum change in length under optimal condition was found to be close to published values for both *Nereocystis* and *Laminaria* (Petrell and Alie, 1995). The equation was validated by comparing actual kelp growth in the field to theoretical kelp growth with environmental inputs.

\[
L_{k2} = \frac{L_{k1} \exp^{G_k(t_2-t_1)}}{1 - \beta L_{k1}(1-\exp^{G_k(t_2-t_1)})} \tag{5}
\]

where $\beta = 1/L_{\text{max}}$

$L_{\text{max}}$ = maximum, average, or obtainable length

for *Nereocystis* 10 m and for *Laminaria* 2.8 m

$L_{k1}$ = initial length at time $t_2$

$L_{k2}$ = new length at time $t_1$

$t_{1-t_2}$ = growth interval time

$G_k$ = actual specific growth rate (% d$^{-1}$)
1.3.8 TISSUE NITROGEN ANALYSIS

Three 2x2 cm size of tissue samples were obtained from three tagged plants of each species to examine whether plants were nutrient deficient during experiment periods. The tissue samples for *Nereocystis* were taken approximately 10 cm away from the junction between bulbs and blades, and the tissue samples for *Laminaria* were obtained approximately 10 cm away from stipes at the beginning and the ending of the growth experiments. They were kept in 30 ml polypropylene bottles and transported to the laboratory at U.B.C. The plants were dried in an oven at 70°C for 24 h, and then ground manually into a homogeneous fine powder. Total carbon and nitrogen were determined as described by Verardo *et al.* (1990), using a Carlo Erba Analyzer NA-1500 at the Oceanography Department, U.B.C.

1.3.9 STATISTICAL ANALYSIS

Statistical analysis was performed with the Sigmastat windows program. The effects of ammonium dilution on the growth of the control (adjacent to a net pen system) and experimental plants (8m away from a net pen system) of *Nereocystis* and *L. saccharina* were tested by two sample t-test. A one-way ANOVA was used to test whether fish culture effluent contributes to significant growth enhancement of kelp among three treatments which were a function of distance from the fish farm, far away from the net pen system, 8 m away from the sea cages and adjacent to the sea cages. A Turkey test was also conducted to isolate the treatment, which differs from the others in growth rates of plants. However, in 1996, a two sample t-test was performed to test ammonium effects on kelp growth between two treatments, far away from and adjacent cages to the net pen system. Furthermore, in order to examine which species is more suitable for integrated culture of salmon/kelp, a one-way ANOVA test was conducted, using the growth rates of laminae of near-farm plants both in 1995 and 1996. A Kruskal-Wallis one-way
ANOVA test was used when no equal variance exits between treatments. In all cases, the null hypothesis was rejected at the 95% probability level ($p < 0.05$).
1.4 RESULTS

1.4.1 ENVIRONMENTAL PARAMETERS

Environmental parameters obtained at the two experimental sites, Wehlis from July-Aug. in 1995 and Cliff Bay from May-July in 1996, were summarized in Table 1. Water temperature (10.0-14.7°C), water visibility (3.5-9.5 m) and salinity (22.3-30.0‰) at both experimental sites were similar and fairly constant during the experimental period between 1995 and 1996 and remained within the levels suitable for the growth of kelp.

However, a pronounced difference in water movement between the sites at 2 m depth was observed (Fig. 2). Generally, water movement at Cliff Bay in 1996 was low (0-15 cm s⁻¹) and averaged as 2.7 cm s⁻¹ since the site was located in a sheltered area. No certain current direction was observed. In contrast, at Wehlis in 1995, current speed ranged from 0-59.1 cm s⁻¹ with high variation in current speed over time (avg. 14 cm s⁻¹) since the site was stationed near the junction with Sutlej Channel known to be a well mixed and turbulent zone caused in part by the tidal jet streaming out of Stuart Narrows from Drury Inlet (PBCMAF, 1987). Most currents were heading toward the northeast or southwest.

Ambient light levels at the experimental sites varied greatly over time. Light levels recorded daily at 2:00 p.m. at Wehlis ranged from 24-450 μmol photon m⁻² s⁻¹ and 20-1025 μmol photon m⁻² s⁻¹ during daytime at Cliff Bay.

1.4.2 NITRATE AND AMMONIUM ANALYSIS

The ambient concentration of nitrate was also considerably influenced by the location of the sites. Water analysis indicated that in 1995 nitrate concentration at both the control (Wehlis Bay) and experimental sites (Simond's Bay) were high (12.3-13.3 μM), which is higher than typical nitrate levels in summer in the sea, whereas the concentrations of NH₄⁺ were less than 2
Table 1. Various environmental parameters measured next to sea cages during the summers of 1995 and 1996.

<table>
<thead>
<tr>
<th></th>
<th>1995 Summer (July-Aug.) at Simond’s Bay</th>
<th>1996 Summer (May-Aug.) at Cliff Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10.0-13.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Irradiance (μmol m² s⁻¹)</td>
<td>24-450</td>
<td>233.8</td>
</tr>
<tr>
<td>Current speed (cm s⁻¹)</td>
<td>0.0-59.1</td>
<td>14.0</td>
</tr>
<tr>
<td>Turbidity (m)</td>
<td>3.5-9.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>25.1-30.0</td>
<td>27.6</td>
</tr>
</tbody>
</table>
Fig. 2. Current speed (cm s\(^{-1}\)) and direction (0=North, 90=East, 180=South and 270=West) next to sea cages at 2 m depth at Simond's Bay from July-August in 1995 and at Cliff Bay from May-August in 1996. Rectangles indicate net pen system locations. Gray lines next to the systems indicate kelp rope locations.
μM at the control site and 1.72-7.5 μM at the experimental site. At Cliff Bay in 1996, water analysis results obtained hourly next to sea cages showed a high variation in NH$_4^+$ concentration over time (Fig. 3). The current direction may account for the fluctuation in NH$_4^+$ concentration over time since water samples were taken at the one side of the net pen system. The concentration of NH$_4^+$ was remarkably influenced by fish feeding activities and ranged between 1-34 μM. In general, 2 hours after fish feeding NH$_4^+$ next to the cages increased to over 5 μM for 14 h, and then reached the typical summer concentration of NH$_4^+$ (< 2 μM). However, the concentration of NO$_3^-$ was less than 5 μM and averaged 1.7 μM (Fig. 4).

1.4.3 C/N RATIO IN TISSUE

Effects of external nitrogen on total C and N contents of *L. saccharina*, *L. groenlandica* and *Nereocystis* cultured near the sea cages (experimental site) and far away from the sea cages (control site) are presented in Table 2. The plants cultivated far from sea cages where there is no influence of fish culture effluent on the growth of plants were assigned as control plants and the plants grown next to sea cages and 8 m away from sea cages were considered to be experimental plants.

The C/N ratios of plants cultured in 1996 were generally higher than those in 1995. The C/N ratios of *L. saccharina* in 1995 were slightly higher than those of *Nereocystis*. Significant differences in C/N ratios were not observed between experimental and control plants both for *L. saccharina* and *Nereocystis*. In 1996, the control plants showed significantly higher C/N ratios ranging from 14.3-37.7, compared to those in 1995. The experimental plants cultivated from June-July in 1996 also showed high C/N ratios (13.7-20.6).

Total tissue nitrogen in the control and experimental plants of *L. saccharina* and *Nereocystis* in 1995 ranged from 1.97-2.25% and 2.33–2.80% of dry weight, respectively. On
Fig. 3. The ambient concentration of ammonium next to sea cages at 2 m depth on May 18, June 18 and July 18-19, 1996. Error bar denotes standard error of hourly samples from triplicates and vertical lines indicate fish feeding times on certain days.
Fig. 4. The ambient concentration of nitrate next to sea cages at 2 m depth on May 18, June 18 and July 18-19, 1996. Error bars denote standard error of hourly samples and vertical lines indicate fish feeding times on certain days.
Table 2. Total C and N contents of three 2x2 cm tissue segments for *Laminaria saccharina*, *L. groenlandica* and *Nereocystis luetkeana* between two consecutive measurements during the experimental period (C and N expressed as % dry weight and as C/N ratio by weight).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%N</td>
<td>%C</td>
<td>C/N</td>
<td>%N</td>
<td>%C</td>
<td>C/N</td>
<td>%N</td>
<td>%C</td>
<td>C/N</td>
</tr>
<tr>
<td><strong>Initial Time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. saccharina</em></td>
<td>2.55</td>
<td>26.84</td>
<td>10.53</td>
<td>1.81</td>
<td>19.41</td>
<td>10.72</td>
<td>1.73</td>
<td>18.94</td>
<td>10.95</td>
</tr>
<tr>
<td><em>N. luetkeana</em></td>
<td>3.65</td>
<td>36.01</td>
<td>9.87</td>
<td>1.93</td>
<td>24.27</td>
<td>12.58</td>
<td>1.68</td>
<td>17.33</td>
<td>10.32</td>
</tr>
<tr>
<td><em>L. groenlandica</em></td>
<td></td>
<td></td>
<td></td>
<td>2.28</td>
<td>21.33</td>
<td>9.36</td>
<td>2.45</td>
<td>23.64</td>
<td>9.65</td>
</tr>
<tr>
<td><strong>In 21 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. saccharina</em></td>
<td>2.25</td>
<td>30.54</td>
<td>13.57</td>
<td>1.24</td>
<td>26.57</td>
<td>21.43</td>
<td>0.87</td>
<td>27.14</td>
<td>31.20</td>
</tr>
<tr>
<td><em>N. luetkeana</em></td>
<td>2.80</td>
<td>25.22</td>
<td>9.01</td>
<td>1.50</td>
<td>21.4</td>
<td>14.27</td>
<td>0.76</td>
<td>24.72</td>
<td>32.53</td>
</tr>
<tr>
<td><em>L. groenlandica</em></td>
<td></td>
<td></td>
<td></td>
<td>1.13</td>
<td>27.7</td>
<td>24.51</td>
<td>0.86</td>
<td>32.40</td>
<td>37.67</td>
</tr>
<tr>
<td><strong>Adjacent to cages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. saccharina</em></td>
<td>1.97</td>
<td>23.78</td>
<td>12.07</td>
<td>2.19</td>
<td>21.91</td>
<td>10.00</td>
<td>1.86</td>
<td>25.51</td>
<td>13.72</td>
</tr>
<tr>
<td><em>N. luetkeana</em></td>
<td>2.42</td>
<td>21.47</td>
<td>8.87</td>
<td>2.39</td>
<td>19.72</td>
<td>8.25</td>
<td>1.49</td>
<td>24.84</td>
<td>16.67</td>
</tr>
<tr>
<td><em>L. groenlandica</em></td>
<td></td>
<td></td>
<td></td>
<td>1.56</td>
<td>23.18</td>
<td>14.86</td>
<td>1.35</td>
<td>27.77</td>
<td>20.57</td>
</tr>
<tr>
<td><strong>8m away</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. saccharina</em></td>
<td>2.08</td>
<td>29.4</td>
<td>14.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. luetkeana</em></td>
<td>2.33</td>
<td>22.51</td>
<td>9.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control = far away from sea cages with no fish culture effluent effect on kelp growth
8 m away = 8 m away from sea cages
the other hand, in 1996, total nitrogen content in tissues of control plants showed distinct nutrient-limitation trends with low N% and high C/N ratio values during the period. Generally, the C/N ratios of control plants ranged from 0.8-1.2%, which were 50% lower than those of experimental plants. In June-July, the N values in tissue of control plants were less than 1% of dry weight, which indicate a 2 to 3 fold change in N content of plants after being transplanting to the control site.

