REDUCING THE ENVIRONMENTAL IMPACT OF SEA-CAGE FISH FARMING THROUGH CULTIVATION OF SEAWEED

Thesis submitted in accordance with the requirements of Open University for the degree of Doctor in Philosophy

by

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July 2006

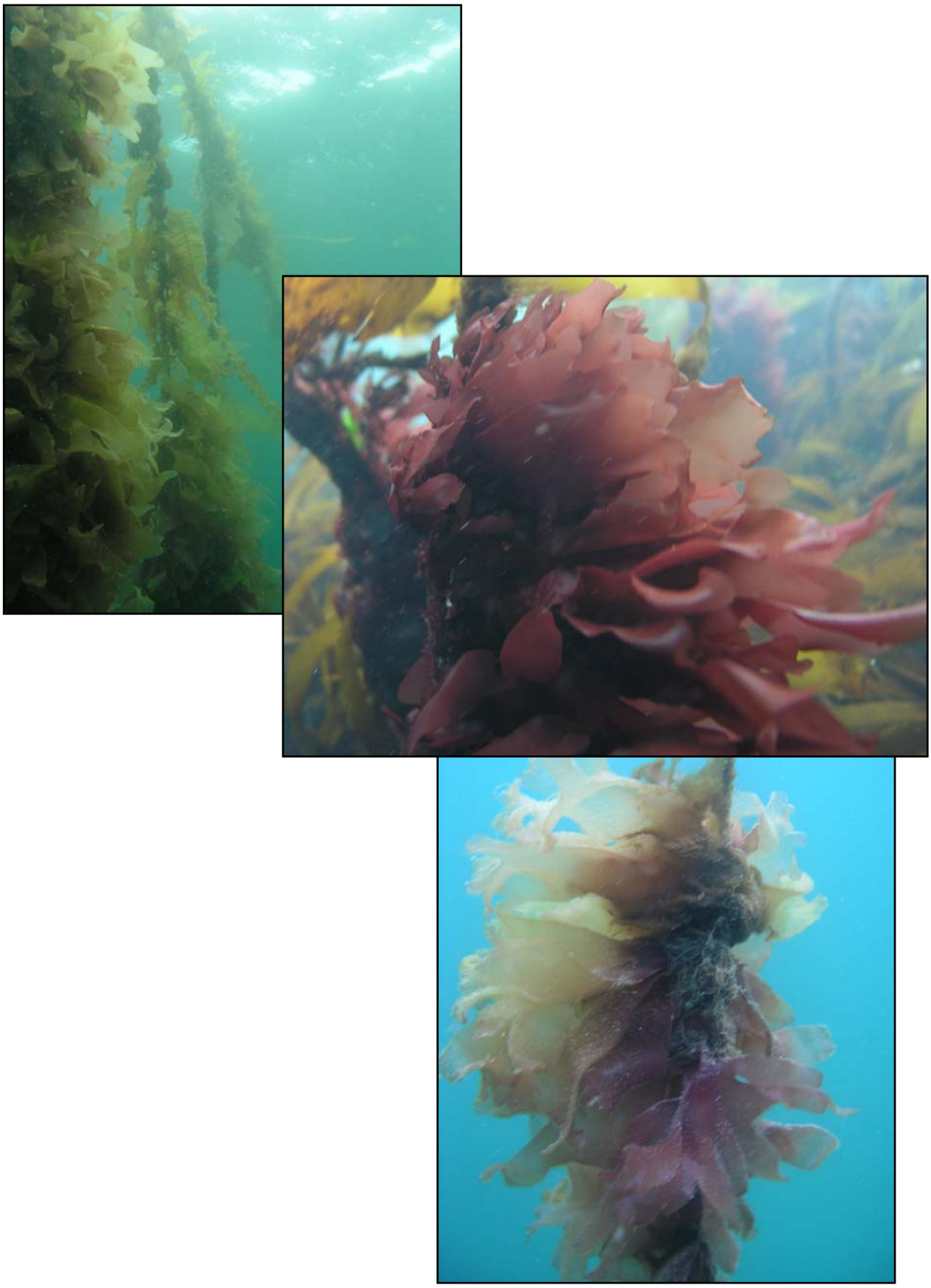
Sponsoring establishment; UHI Millennium Institute.
Academic Partner; Scottish Association for Marine Science (SAMS).

Upper: Loch Duart Ltd. headquarters in Badcall Bay, NW Scotland.

Middle: From Loch Duart Ltd. Headquarters looking west across Badcall Bay.

Lower: Looking south across the head of Badcall Bay.

Upper: Rachel Miller and *Laminaria saccharina* harvest 2004.
Middle: *Laminaria saccharina* droppers on longline 2005.
Lower: Nick Joy and *Laminaria saccharina* harvest 2004.