1.4.4 MORTALITY AND GROWTH RATE

There was a great loss of tagged plants on several occasions: handling, strong water movement, the colonization of epiphyte and fish feed organic particles, kelp grazers, and inefficient holdfast fixation on kelp lines. The plants (63-73%) were lost in the summer of 1995 mainly due to strong current (max. 57 cm s\(^{-1}\) at the experimental site) and handling. Plants were frequently lost due to a breakage of the stipe or a breakage between stipe/bulb and lamina. In contrast, in the summer of 1996, the control and experimental plants (10-60%) were lost mostly due to severe bryozoan colonization (*Membranipora membranacea*, a member of invertebrate), which increased blade loss during the onset growth. In addition, in the summer of 1995 next to sea cages at 1 m depth, *Nereocystis* was all deteriorated when exposed to strong sunlight. From June to July of 1996, the de-pigmentation and severe erosion of distal lamina in the control plants resulted in a shortening of the laminae during the time presumably due to the nitrogen-limitation stress and heavy colonization by bryozoan. However, fewer plants were lost in 1996 due to strong currents and handling because each holdfast was wrapped with a rubber band.

The growth rates in terms of laminar increment in length and width, biomass increment and total length increment during 1995 and 1996 summer growth study periods, are summarized in Table 3, 4 and 5.
Table 3. Differences in growth of kelp (Laminaria saccharina and Nereocystis luetkeana) during July-June in 1995.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Laminaria saccharina</th>
<th>Nereocystis luetkeana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant n</td>
<td>Blade Length Growth Rate (cm d⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>2.50 (±1.38)</td>
</tr>
<tr>
<td>Next to cage</td>
<td>12</td>
<td>5.84 (±0.86)</td>
</tr>
<tr>
<td>1 m depth</td>
<td>12</td>
<td>3.75 (±1.57)</td>
</tr>
<tr>
<td>2 m depth</td>
<td>12</td>
<td>4.86 (±1.95)</td>
</tr>
<tr>
<td>8 m from cage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N/A: Not applicable
Like superscript in a column indicates insignificant difference (p<0.05)
Table 4. Differences in growth of kelp (Laminaria saccharina, L. groenlandica and Nereocystis luetkeana) during May-June in 1996.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Plant n</th>
<th>Blade Specific Growth Rate in Length (% d⁻¹)</th>
<th>Blade Specific Growth Rate in width (% d⁻¹)</th>
<th>Total length Specific Growth Rate (% d⁻¹)</th>
<th>Specific Growth in terms of Mass (% d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminaria saccharina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>1.68 (±1.01)ᵃ</td>
<td>0.76 (±0.46)ᵃ</td>
<td>1.58 (±0.92)ᵃ</td>
<td>4.12 (±1.85)ᵃ</td>
</tr>
<tr>
<td>Next to cage</td>
<td>15</td>
<td>3.70 (±1.09)ᵇ</td>
<td>2.77 (±0.73)ᵇ</td>
<td>3.51 (±1.03)ᵇ</td>
<td>7.78 (±2.18)ᵇ</td>
</tr>
<tr>
<td>Nereocystis luetkeana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>1.42 (±0.73)ᵃ</td>
<td>N/A</td>
<td>0.96 (±0.45)ᵃ</td>
<td>3.74 (±0.88)ᵃ</td>
</tr>
<tr>
<td>Next to cage</td>
<td>14</td>
<td>2.80 (±1.25)ᵇ</td>
<td>N/A</td>
<td>2.15 (±0.91)ᵇ</td>
<td>5.47 (±1.58)ᵇ</td>
</tr>
<tr>
<td>Laminaria groenlandica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>1.48 (±0.71)ᵃ</td>
<td>0.62 (±0.32)ᵃ</td>
<td>1.42 (±0.65)ᵃ</td>
<td>5.15 (±2.17)ᵃ</td>
</tr>
<tr>
<td>Next to cage</td>
<td>11</td>
<td>2.70 (±0.79)ᵇ</td>
<td>2.21 (±0.30)ᵇ</td>
<td>2.62 (±0.74)ᵇ</td>
<td>7.25 (±0.89)ᵇ</td>
</tr>
</tbody>
</table>

N/A: Not applicable
Like superscript in a column indicates insignificant difference (p<0.05)
Table 5. Differences in growth of kelp (*Laminaria saccharina*, *L. groenlandica* and *Nereocystis luetkeana*) during June-July in 1996

<table>
<thead>
<tr>
<th>Sites</th>
<th>Plant n</th>
<th>Blade Specific Growth Rate in Length (% d&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Blade Specific Growth Rate in width (% d&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Total length Specific Growth Rate (% d&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Specific Growth in terms of Mass (% d&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laminaria saccharina</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
<td>4.08 (±0.74)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 (±0.55)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.41 (±0.71)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.73 (±1.39)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Next to cage</td>
<td>14</td>
<td>4.79 (±0.34)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.67 (±1.00)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.10 (±0.31)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.94 (±1.39)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Nereocystis luetkeana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>-2.03 (±0.45)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A</td>
<td>-0.93 (±0.26)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.02 (±0.14)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Next to cage</td>
<td>7</td>
<td>2.76 (±1.08)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
<td>1.76 (±0.61)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.43 (±2.22)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Laminaria groenlandica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>1.68 (±0.70)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 (±0.52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56 (±0.66)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.89 (±1.59)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Next to cage</td>
<td>7</td>
<td>2.16 (±0.52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 (±0.55)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.96 (±0.44)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.72 (±1.43)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N/A: Not applicable
Like superscript in a column indicates insignificant difference (p<0.05)
All three species, *L. saccharina*, *L. groenlandica* and *Nereocystis* exhibited a pronounced response to nitrogen availability in developing sporophytes during the experimental periods. In the summer of 1995 when ambient concentration of NO$_3^-$ was high (> 10 μM), significant differences in growth rates were not observed between control and experimental plants for *Nereocystis*, but for *L. saccharina*. The growth rates of *Nereocystis* were generally higher than those of *L. saccharina*. Maximum specific growth rates in length and biomass were achieved from control plants of *Nereocystis* as 6.57 and 6.58% of wet weight, suggesting that the plants were not nitrogen-limited during the period. On the other hand, the growth rates of experimental plants for *L. saccharina* showed significantly higher blade growth rates in length (5.84 and 4.86% d$^{-1}$) and width (3.75 and 4.39% d$^{-1}$) than those of control plants (2.50 and 1.38% d$^{-1}$, respectively). However, no significant difference between the experimental plants adjacent to sea cages and 8 m away from sea cages was found except in biomass, but the better growth was achieved by the plants next to sea cages at 2 m depth.

During the summer of 1996, the growth of the plants for two consecutive 4-weeks from May to July varied over time, and significant differences in growth rates were obtained between control and experimental plants due to nitrogen availability. The specific growth rates in length of experimental plants including *L. groenlandica* ranged from 2.70 to 3.79% d$^{-1}$ from May-June and 2.16-4.79% d$^{-1}$ from June-July. During June-July, *Nereocystis* control plants encountered severe distal erosion, which resulted in minus growth. *L. saccharina* control plants showed 4.08% d$^{-1}$ growth in length during the time. However, the laminae of the *L. saccharina* control plants were considerably thinner and de-pigmented to light brown, indicating that pigments were rapidly mobilized as a nitrogen source to sustain the growth in nitrogen-deficient water. This suggestion is supported by the high C/N ratio of 31. On the other hand, *L. saccharina* experimental plants were glossy and darkly pigmented and with a lower C/N ratio (13.7). In general, the growth of *L. groenlandica* was slower than other species over the time, but higher
growth in biomass was achieved presumably due to the thicker growth of the lamina.

The investigation of the growth in width for Nereocystis was not possible since the blades debladed. The deblading of Nereocystis varied over the experimental period. The mean number of blades for Nereocystis at the end of growth studies in 1995 and 1996 ranged from 5 to 12.

The growth rates of stipes were generally as low as 0.5-1.1% d\(^{-1}\) for Laminaria, 0.2-2.1% d\(^{-1}\) for Nereocystis and 0.6-1.0% d\(^{-1}\) for L. groenlandica. Maximum growth rate in length of stipes for Nereocystis was recorded as 2.2% d\(^{-1}\) in the summer of 1995. The increase in bulb diameter was less than 1.3% d\(^{-1}\) over the experimental period.

In 1996, most of plants suffered from the heavy infestation by the bryozoan, *M. membranacea*, whereas only a few plants did in 1996. Little infection by other epiphytes was observed. Bryozoan infections were generally observed on older tissue of the plant, on the bulb and the stipe in Nereocystis, and mostly on the lamina in L. saccharina and L. groenlandica. A high percentage of bryozoan coverage was observed in L. groenlandica, which had a slower growth. In addition, debris on the blade of plants near sea cages was considerably more due to the stagnant water movement.

During growth studies, sporophyte reproduction in L. saccharina and L. groenlandica did not occur whereas a few sorus formations were observed on the blades of experimental plants in Nereocystis both during 1995 and 1996 growth studies.

**1.4.5 COMPARISON OF ACTUAL GROWTH TO THEORETICAL GROWTH**

The actual growth rates were compared with the theoretical growth rates using Eqs 1-3. Actual fish farm data during the times were used for parameters in the equations. During the study periods, fish feeding rates, F, were 0.0096 at Simond’s Bay and 0.0049 (kg of feed kg\(^{-1}\) d\(^{-1}\)) at Cliff Bay and the number of fish were 46,765 and 127,915, respectively. Average fish size was 2.6 kg at both sites.
The ammonia produced is converted to ammonium concentration (\(\mu\text{M}\)) using current velocity and the flow area of the sea cages by Eq. 2 and 3. Ammonium concentration near the fish farms dramatically decreased with the increase of current velocity (Figs. 5B and 6B). Low current velocity (1 cm s\(^{-1}\)) was used to determine growth rate of kelp since ammonium was concentrated during the slack tide. However, ammonium at Cliff Bay did not reach sufficient concentration for kelp growth even during slack tide when current was flowing through the side with the largest number of cages to kelp (Fig. 5A). This type of the system did not maximize \(\text{NH}_4^+\) concentration. In contrast, ammonium concentration at Simond’s Bay was sufficient for the growth of kelp when current was passing through the smallest number of cages to kelp (Fig. 6A). Eq. 4.2 was used since water temperatures at the sites were between 10 to 15°C. Expected theoretical growth rates by Eq. 4.2 for \(L.\text{saccharina}\) and \(Nereocystis\) were 15% d\(^{-1}\) at Simond’s Bay in 1995 and 4.1% d\(^{-1}\) at Cliff Bay in 1996, respectively. The theoretical growth rates of kelp were approximately 2-3 fold higher than the actual growth rate at Simond’s Bay in 1995 (Table 3). However, at Cliff Bay in 1995, theoretical and actual growth rates were similar in \(L.\text{saccharina}\) (3.70% d\(^{-1}\) and 4.79% d\(^{-1}\) during May-July). However, lower actual growth rates for \(Nereocystis\) (2.80 and 2.70% d\(^{-1}\)) were observed during the time (Table 4 and 5).

The logistic growth equation Eq. 5 for comparing field-growth to theoretical kelp growth was validated with the inputs of actual growth rates. In general, actual kelp length was slightly higher than theoretical kelp length. The growth model Eq. 5 appeared to be valid for \(Nereocystis\) with no significant difference between actual and theoretical growth in length (\(p < 0.05\)). However, for \(L.\text{saccharina}\), significant differences were observed between actual and theoretical growth in length for the plants at Cliff Bay in 1996 (\(p > 0.05\)), but not for plants at Simond’s Bay in 1995 (\(p < 0.05\)). Actual growth in length for \(L.\text{groenlandica}\) was significantly lower than expected (\(p > 0.05\)).
Fig. 5. Theoretical changes in ammonium concentration next to sea cages at Simond’s Bay depending on current speed and direction. Current A flows through the side of largest numbers of cages whereas Current B flows through the side of smallest number of cages. A vertical line in Graph B denotes average current speed during the time.
Fig. 6. Theoretical changes in ammonium concentration next to sea cages at Cliff Bay in 1996 depending on current speed and direction. Current A flows through the side of largest numbers of cages whereas Current B flows through the side of smallest number of cages. A vertical line in Graph B denotes average current speed during the time.
Fig. 7. Currents flowing within the above angles were assumed to bring fish culture effluent to kelp. Data within the angles were counted to estimate the benefit of fish culture effluent as fertilizer during the summer of 1995 and 1996. Gray lines next to sea cages indicate kelp ropes and arrows denote current directions.
1.4.6 ESTIMATION OF BENEFIT OF FISH CULTURE EFFLUENT FOR KELP GROWTH

The benefit of fish culture effluent as fertilizer for kelp growth during the time was determined by current data (speed and direction) sampled by a S4 current meter at 2 m depth next to sea cages every 30 minutes during the experimental periods. Current data, which brought fish culture effluent to kelp, were counted satisfying the following conditions:

(1) Currents flowing within certain angles were assumed to bring fish culture effluent to kelp (Fig. 7).

(2) Ample current speed for kelp growth were considered to be from 0 to 10 cm s\(^{-1}\) since a fast current (over 10 cm s\(^{-1}\)) leads to high dilution of NH\(_4^+\).

(3) Light was available for kelp from 6:00 a.m. to 9:00 p.m. in summer.

(4) Kelps were fertilized by the fish farm for 4 h from 10:00 a.m. to 2:00 p.m. when fish are fed at 8:00 a.m.

(5) Other environmental parameters such as temperature, salinity and water visibility were not limiting factors since they were within suitable levels for kelp growth.

Percent of current data fulfilling all the conditions at both sites was less than 4% during the time (Table 6).
Table 6. The estimation of the benefit of fish culture effluent as fertilizer for growth of kelp at the sites during the time, based on current meter readings (current direction and speed). Data were sampled every 30 min during the summer of 1995 and 1996.

<table>
<thead>
<tr>
<th></th>
<th>1995</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At Simond’s Bay</td>
<td>At Cliff Bay</td>
</tr>
<tr>
<td>Data (n)</td>
<td>1792</td>
<td>210</td>
</tr>
<tr>
<td>Avg. Current Speed (cm s⁻¹)</td>
<td>14.0 (±9.2)</td>
<td>2.7 (±2.4)</td>
</tr>
</tbody>
</table>

**CONDITIONS**

<table>
<thead>
<tr>
<th></th>
<th>1995</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. LT &amp; PCD</td>
<td>14.2 %</td>
<td>14.3 %</td>
</tr>
<tr>
<td>II. LT &amp; PCD &amp; ACS</td>
<td>5.9 %</td>
<td>14.3 %</td>
</tr>
<tr>
<td>III. LT &amp; PCD &amp; ACS &amp; FER</td>
<td>3.1 %</td>
<td>3.3 %</td>
</tr>
</tbody>
</table>

LT : Light available from 6:00 a.m. to 9:00 p.m.
PCD : Positive current direction for the growth of kelp
ACS : Ample current speed from 0 to 10 cm s⁻¹
FER : Fertilization period from 10:00 a.m. to 2:00 p.m. after fish feeding daily at 8:00 a.m.
Three kelp, *Laminaria saccharina*, *L. groenlandica* and *Nereocystis*, which are commonly observed near a fish farm or on fish farm nets in Northwest Pacific Ocean, were used for the growth studies in summers of 1995 and 1996.

Environmental data such as temperature, salinity and water visibility collected next to salmon sea cages during study periods were within suitable levels for the growth of the kelps: temperature as 10.0-14.7°C; salinity as 22.3-30.0‰; water visibility as 3.5-9.5 m. Petrell *et al.* (1993) also reported that many Atlantic salmon sea-cage sites in British Columbia, Canada meet comparable environmental criteria with those of *L. saccharina* and *Nereocystis*. However, *L. saccharina* is known to grow well at temperature up to 20°C (Druehl, 1967), which was not observed at any of the sites. During the experimental periods, light intensities at 2 m water depth were sufficiently high to support photosynthesis of all three species of kelp and ranged from 20-1025 μmol photon m⁻² s⁻¹ during daytimes, which may reach the saturation levels for the light requirement of plants. Grevby *et al.* (1989) reported that for *L. saccharina* 45 μmol photon m⁻² s⁻¹ is 50% of the light saturation for growth and leads to a growth rate of approximately 4.0 cm d⁻¹. With 42-56 μmol photon m⁻² s⁻¹ the blade of three European *Laminaria* spp., *L. saccharina*, *L. digitata* and *L. hyperborea* attained about 70% of their maximum photosynthetic rate and above about 150 μmol photon m⁻² s⁻¹, photosynthesis was light-saturated in all three species (Lüning, 1979). Merrill and Gillingham (1991) suggested that in culture of *Nereocystis* the determination of the depth at which kelp long lines are deployed should be considered since the effect of depth can significantly influence the growth rate of plants and the eventual total biomass produced. The depth of kelp lines may be adjustable by growth strategies (Druehl, 1981) and quality control of final products since the range of irradiance under water is wide in summer. The adjustment may also be involving in convenient to fish farmers. The recommended
depths of kelp lines in British Columbia, Canada were 2 to 3 m in winter and 6 to 7 m in summer (Petrell et al., 1993).