Upper: *Palmaria palmata* droppers on longline 2005.
Middle: *Palmaria palmata* on *Laminaria hyperborea* stipe 2004.
Lower: Close up of *Palmaria palmata* on dropper 2005.

Upper: *Palmaria palmata* harvest 2004.

Middle: *Palmaria palmata* on frame 2005.

Lower: *Palmaria palmata* heavily fouled with bryozoans, mussels and other August 2005.

ABSTRACT

Cultivation of *Laminaria saccharina* (Linnaeus) Lamouroux and *Palmaria palmata* (Linnaeus) Kuntze was trialled at three fish farm sites in north-west Scotland. Results show that seasonal yields of *L. saccharina* were enhanced by 50 % and *P. palmata* by 63 % when cultured adjacent to fish farm cages compared to environmentally similar sites away from the farms. Yields of *P. palmata* were further enhanced under conditions of optimal water movement.

Ammonium concentrations in the seawater 0 to 50m away from the fish cages were found to be 2 - 3 μM greater than ambient. Enhanced concentrations of ammonium could be detected 200 to 300 m from the cages although the distribution is heavily influenced by local hydrography.

Nitrogen content of *L. saccharina* and *P. palmata* cultured adjacent to the salmon cages in summer was higher than in seaweeds cultured at reference sites away from fish cages.

Stable nitrogen isotope analysis indicates that the nitrogen in seaweeds grown next to salmon cages is derived from the fish farm and farm derived nitrogen is likely to be widely dispersed in the lochs where cages are situated.

A preliminary economic analysis showed that growing seaweeds commercially, in particular *P. palmata*, may be at worst cost neutral, with profitability increasing as a result of enhanced production through increased nutrient availability adjacent to fish farms. A one hectare seaweed farm producing 600 tonnes wet weight over two years

(300 tonnes per year) of *P. palmata* could potentially absorb up to 30 % of nutrients generated from a 500 tonnes salmon production unit.

As farm origin nitrogen is evident in biota at distances of up to one kilometre away from the cages, cultured macroalgae would not have to be sited close to cages to result in net nitrogen removal facilitating the siting of algal farms in areas more suited for individual species requirements while still maintaining bioremediation benefits.

ACKNOWLEDGEMENTS

First of all, I would like to thank Dr Maeve Kelly and Professor Mathew Dring for giving me the opportunity to take on this project and for being supportive supervisors throughout.

Dr Lynn Browne introduced me to the vagaries of culturing *Palmaria* and my trips to Northern Ireland would not have been as rewarding without her input.

Liz Cook was a source of inspiration and I am very grateful for her assistance in the field.

I would also like to thank others who assisted in the field including Martin Lilley, Tony Robson, Jenny Kakkonen, Richard Shucksmith, Andreas Schuenhoff, Coleen Suckling and Benedikt Franz.

The two Environmental officers, Rachel and Hazel, as well as field staff, in particular, Willie and Ian at Loch Duart Ltd were good company and very helpful. The project is indebted to Loch Duart Ltd as the active industrial partner and Nick Joy for his vision.

Special thanks for technical aspects of the thesis go to Tim Brand, Chris Cromey, Keith Davidson and Charlie Scrimgeour.

I am appreciative also for the contributions by Jenny Kakkonen and Angela Sandilands to nutrient uptake experiments and to fellow PhD students for companionship and empathy. Adam and Susannah have been good friends.

I am grateful to my parents for their support and patience with my prolonged educational journey.

The project would not have been possible without my wife Fiona and is dedicated to my children, Ben and Sophie, who are still tolerant of a father who studies seaweed for a living.

GLOSSARY

ADCP	Acoustic Doppler Current Profiler
ANOVA	Analysis of Variance
CTD	Conductivity-Temperature-Depth Instrument
Diadromous	Regularly migrating between freshwater and seawater e.g. salmon.
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
ECE	Equilibrium Concentration Enhancement
EcoQOs	Ecological Quality Objectives related to Eutrophication set by OSPAR
FRS	Fisheries Research Services
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic. The "OSPAR Convention" was opened for signature at the Ministerial Meeting of the Oslo and Paris Commissions in Paris on 22 September 1992.
PES	Provosali's Enrichment Solution
RID	Riverine Inputs and Direct Discharges
SAMS	Scottish Association for Marine Sciences
SEPA	Scottish Environmental Protection Agency

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CHAPTER 1

REDUCING THE ENVIRONMENTAL IMPACT OF SEA-CAGE FARMING THROUGH CULTIVATION OF SEAWEEDS.

Introduction

Fish cage farming today is a significant industry, expanding in many parts of the world. In 2002 total world fish aquaculture production was nearly 26 million tonnes. From a world production of about 100,000 tonnes in the early 1950s, total marine and diadromous fish farm production has increased to about 3.7 million tonnes in 2004 (Figures 1.1 & 1.2, FishStat Plus, FAO 2006).

Fish farming in the UK has increased from an annual production of 598 tonnes in 1980 (Tett and Edwards 2002) to 169,736 tonnes in 2003 (Smith *et al.* 2005), a 283 fold increase in production over 23 years. Over 90% by value and by volume of the UK's aquaculture takes place on the west coast of Scotland. Production is dominated by Atlantic salmon (*Salmo salar*) reared in cages at sea, with a much smaller production of rainbow trout (*Onchorhynchus mykiss*). The last two years which has seen the first declines in salmon production. In 2004, salmon production was 158,099 tonnes, and rainbow trout production 6,352 tonnes, both reductions from the previous year, 7% and 10% respectively, with further reductions expected for 2005 (Smith *et al.* 2005).

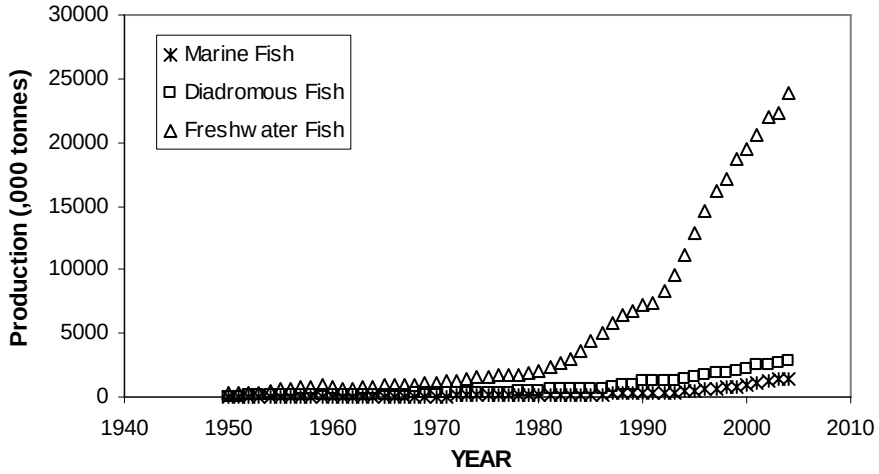

Figure 1.1 World aquaculture fish production 1950 – 2004 (FAO: Fishstat Plus 2006, www.fao.org/fi/statist/fisoft/fishplus.asp also earthtrends.wri.org).

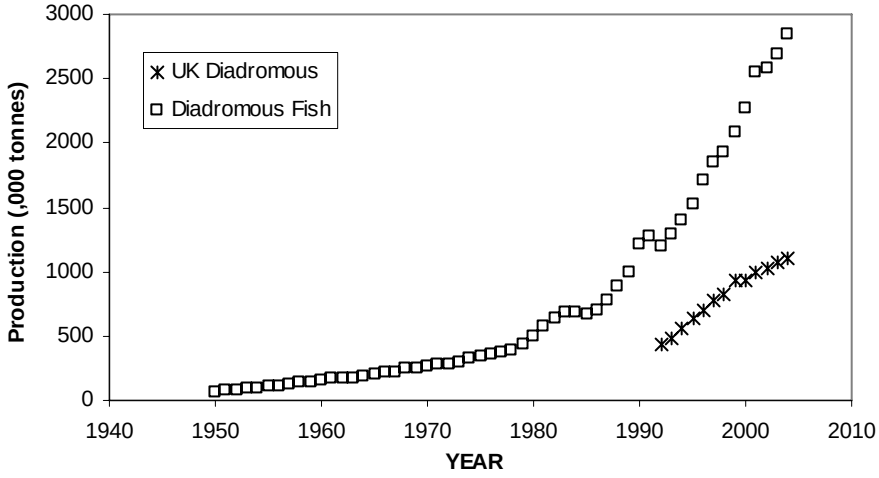

Figure 1.2 World and U.K. Diadromous aquaculture fish production 1950 – 2004 (FAO Fishstat plus 2006).

Salmon are typically fed energy rich diets high in protein and lipids. The principal end product of protein metabolism in fish is ammonia-nitrogen, which is toxic and therefore either excreted rapidly or converted to less toxic substances (Porter *et al.* 1987). A range of other nitrogenous compounds may also be excreted (including urea, trimethylamime, creatine and creatinine), and the chemistry of these compounds in sea water is complex (Handy and Poxton 1993b). If the amount of protein ingested is higher than the organisms' requirement or if the amino acid balance is poor, deamination occurs and nitrogen is excreted passively, mainly as ammonia, across the gills.

In general between 52 and 95% of the nitrogen, 85% of the phosphorus and 80 – 88% of the carbon input to a marine fish farm may be lost to the environment as feed wastage, fish excretion, faeces production and respiration (Wu 1995). This equates to 95 – 102 kg N and 9.5 kg P per tonne of fish produced (see figure 1.3, Hall *et al.* 1992; Subandar *et al.* 1993).