Total tissue nitrogen of plants is strongly correlated to external N concentration levels (Chapman et al., 1978; Wheeler and North, 1980) and also shows the seasonal variations with lower values during summer months and higher values in the winter months due to nitrogen seasonality in nature (Chapman and Craigie, 1977; Wheeler et al., 1984; Rosell and Srivastava, 1985; Henley and Dunton, 1995). In this study, the N content in tissues of experimental plants during summer of 1995 and 1996 ranged from 1.35-2.80% of dry weight. Such values are generally considered to be as high as those of plants growing in winter. At the end of the second growth study from June-July in 1996, however, the N content in tissues of the three species at the control site significantly declined and reached minimum levels below 1% of dry weight, indicating that internal N-reserves were utilized (Gerard, 1982). Similar results were obtained for L. saccharina (0.83%) and M. integrifolia (0.85%) in June (Druehl, 1980a). The internal N accumulation of the control plants was not observed and N contents were also slightly lower (1.35-1.86% of dry weight) than those of the experimental plants (1.56-2.39%) during the first growth study from May-June in 1996, suggesting that N reserves were used (Fig. 8). During the first growth study period in 1996, the control plants of L. saccharina were remarkably depigmented to light brown (0.87% of N content in tissue) due to the utilization of pigment-protein complexes as a nitrogen source. However, the experimental plants were darkly pigmented with 1.86% of N content in tissue. DeBoer (1981) observed marked decreases in pigment content under nitrogen deficiency, and Smith et al. (1983) also suggested that pigments may serve a secondary role of nitrogen storage. Lobban et al. (1985) suggested that the rapid decline of N reserves could happen for growth of plants during higher temperatures, increased irradiance, and low external nitrogen, which are common during late spring and summer months.
Fig. 8. C and N contents in 2x2 cm tissue samples at the beginning and the end of the growth periods.

IT = at the beginning of growth periods
OM = adjacent to a net pen system
8M = 8 m away from a net pen system
CL = Control site (Distal site)
In the summer of 1995, the C/N ratios of the control and experimental plants for *L. saccharina* and *Nereocystis* were similar due to the effect of the high ambient concentration of NO$_3^-$ (over 10 μM) on the N reserves in the tissues. On the other hand, in the summer of 1996, the C/N ratios of experimental plants were significantly lower than those of control plants, suggesting that the experimental plants had significant benefit of NH$_4^+$ from the sea cages. High C/N ratios of the control plants may indicate that total N contents in tissue of plants were used to support new tissue production in the absence of external N-supply after transplanting to remote areas from sea cages.

Growth of *Laminaria* species has been investigated in field and laboratory experiments by many researchers: specific growth rates were reported as 2 cm d$^{-1}$ during summer (Nicholson, 1970) and 3-6% d$^{-1}$ (Kain, 1987) for mature *Nereocystis*, as 10 cm wk$^{-1}$ during June for *L. saccharina* (Lüning, 1979), as 4.1 cm wk$^{-1}$ during May for *L. digitata* (Lüning, 1979), as 4.5 cm d$^{-1}$ and 3.45 cm d$^{-1}$ (Baik and Pyen, 1973) for *L. japonica*, 7-12% d$^{-1}$ for *M. pyrifera* (Wheeler and North, 1980). Yarish and Egan (1987) observed a maximum growth rate of 2.53 cm d$^{-1}$ in May and early June, but in August, little growth or no growth (0.15 cm d$^{-1}$). During the growth studying periods, the growth of all three speices, *L. saccharina*, *L. groenlandica* and *Nereocystis* were significantly influenced by external N concentration in seawater as reported for kelp by other researchers (Chapman and Craigie, 1977; Chapman *et al.*, 1978; Sjøtun, 1993). During the summer of 1995, the ambient concentration of NO$_3^-$ was over 10 μM both at control and experimental sites and constant until the end of the growth study. This resulted in a similar growth enhancement both of control and experimental plants for *Nereocystis*, indicating no significant effect of NH$_4^+$ released from the fish farm on the growth of plants. However, the fact that higher growth rate with lower C/N ratio in *L. saccharina* next to sea cages than that in *L. saccharina* 8 m away from sea cages, may indicate that *L. saccharina* requires more nitrogen for the growth than *Nereocystis*. This result could be explained by the higher C/N ratio (12-14.1) for
L. saccharina than those for Nereocystis (8.9-9.7). Chapman et al. (1978) reported that L. saccharina required constant exposure at 10 μM NO₃⁻ to maintain maximum growth and build internal N-reserves simultaneously. The highest growth rates were recorded as 6.57% d⁻¹ from Nereocystis control plants and 5.84% d⁻¹ from next-to-sea-cages L. saccharina plants.

In the growth study in 1996, the pronounced difference in the effect of external N supply on growth of plants were observed. After being transplanted to the control site where the N concentration in seawater was low, 1.7 μM for NH₄⁺ and less than 3.5 μM for NO₃⁻, the growth of all three species was significantly influenced by low external N availability. The growth data obtained in 1996 suggest that the experimental plants next to sea cages were also slightly nitrogen-limited, even though plants showed a significant difference in growth compared to control plants.

The specific growth rates of stipes of Laminaria were generally low and ranged from 0.5-1.1% d⁻¹. The growth rates in stipes of Nereocystis were considerably lower (0.2-2.1% d⁻¹) probably because they were exposed to full or near full daylight. Similarly, Nereocystis grew at 9 mm d⁻¹ when plants were held near the sea surface (Kain, 1987). The elongation rate of the stipe for Nereocystis is known to be strongly correlated to water depth, which determines irradiance availability: 7.8 cm d⁻¹ at 4 m, and 5 cm d⁻¹ at 2 m below mean low tide (Duncan, 1973). Duncan and Foreman (1980), however, reported that mature stipes can elongate at up to 25 cm d⁻¹.

The great loss of experimental or commercial plants in the field has been reported due to various factors: nutrient stress, strong wave action, handling (Torkko, 1987; Sjøtun, 1993), bryozoans (Lüning, 1979, Tokko et al., 1987; Merrill and Gilingham, 1991; Hurd et al., 1994a), kelp grazers (Druehl, 1980a, 1981; Gagne et al., 1982; Yarish and Egan, 1987), erosion, other epiphytes (Druehl, 1981). In this study, however, the bryozoan, M. membranacea was the most damaging fouling organism on the three species and rendered the plants virtually useless for
food-grade products. However, limited information has been elucidated about the effects of bryozoan colonization on kelp. In general, the colonization of bryozoa varied between individual plants, blades and stipes due to temporal variation (Lobban, 1978) and increased blade loss occurred during the onset of growth (Lüning, 1979). The bryozoa were mostly observed on *L. groenlandica* at 2-4 m depth (Druehl, 1981). Hurd *et al.* (1994a) reported that bryozoa on kelp inhibited NO$_3^-$ and NH$_4^+$ removals from seawater but provided a source of NH$_4^+$ through excretion. In *L. japonica*, a low fertility rate was observed when the presence of epiphytes and bryozoa covered the blade (Torkko *et al.*, 1987). Druehl (1980b) suggested more wave-exposed areas as a good kelp farm site since epiphytism and blade damage could decrease. Mizuta *et al.* (1994) suggested that when N storage was exhausted, insoluble nitrogen might be largely utilized to satisfy the nitrogen demand of tissues by the catabolic processes, which may result in severe erosion of apical tissues in summer months.

The theoretical growth rates (15% d$^{-1}$) were approximately 2-3 fold higher than actual growth rate of kelp (approximately 5-6% d$^{-1}$) at Simond’s Bay in 1995. Inorganic nitrogen at the site was sufficient for the growth of plants not only because the high concentration of NH$_4^+$ during slack tide was generated by fish excretion, but also because the ambient concentration of NO$_3^-$ was consistently high (over 10 μM). However, the actual growth rates never reached the maximum growth rate (15% d$^{-1}$) expected by Eq. 4. Petrell *et al.* (1993) also obtained in a raceway culture system 25% lower actual growth rate (9% d$^{-1}$) than expected (12% d$^{-1}$) at temperatures less than 10°C with an average concentration of dissolved inorganic nitrogen equal to 9.7 μM. The fact that the equation was developed based on the result from laboratory culture experiments may overestimate growth rates of kelp. For example, Boton and Lüning (1982) obtained high growth rates (15-18 % d$^{-1}$) for *L. saccharina* at 10-15°C in enriched culture medium. However, extrapolation from laboratory to field condition is generally known to be fraught with problems since culture conditions certainly do not simulate water turbulence in the
sea, which influence uptake by kelp and the dilution of nutrients. It is also considered that lower growth in the field is due to competition for nitrogen from bacteria, microalgae and other macroalgae. However, when the constant in Eq. 4 (1.7) was substituted by 0.7, the growth rates were similar to the actual growth rates. From the results of the growth study, the logistic Eq. 5 appeared to be valid for *Nereocystis*. However, for *L. saccharina*, a significant difference was observed between actual and theoretical growth rates at Cliff Bay, but not at Simond's Bay. Theoretical growth in length for *L. groenlandica* was significantly lower than expected (p > 0.05). Eq. 5 may not suitable to estimate the growth for *L. groenlandica*, which is generally known to have slower growth than *L. saccharina* and *Nereocystis*. Druehl (1988) reported that *L. groenlandica* generally requires 18 months of culture before the harvesting period. It is, however, difficult to determine if the equation is valid to estimate growth rate of the kelps since high variations in growth observed between individual plants. It is strongly recommended that the growth model should be examined at varied environmental conditions and sites with large number of plants.

In this study, all three species showed that they were fertilized by fish culture effluent during the summer when nitrogen is limited in the sea. However, it is still difficult to determine how much a fish farm can contribute to kelp growth in field. Different environmental conditions such as currents, external N concentration and temperature may lead to different results at different sites since many unknown factors may exist in the uncontrolled environment.

In the summer of 1995, plants were not significantly nitrogen-limited since a high ambient concentration of NO$_3^-$ (over 10 µM) resulted in a significant growth enhancement. This result suggests that a farm site located in upwelling area of nutrient-rich water, or well-mixed zone may be a way to enhance the growth of kelp during a summer nitrogen-deficient period. However, fish farms are generally sited away from upwelling areas to avoid detrimental algal blooms (Petrell and Ali, 1995).
2. Ammonium and Nitrate Uptake by *Laminaria saccharina* and *Nereocystis luetkeana* Originating from a British Columbian Salmon Sea Cage Farm

2.1 INTRODUCTION

Nitrogen is a crucially important element for life and one of the most abundant elements in the biosphere. However, it is considered to be in limited supply mainly because 99.96% of the nitrogen is in the form of N\textsubscript{2} with only 0.04% in a combined form (Lobban *et al.*, 1985; Vymazal, 1994). The major nitrogen source for algae is inorganic nitrogen such as ammonium, nitrate and urea (Lobban *et al.*, 1985; Sze, 1986). The application of ammonium and nitrate during a nitrogen deficient period (Tseng, 1981; Yamada and Iwasaki, 1964) and the use of animal-waste water as nitrogen fertilizer (Przytocka-Jusiak *et al.*, 1984; Aziz and Ng, 1992; Petrell *et al.*, 1993; Buschmann *et al.*, 1996) have been introduced for the effective management of algal cultivation.

The studies on nutrient uptake of algae have been conducted both with laboratory and field cultures because nutrient uptake may be used as a good approximation of growth of algae (Lobban *et al.*, 1985). Nitrogen uptake rates by algae depend on inorganic nitrogen sources and concentration, and on environmental factors such as irradiance, temperature and water movement (Haines and Wheeler, 1978; Lobban *et al.*, 1985).

In addition, significant influences on uptake rate of nutrients have been reported by biological factors: the various type of tissue (Topinka, 1978), the age of the plant (Harrison *et al.*, 1982), its nutritional past history or nitrogen status of the thallus (D’Elia and DeBoer, 1978, Kopczak, 1994), and interplant variability (Gerard, 1982; Kopczak, 1994). Kelps (Laminariales) have a strong seasonal variation in growth, that is, rapid growth in the late winter and early spring when inorganic nitrogen is available.
The highest uptake rate was usually observed for the thallus of a whole plant (Topinka, 1978), but the spatial variation of nitrogen uptake within the thallus of *Macrocystis* was also reported by Kopczak (1994). Young tissue with high metabolic activity needs greater nitrogen requirements than old tissue (Lobban *et al.*, 1985). Harrison *et al.* (1986) reported that the ammonium and nitrate uptake rates of first-year of *L. groenlandica* plants were three times higher than those of second and third year plants.

It has been well demonstrated that nitrogen uptake of macroalgae increases when nitrogen availability decreases and when N content of tissue is low in nature. Kopczak (1994) found that nitrate uptake of *Macrocystis* was significantly higher in N-starved tissue than in N-replete tissue, and D’Elia and DeBoer (1978) also observed that when two red algae, *Gracilaria foliifera* and *Agardhiella subulata*, were grown under nitrogen-limited conditions they showed higher C/N ratios and uptake rates of ammonium than for plants grown in a nitrogen-rich condition.

The suppression of nitrate uptake by ammonium (Hanisak and Harlin, 1978; Pistorius *et al.*, 1978), the increase of ammonium uptake by nitrate supply (Haines and Wheeler, 1978; Mizuta and Maita, 1991), and preferential uptake of ammonium by phytoplankton (Conway, 1977; Schuler *et al.*, 1953) have been demonstrated both under laboratory and field studies. On the other hand, linear uptake rates of ammonium were also reported over a range of ammonium concentration for kelps, *Macrocystis pyrifera* (Haines and Wheeler, 1978) and *L. groenlandica* (Harrison *et al.*, 1986). Chapman and Craigie (1977) found that *L. longicurris* could store NO$_3^-$ in the algal tissue in March at concentrations 28,000 times that of the surrounding water. In addition, marine algae, *Valonia* and *Halicystis*, can accumulate nitrate 2,000 times and 500 times over the nitrate value of seawater, respectively (Jacques and Osterhout, 1938). Rosenberg and Ramus (1984) found that nitrogen uptake capacity of seaweeds is a direct function of surface
area-to-volume ratio (SA/V), where N storage capacity varies inversely with SA/V. (Rosenberg and Ramus, 1982).

Previous studies for the uptake rates of nitrogen by Laminariales have been reported by other researchers: Hurd et al. (1994b) reported that the uptake rate of ammonium and nitrate for *Macrocystis* and *Nereocystis* ranged from 3.04-6.55 μmol g dry wt⁻¹ h⁻¹ by a low-volume flow tank experiment; Harlin and Craigie (1978) noted the maximum uptake of NO₃⁻ by *Laminaria longicruris* ranged between 7 and 10 μmol g dry wt⁻¹ h⁻¹ at 15°C under 1800 μW cm⁻² of irradiance; Mizuta and Maita (1991) observed the enhancement of ammonium uptake by *Laminaria japonica* by NO₃⁻ supply (20 μM) from 8.2 to 11.62 μmol g dry wt⁻¹ h⁻¹; Haines and Wheeler (1978) reported the maximum uptake of NH₄⁺ and NO₃⁻ by *Macrocystis pyrifera* as 23.8 and 30.5 μmol g dry wt⁻¹ h⁻¹, respectively; Braga and Yoneshigue-Valentin (1996) found the maximum uptake rates of NH₄⁺ and NO₃⁻ by the laboratory-grown young sporophytes of *Laminaria abyssalis* to be 5.0 and 2.0 μmol g dry wt⁻¹ h⁻¹, respectively.

It has been proposed that the plants in a nitrogen rich area may have different nitrogen uptake rates, compared to the plants in a nitrogen-limited area. Carpenter and Guillard (1971) reported that clones of single species of phytoplankton from areas of different nutrient availability have different nutrient kinetics. In addition, Chapman et al. (1978) have shown that *L. saccharina* grown in nutrient-rich seawater has a higher $K_s$ value, ca. 1.4 μM NO₃⁻, which is very similar to that of coastal phytoplankton, and suggested that kelp in nutrient-poor areas have lower $K_s$ values.
2.2 OBJECTIVES

This study was conducted to provide information on nitrogen uptake by kelps originating from a salmon fish farm where high concentrations of ammonium are available as a nitrogen source.

The hypothesis is:

The kelps may have different nitrogen uptake rates, compared to the plants in a nitrogen-limited area.

The objectives of this study were:

1) To examine the uptake rates of NH$_4^+$ and NO$_3^-$ by *Laminaria saccharina* and *Nereocystis luetkeana* in simulated concentrations of salmon culture effluent, which are available adjacent sea cages.

2) To examine if the preferential uptake of NH$_4^+$/NO$_3^-$ over NO$_3^-$/NH$_4^+$ exists for both species.

3) To test if the inhibition of NH$_4^+$ uptake over NO$_3^-$ uptake by both species exists.

4) To determine the maximum uptake rates of NH$_4^+$ and NO$_3^-$ in the concentrations tested.
2.3 MATERIALS AND METHODS

2.3.1 PLANT MATERIALS

In August of 1996, juvenile *Nereocystis luetkeana* and *Laminaria saccharina* were collected on a fish farm operated by BC Packers Ltd. and located at Cliff Bay near Tribune Channel, B.C., Canada. This site was known to have an environment of relatively low wave stress and slow current, fairly constant temperature at around 12.7°C and salinity near 26.0% at 2 m depth in summer (PBCMAF, 1987). Tissue segments (about 10 x 10 cm) were cut from portions located 5-15 cm from the stipe-blade for *L. saccharina* and from the bulb-blade for *Nereocystis*. These tissue segments, kept in dark in a cooler filled with seawater obtained next to sea cages and ice packs in dark, were transported to a growth chamber in the Department of Botany, Univ. of British Columbia, B.C., Canada. They were maintained in the cooler at 12°C under 35 pmol photon m\(^{-2}\) s\(^{-1}\) for approximately 24 h. The day before the experiment began, microorganisms and visible epiphytes were removed by gently brushing, wiping and washing with filtered seawater. The seawater (salinity 24.5% and pH 7.59) used in the experiment was obtained from Point Atkinson, West Vancouver, B.C., Canada and then filtered through 0.45 μm membrane filters. The segments were re-trimmed at 7x7 cm, and maintained in an ice cooler filled with filtered nitrogen-poor seawater (< 3 μM of NH\(_4^+\) and NO\(_3^-\)) at 12°C under 135 pmol photon m\(^{-2}\) s\(^{-1}\) from white fluorescent lights for 24 h. Three 2x2 cm segments from 10x10 cm of tissue segments per species were prepared for the analysis of the C/N ratio in the tissues. Just before the experiment began, mucus from the segments was removed by gently wiping with a paper towel and rinsing with filtered seawater.

The nitrogen uptake experiment was conducted in 1 L-beaker vessels filled with 700 ml of filtered seawater in four combinations of NH\(_4^+\) and NO\(_3^-\): 15 μM NH\(_4^+\) only; 20 μM NH\(_4^+\) plus 10 μM NO\(_3^-\); 20 μM NH\(_4^+\) plus 20 μM NO\(_3^-\); 40 μM NH\(_4^+\) plus 30 μM NO\(_3^-\). These
combinations were made to simulate the concentration of NH$_4^+$ and NO$_3^-$ near a fish farm in summer. Triplicates were conducted for each combination. The culture medium was supplemented with NH$_4$Cl and KNO$_3$. The tissue segments were randomly selected and replaced in 12-culture vessels. Thirteen culture vessels including a culture vessel without a tissue segment as control to monitor microbial activity were positioned on a orbital shaker and agitated at around 125 rpm under 135 µmol photon m$^{-2}$ s$^{-1}$ of irradiance. Two peristatic pumps were used to obtain 12 samples simultaneously at each sampling period. Twelve of 13 samples were simultaneously obtained by two peristatic pumps through 70 cm long and 1.7 mm I.D. tubes, and a sample was taken by a 10 ml pipette directly from a control vessel, which contained 20 µM NH$_4^+$ plus 20 µM NO$_3^-$ in the culture medium. However, sampling from the control was conducted only at the beginning and end of the experiment. The volume of each sample was 7 ml and about 2 ml of culture medium were discarded before sampling to remove previous residue of culture medium inside the tubes.

The concentrations of NH$_4^+$ and NO$_3^-$ were measured every 30 min for 3 h. Uptake rates expressed on a dry weight basis were estimated according to the following equation:

\[ V = \frac{\Delta n}{(\Delta t \times \chi)} \]

Where:

- \( V \) = uptake rate (µg N g$^{-1}$ dry weight h$^{-1}$)
- \( n \) = NH$_4^+$ or NO$_3^-$ content of the culture medium (µM)
- \( t \) = time (h)
- \( \chi \) = average dry weight of 7x7 cm tissue segments

2.3.2 C/N RATIO ANALYSIS

Three 2x2 cm sized tissue samples were randomly obtained from 10x10 cm tissue segments of each species to examine the nitrogen history of plants growing near the fish farm.
They were kept in 30 ml polypropylene bottles and transported to a laboratory at U.B.C. The tissue segments were dried at 70°C for 24 h, and ground manually into a homogeneous fine powder. Total content of carbon and nitrogen was determined as described by Verardo et al. (1990), using a Carlo Erba Analyzer NA-1500 at the Oceanography Department, U.B.C.

2.3.3 ANALYSIS OF AMMONIUM AND NITRATE NEXT TO SEA CAGES

In order to trace the nitrogen history of plants, hourly water samples for ammonium and nitrate were obtained in triplicate near a fish farm from 2 m depth next to one end of a sea cage system through a 1 μm G/FF syringe filter on May 18th, June 18th and July 18-19th in 1996. The samples were immediately poisoned with 0.01 ml chloroform/10 ml seawater to improve the preservation of samples, and stored in 30 ml polypropylene bottles as described by Dosdat et al. (1995).

Samples were analyzed by a Technicon Autoanalyzer II using standard techniques (Harrison et al., 1986) at the Department of Chemical & Bio-Resource Engineering, U.B.C., Vancouver, Canada.

2.3.4 STATICAL ANALYSIS

The consumption of NH₄⁺ and NO₃⁻ in culture medium (20 μM of NH₄⁺ plus NO₃⁻, respectively) by L. saccharina and Nereocystis every 30 min for a 3 h incubation was used for statistical analysis. Significant differences in NH₄⁺ and NO₃⁻ uptake by L. saccharina and Nereocystis were tested at concentrations of 20 μM of NH₄⁺ plus 20 μM of NO₃⁻, using a paired sample t-test. Two-sample t-test was also used to examine significant differences in uptake of NH₄⁺ and NO₃⁻ between species. In cases where the test of data normality failed, non-parametric t-tests (Wilcoxon signed rank test and Mann-Whitney Rank sum test) were introduced. In all cases, the null hypothesis was rejected at the 95% probability level (p < 0.05).
2.4 RESULTS

The concentrations of $\text{NH}_4^+$ and $\text{NO}_3^-$ next to sea cages over time are presented in Fig. 3. The concentration of $\text{NO}_3^-$ was fairly constant at around 1.7 $\mu$M, which is similar to typical concentrations of nitrate in the sea during summer (Fig. 4). The concentration of $\text{NH}_4^+$ depended on fish feeding activities and ranged between 1-34 $\mu$M. Unlike the variation of $\text{NO}_3^-$ concentrations, however, the variation of $\text{NH}_4^+$ concentrations was high. In the $\text{NH}_4^+$ analysis of fish culture effluent obtained next to the sea cages 2 hours after fish feeding, $\text{NH}_4^+$ next to cages increased, remained high (> 5 $\mu$M) for 14 h and then became the typical summer-background concentrations of $\text{NH}_4^+$ (< 2 $\mu$M). The concentration of ammonium next to a sea cage system was strongly correlated to fish feeding activity (Fig. 3).

The average wet weight of the 7x7 cm tissue segments for $L. \text{saccharina}$ was similar to that of $\text{Nereocystis}$ being 4.63 and 4.48 g, respectively, but the average dry weights for $\text{Nereocystis}$ was about half that of $L. \text{saccharina}$ being 0.34 g. C/N ratios of the three tissue samples from both species of plants were averaged due to no significant differences between the individual tissue segments. C/N ratios of $L. \text{saccharina}$ and $\text{Nereocystis}$ were 10.3 and 12.7 respectively (Table 7).

The typical time course of decreases of $\text{NH}_4^+$ and $\text{NO}_3^-$ concentrations in the medium is shown in Fig. 9 and the uptake rates of $\text{NH}_4^+$ and $\text{NO}_3^-$ by tissue segments of both species over the 3-hour incubation time are summarized in Table 8. The pattern of ammonium and nitrate uptake by $L. \text{saccharina}$ was similar to $\text{Nereocystis}$. Uptake rates were varied over time, but generally, the uptake rates of ammonium and nitrate over the first hour of exposure to all combinations of substrate were higher than those over the rest of the incubation time. The noticeable variation in uptake between triplicates was observed in $L. \text{saccharina}$, especially at a higher concentration of substrate. The surge uptake by both species was generally observed...
during the first hour of the incubation. The highest uptake rates were observed at the highest concentration in the culture medium (40 \( \mu M \) \( NH_4^+ \) plus 30 \( \mu M \) \( NO_3^- \)) for a 1-h incubation time for \( L.\ saccharina \) for \( NH_4^+ \) being 14.8 and for \( Nereocystis \) for \( NO_3^- \) being 27.2 \( \mu mol\ g\ dry\ wt^{-1}\ h^{-1} \). In general, uptake rates by \( Nereocystis \) exceeded those of \( L.\ saccharina \). Mean uptake rates of \( NH_4^+ \) and \( NO_3^- \) by \( L.\ saccharina \) ranged from 6.0-8.9 and 4.6-10.6 \( \mu mol\ g\ dry\ wt^{-1}\ h^{-1} \), respectively and for \( Nereocystis \) from 6.6-9.3 for \( NH_4^+ \) and 6.1-17.0 \( \mu mol\ g\ dry\ wt^{-1}\ h^{-1} \) for \( NO_3^- \), each. Both \( L.\ saccharina \) and \( Nereocystis \) exhibited the trend of saturation in uptake rate of ammonium at around 10 and 13 \( \mu mol\ g\ dry\ wt^{-1}\ h^{-1} \), respectively, without effect by the presence of \( NH_4^+ \). In contrast, the uptake rates of \( NO_3^- \) were generally enhanced with the increase in the concentration of the substrate (Fig. 10). In a control vessel after a 3-hour incubation time, the concentration of \( NH_4^+ \) slightly increased up to 2.3 \( \mu M \), whereas a decrease of 3.6 \( \mu M \) \( NO_3^- \) was observed.

In this study, the suppression of \( NH_4^+ \) or \( NO_3^- \) uptake by both species was not observed in all culture media over time. \( L.\ saccharina \) exhibited no significant differences merely in uptake of ammonium and nitrate in comparison with the disappearance over time of 20 \( \mu M \) \( NH_4^+ \) plus 20 \( \mu M \) \( NO_3^- \) in the medium (p > 0.05), although nitrate uptake rate exceeded ammonium uptake. \( Nereocystis \), however, showed a significant difference in ammonium uptake at the same concentration of \( NH_4^+ \) plus \( NO_3^- \) (p < 0.05). \( NH_4^+ \) uptake for \( L.\ saccharina \) and \( Nereocystis \) was not significantly different (p > 0.05). However, significant differences were observed in nitrate uptake between the two species (p < 0.05). The uptake of nitrate by \( L.\ saccharina \) was significantly lower (approximately 45% of \( Nereocystis \)).
Table 7. The wet and dry weights (g) and total C and N contents of 7x7 cm tissue segments for *Laminaria saccharina* and *Nereocystis luetkeana* (C, N expressed as % dry weight and as C:N by weight; Mean values ± SE for wet (W) and dry (D) weights, and W/D; n=6 for wet and dry weights and n = 3 for C:N ratios by weight).

<table>
<thead>
<tr>
<th>Species</th>
<th>Wet (g)</th>
<th>Dry (g)</th>
<th>W/D</th>
<th>Total N</th>
<th>Total C</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Laminaria saccharina</em></td>
<td>4.63±0.80</td>
<td>0.66±0.12</td>
<td>7.28±0.79</td>
<td>2.26</td>
<td>23.36</td>
<td>10.34</td>
</tr>
<tr>
<td><em>Nereocystis luetkeana</em></td>
<td>4.48±0.45</td>
<td>0.34±0.04</td>
<td>13.30±0.82</td>
<td>1.86</td>
<td>23.64</td>
<td>12.71</td>
</tr>
</tbody>
</table>
Fig. 9. Time course of decrease in nitrate and ammonium in four combinations of culture medium for 7x7 cm tissue segments from first-year plants of Laminaria saccharina and Nereocystis luetkeana at 12°C under 125 rpm agitation and 135 μmol photons m⁻² s⁻¹ of irradiance.
Table 8. Ammonium and nitrate uptake rates in µmol g⁻¹ dry wt h⁻¹ (± SE where n = 3) for *L. saccharina* and *Nereocystis luetkeana* in four combinations of NH₄⁺ and NO₃⁻ during a 3 h incubation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Incubation Time (h)</th>
<th>15 µM [NH₄⁺]</th>
<th>0 µM [NO₃⁻]</th>
<th>20 µM [NH₄⁺]</th>
<th>10 µM [NO₃⁻]</th>
<th>20 µM [NH₄⁺]</th>
<th>20 µM [NO₃⁻]</th>
<th>40 µM [NH₄⁺]</th>
<th>30 µM [NO₃⁻]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. saccharina</em></td>
<td>0 - 1</td>
<td>13.4 (±4.06)</td>
<td>&lt; 2</td>
<td>7.86 (±2.20)</td>
<td>1.43 (±0.95)</td>
<td>10.1 (±2.36)</td>
<td>8.17 (±4.27)</td>
<td>14.8 (±8.38)</td>
<td>18.7 (±1.37)</td>
</tr>
<tr>
<td></td>
<td>1 - 2</td>
<td>4.07 (±0.86)</td>
<td></td>
<td>5.15 (±4.64)</td>
<td>6.88 (±0.83)</td>
<td>4.07 (±3.22)</td>
<td>3.73 (±0.43)</td>
<td>2.54 (±3.62)</td>
<td>7.37 (±0.57)</td>
</tr>
<tr>
<td></td>
<td>2 - 3</td>
<td>5.86 (±2.59)</td>
<td></td>
<td>13.6 (±5.56)</td>
<td>5.49 (±1.39)</td>
<td>3.89 (±1.28)</td>
<td>4.32 (±1.50)</td>
<td>4.92 (±3.08)</td>
<td>5.71 (±0.77)</td>
</tr>
<tr>
<td><em>Nereocystis luetkeana</em></td>
<td>0 - 1</td>
<td>12.7 (±4.62)</td>
<td>&lt; 2</td>
<td>9.81 (±4.15)</td>
<td>10.3 (±3.07)</td>
<td>12.1 (±5.87)</td>
<td>17.7 (±2.11)</td>
<td>13.0 (±0.85)</td>
<td>27.2 (±2.41)</td>
</tr>
<tr>
<td></td>
<td>1 - 2</td>
<td>3.42 (±2.46)</td>
<td></td>
<td>10.8 (±3.42)</td>
<td>7.11 (±2.42)</td>
<td>3.95 (±0.69)</td>
<td>8.77 (±0.47)</td>
<td>7.53 (±0.33)</td>
<td>15.0 (±1.61)</td>
</tr>
<tr>
<td></td>
<td>2 - 3</td>
<td>3.86 (±1.22)</td>
<td></td>
<td>6.93 (±0.38)</td>
<td>&lt; 1</td>
<td>3.68 (±0.45)</td>
<td>7.70 (±1.24)</td>
<td>7.46 (±0.51)</td>
<td>8.72 (±0.64)</td>
</tr>
</tbody>
</table>
Fig. 10. Ammonium and nitrate uptake rates for *Laminaria saccharina* and *Nereocystis luetkeana* in four culture media containing different concentrations of NH$_4^+$ and NO$_3^-$ for the first hour of a 3 h incubation.

- $\bullet$ NH$_4^+$ uptake rates (only 15 $\mu$M NH$_4^+$ with ambient conc. NO$_3^-$)
- $\blacksquare$ NH$_4^+$, $\Box$ NO$_3^-$ uptake rates (20 $\mu$M NH$_4^+$ plus 10 $\mu$M NO$_3^-$)
- $\triangle$ NH$_4^+$, $\triangle$ NO$_3^-$ uptake rates (20 $\mu$M NH$_4^+$ plus 20 $\mu$M NO$_3^-$)
- $\blacklozenge$ NH$_4^+$, $\lozenge$ NO$_3^-$ uptake rates (40 $\mu$M NH$_4^+$ plus 30 $\mu$M NO$_3^-$)
2.5 DISCUSSION

In temperal coastal waters during late spring and throughout the summer, the main factor limiting algal production is the availability of inorganic nitrogen, although irradiance and temperature may be optimal for photosynthesis and growth at the time (Hanisak, 1983; Chapman, 1977; Chapman and Craigie, 1978; Wheeler et al., 1984). *L. saccharina* and *Nereocystis luetkeana* are typically found on fish farm nets, exposed to ammonium released from the fish farm. Utilizing ammonium by algae is considered metabolically as an energy-saving process since ammonium can be directly incorporated into amino acids, whereas nitrate must be reduced to nitrite and finally to ammonium prior to the incorporation into organic compounds (Lobban et al., 1985). In general, around a fish farm, the concentrations of ammonium are determined by total fish mass, fish size, final stocking density, feeding rate, mortality and the volume and number of sea cages (Petrell et al., 1993). However, the ammonium produced mostly by fish excretion may not be 100% available to plants due to currents, depending on which side of a net pen system the plants are located. Plants are considered to have the benefit of pulse-supply ammonium by fish excretion after feeding events.

The C/N ratio is known to be inversely correlated with the concentration of nitrogen surrounding seaweeds (Wheeler and North, 1980; Lobban et al., 1985; Friedlander and Ben-Amotz, 1991). The plants used for this study were probably somewhat nitrogen-limited because C/N ratios of 7x7 cm tissue segments were slightly greater than 10 (Table 1). However, the C/N ratios of tissue segments of *L. saccharina* and *Nereocystis* grown next to sea cages were somewhat lower than typical C/N ratios of macroalgae reported during the summer. For example, the C/N ratio of *Gracilaria conferta* ranged from 18 to 48 during this period (Friedlander and Ben-Amotz, 1991). In members of the Laminariales, on June-July 1981, the highest values of C/N ratios were recorded as 37 for *Macrocystis integrifolia*, 24 for *Nereocystis*
lutescens (Roell and Srivastava, 1985), and 31 for L. saccharina on July-Aug., 1982 (Sjøtun, 1993). It seems likely that ammonium excreted by fish had been taken up by both species of plants as major sources of nitrogen and accumulated in tissue as N-reserves.

In this study, L. saccharina and Nereocystis took up ammonium and nitrate simultaneously as previously reported for the Laminariales. Laminaria groenlandica utilized NH$_4^+$ and NO$_3^-$ simultaneously and uptake rates were identical and equal to uptake rates when only nitrate or ammonium was present in the medium (Harrison et al., 1986). Young sporophytes of the Brazilian kelp, Laminaria abyssalis, took up NH$_4^+$ and NO$_3^-$ simultaneously, although NH$_4^+$ was taken up rapidly (Braga and Yoneshigue-Valentin, 1996). Thomas and Harrison (1987) also suggested that simultaneous uptake of ammonium and nitrate is advantageous in acquiring greater amounts of nitrogen per unit time.

Generally, uptake rates of both species were a function of the concentrations of ammonium and nitrate in the medium, although uptake rates of substrates varied over time (Table 2). The rapid disappearance of ammonium and nitrate by both species was highest for the first hour of exposure to the medium. These similar patterns were also reported in Macrocystis pyrifera (Harlin and Wheeler, 1978), in L. saccharina (Chapman et al., 1978) and in L. groenlandica (Harrison et al., 1986) and suggested to be due to a combination of active transport plus a diffusion component. The highest uptake rates were observed at the highest substrate concentrations, 40 μM NH$_4^+$ plus 30 μM NO$_3^-$, over an incubation period of 1 hour for L. saccharina and Nereocystis, ammonium and nitrate uptake rates 14.8 and 27.2 μmol g dry wt$^{-1}$ h$^{-1}$. The saturation of ammonium uptake was observed both by L. saccharina and Nereocystis at around 10 and 13 μmol g dry wt$^{-1}$ h$^{-1}$, respectively, whereas the consumption of nitrate was continued to increase up to the highest concentration of nitrate in this study (30 μM nitrate). Ammonium saturation with increasing concentration of substrate suggests that a carrier is involved through active transport, whereas a linear increase of nitrate uptake may represent a
second transport mechanism (Lobban et al., 1985). The linear increase in nitrate uptake by *L. saccharina* over 10 μM nitrate agrees with Chapman et al. (1978). Harrison et al. (1986) also reported that the NO$_3^-$ uptake rate of *L. groenlandica* was maximal above 60 μM when 185 μM NO$_3^-$ was added. In this study, no effect of NH$_4^+/NO_3^-$ on the rate of NO$_3^-$/NH$_4^+$ was observed both for *L. saccharina* and *Nereocystis* and this agrees with other reports by Harlin (1978) for *Laminaria longicurris* and Subandar et al. (1993) for *L. saccharina*, but differs from that for *L. groenlandica* where NO$_3^-$ uptake was suppressed in the presence of NH$_4^+$ only for the first 30 min of the incubation. Hanisak and Harlin also (1978) reported that NO$_3^-$ uptake by the green macroalga, *Codium fragile*, was totally suppressed by a 10-fold excess of NH$_4^+$.

The variability in nutrient uptake has been discussed and suggested to be due to different methods in measurements and cultures (Harrison and Dreuhl, 1982; Probyn and Chapman, 1982; Hurd et al., 1994b), algal biomass to volume of the medium (Harrison and Druehl, 1982; Hurd et al., 1994b), N-status in tissue of plants (D’Elia and DeBoer, 1978; O’Brien and Wheeler, 1987; Kopczak, 1994) and interplant variations (Harrison and Druehl, 1982; Gerard, 1982; Kopczak, 1994). Any comparison of these results of nitrogen uptake with other researchers may be difficult of different methods used. Firstly, the medium volume in a culture vessel was small (only 700 ml). This biomass-to-seawater volume ratio (approximately 4.5 g:0.7 L) to trace the disappearance of substrate was slightly higher than 5 g seaweed L$^{-1}$ suggested by Harrison and Druehl (1982). Secondly, all tissue segments are from first-year plants, which could have a higher N uptake than older kelp. Harrison et al. (1986) observed 2-3 times higher NH$_4^+$ and NO$_3^-$ uptake rates for first-year *L. groenlandica* than for two or three years old plants.

This is the first study of nitrogen uptake by macroalgae originating from a fish farm where inorganic nitrogen is available year around. It is concluded from the results that the nitrogen requirements for growth, or at least to overcome the nitrogen deficient status for metabolism of *L. saccharina* and *Nereocystis* during summer may be met with NH$_4^+$ (10-20 μM)
released for 2-3 h from a fish farm. This fact corresponded with the dominance of both species next to sea cages during the nitrogen-deficient period. It is not clear that *Laminaria saccharina* and *Nereocystis* grown next to fish sea cages may adapt themselves to high external ammonium to take advantage of their potential for high uptake of ammonium and nitrate during a nitrogen-deficient period. Further study, however, is needed to determine the differences in nitrogen uptake and the sizes of internal pools for N-storage between populations that have different past nitrogen histories.
OVERALL DISCUSSION

In this study, kelp growth was enhanced by fish culture effluent during a summer nitrogen deficient period. Kelp at a location near a fish farm may have ammonium pulse-supplied by currents or diluted during a slack tide condition. Current conditions, depending on the farm location, may be one of most important environmental factors to provide ammonium to kelp near a fish farm. From the current data in this study, the probability that kelp is fertilized at a location could be low (Table 6). It is very difficult to relate current conditions to the growth of kelp near a fish farm since current conditions vary highly, depending on a location and time. Petrell and Alie (1995) suggested that slack current condition should last for 3 hours to provide ample time for nutrient uptake (Petrell and Alie, 1995). At Simond's Bay in 1995, slack current condition (current speed of > 3 cm s\(^{-1}\)) was very short, or sometimes barely existed since the site was located near a well mixed zone. In contrast, at Cliff Bay in 1996, the current speed was generally > 3 cm s\(^{-1}\). In low current, which is a similar case at Cliff Bay, ammonium uptake by kelp would be low due to the size of diffusion boundary (Petrell and Alie, 1995). This could lead to lower kelp growth, compared to that at Simond's Bay in 1995. In a nutrient deficient area where plants depend on ambient inorganic nitrogen, a high current speed may be required for good growth of seaweed (Matumoto, 1959). Fast water movement may prevent the accumulation of a high ammonium concentration near a salmon farm due to high dilution. Locating a kelp farm where current passes through the side with the smallest number of sea cages could provide a higher concentration of ammonium for kelp growth (Figs. 5 and 6).

It seems more efficient to fertilize kelp with nitrate than ammonium since nitrate uptake by both species linearly increased without the effect of external concentration of ammonium in the medium (Fig. 10). In contrast, ammonium uptake was saturated approximately at 11-13 µM, which is generally available each day next to sea cages after fish feeding. Consequently, a site
where the ambient concentration of ammonium is high (near 15 μM) in the sea during summer may be most suitable both for *L. saccharina* and *Nereocystis*. A high concentration of nitrate is generally found near local mixing, land runoff, or water from below the thermocline (Neushul *et al.*, 1992). Applying nitrate fertilizer to the kelp could be a way to enhance the yield during a nitrogen-deficient period.
CONCLUSION

The main purpose of these 2 studies was to test the hypothesis: a salmon farm can fertilize kelp during summer when only nitrogen is a limiting factor for the growth of kelp. The following conclusions can be made:

1. *Laminaria saccharina* benefits more than *Nereocystis luetkeana* on a fish farm. This is because, of the two species, *Nereocystis* prefers NO$_3^-$ over NH$_4^+$, and NH$_4^+$ is the principal inorganic nitrogen source stemming from the fish farm. The growth of *L. saccharina* on a salmon farm is higher than that of *Nereocystis* if the ambient concentration of NO$_3^-$ is low.

2. In general, the theoretical growth rate was higher than the actual growth rate. Actual growth rates were 2-3 fold lower than expected at Simond's Bay in 1995 where the ambient NO$_3^-$ level (over 10 μM) was sufficient for kelp growth. Differences in expected values were associated with the fact that the theoretical growth model was developed based on the results from laboratory culture experiments. The logistic growth equation 4 was valid for *Nereocystis luetkeana* (p < 0.05). However, for *L. saccharina*, significant differences were observed between actual and theoretical growth in length for the plants at Cliff Bay in 1996 (p > 0.05), but not for plants at Simond's Bay in 1995 (p < 0.05). The actual growth in length for *L. groenlandica* was significantly lower than expected by the theoretical growth equation (p > 0.05).

3. In 1995 when ambient concentration of NO$_3^-$ was high (over 10 μM), the growth of *Nereocystis* was not influenced by NH$_4^+$ supply from a fish farm. In contrast, for *L. saccharina* significantly higher growth was observed near the fish farm (Table 3). However,
no significant dilution effect of NH$_4^+$ on the growth of plants adjacent to or 8 m away from the sea cages was observed for both L. saccharina and Nereocystis. Generally, at low ambient concentration of NO$_3^-$ (> 5 μM) in 1996, the growth of a near-fish farm plants in length, width and wet weight was significantly higher than that of control plants due to NH$_4^+$ supply from the fish farm (Table 4 and 5). The C/N ratios in the plant tissues generally increased with distance from the sea cages.

4. Light intensity available during the summer periods was sufficiently high to support photosynthesis of all three species of kelp. The depth adjustment of kelp ropes can be made over wide range depending on growth and product strategies.

5. The bryozoan, Membranipora membranacea, was the most damaging epifauna during the study period. In the summer of 1996, all three species had a severe infection at a low current site (avg 2.7 cm s$^{-1}$).

6. Both L. saccharina and Nereocystis exhibited the trend of saturation in uptake rate of NH$_4^+$ at around 10 and 13 μmol g dry wt$^{-1}$ h$^{-1}$. However, NO$_3^-$ uptake both by L. saccharina and Nereocystis increased linearly up to the highest dissolved inorganic nitrogen level (40 μM NH$_4^+$ plus 30 μM NO$_3^-$) tested in this study.

7. The suppression of NH$_4^+$ or NO$_3^-$ uptake by both L. saccharina and Nereocystis was not observed in all culture media over time. Only Nereocystis showed a preference for NO$_3^-$ when both 20 μM NH$_4^+$ and 20 μM NO$_3^-$ were present in the medium.
SUGGESTIONS FOR FUTHER STUDY

While it was revealed in this study that fish culture effluent can fertilize kelp and enhance the growth during the nitrogen-deficient period, further research should concentrate on the following aspects:

1. The implementation of the integrated culture of salmon/kelp is needed on a large commercial scale.

2. Comparison of total kelp production from integrated culture of kelp/salmon to that from monoculture of kelp is needed through a complete cycle to access the actual feasibility.

3. The physical and biological effects of bryozoans on the growth of kelp should be examined, and a cultivation strategy should be developed since its damage renders kelp virtually useless for final products.

4. Determine if ample current speed can reduce epifauna colonization on kelp. This result will be helpful to select a good farm site.

5. The analysis of the composition of kelp cultivated near a salmon farm and comparison to the composition of kelp growing at the distal area to examine any quality improvements for kelp products.

6. Determine the effects of multi-harvesting of kelp on the growth.

7. Measure nitrogen uptake in a simulated tidal current to determine when optimal uptake occurs by plants.

8. Examine NH$_4^+$ concentration change and dilution along with current speed and direction over time at different positions.

9. Kelp growth models need to be reexamined at varied environmental conditions and sites.
10. Examine the effects of ammonium/nitrate uptake on kelp growth and internal nitrogen storage.
REFERENCES


Warrer-Hansen, I. 1979. Fish farms are having to watch their waste. Fish Farming Int. 6: 32-34.