The rapid growth in sea-cage fish farming in the UK, particularly in Scotland and Ireland, and the extent to which intensive aquaculture results in degradation of the surrounding environment has been the subject of continued speculation and investigation (Gowen and Bradbury 1987; Gillibrand *et al.* 2002; Heath *et al.* 2002; Tett and Edwards 2002; Gubbins *et al.* 2003; Rydberg *et al.* 2003; Smayda 2006; Beveridge 1984). The quantity of nutrient wastes discharged at fish farms becomes evident when compared to muncipal loadings. For example, 8,700 t of the 10,000 t of salmon produced in Scotland in 1986 were farmed on the West and North coasts and the Hebridean Islands (WNH; World Natural Heritage region). The amount of nutrients discharged from fish farms in the WNH region that year was estimated to have exceeded that in the waste from the human population in that region (Tett and Edwards 2002). The WNH salmon production increased to 25,000 t in 1990 and 81,000 t in 2000. Tett and Edwards (2002) calculated that the regional WNH nutrient loading from fish farms in the 15-year period from 1985-2000 increased from parity with domestic waste delivery to exceeding the latter by 9-fold. MacGarvin (2000) conjectured that, in the year 2000, Scottish aquaculture released approximately 7,500 tonnes nitrogen and 1,240 tonnes phosphorus, amounts comparable to the annual sewage input of approximately 3.2 million and 9.4 million people, respectively. In 1997, Scotland's population was 5.1 million.

There is now a consensus that at least 80% of the total dissolved inorganic nitrogen (DIN) from fish farming is plant-available as potentially eutrophicating substances (Persson 1992; Troell *et al.* 1997; Black 2001). The remainder is lost as particulate organic waste, a proportion of which is also likely to end up in the water column as dissolved nutrients (Hall *et al.* 1992; Wu 1995). In coastal waters the two most important elements in algal metabolism are nitrogen and phosphorus because they are most likely to limit growth rate under 'natural' conditions. As phosphorus is in excess in the open ocean, the principal limiting factors for algal productivity are N and light (Dring 1991; Lobban and Harrison 1996). Fish excreta and waste fish food have a N:P ratio close to 7:1 w/w (Aure and Stigebrandt 1990) and hence provide well balanced nutrients for algal growth.

The Scottish Environmental Protection Agency (SEPA) regulates fish farming activity by assessing the local hydrographic features and tonnage of fish produced on site, balancing this against the need to allow for free movement of additives, such as feed pellets, fish excretion and fish faeces, which mix in the water column and are advected by tide and wind-induced currents to mix within the surrounding coastal waters (Falconer and Hartnett 1993; Wu 1995). Hence, with the exception of the area of seafloor immediately below the cages, gross impacts in the immediate vicinity of the farm are largely prevented, but the farming activity still results in the net input of nutrients to the surrounding seas (Gowen and Bradbury 1987; Aure and Stigebrandt 1990; Pitta *et al.* 1998; Karakassis *et al.* 2001; Doglioli *et al.* 2004; Islam 2005; Pitta *et al.* 2005; Tsapakis *et al.* 2006).

Changes in nutrient ratios in inshore waters due to anthropogenic input have been linked to harmful algal blooms (HABs, Berry, 1996). However, Smayda (2006) reported that, despite the 283-fold increase in farmed salmon production that has occurred in Scotland over a period of 23 years, accompanied by a more than 23-fold increase in annual N and P waste loadings from fish farms since 1985, there is no evidence that blooms of the indigenous HAB flora have increased in frequency or that novel HAB species have newly expanded into Scottish coastal waters. These conclusions agree with those arrived at by Tett and Edwards (2002), and by Rydberg *et al.* (2003) concerning possible evidence for HABs in Scottish waters.

The possibility of nutrients contributing to eutrophication in Scottish waters is not as equivocal, however, as it not clear how wastes are distributed within lochs. Most research has been aimed at immediate sedimentation underneath the farm i.e. on uneaten food plus faecal pellets (Cromey *et al.* 1998; Cromey *et al.* 2002; Nickell *et al.* 2003). However, the bottom underneath a farm recovers within 2-4 years (Holmer and Kristensen 1996; Karakassis *et al.* 1999; Brooks *et al.* 2003; Macleod *et al.* 2004) after the farm is removed. The potential for eutrophication of the environment as a result of nutrients originating from fish farms has been recognized by the regulatory authorities in Scotland and the Equilibrium Concentration Enhancement (ECE) for a loch is considered when determining salmon farm biomass consents. The ECE is the ratio between nutrients added via fish farming and the averaged water flux estimated as the ratio between the volume of the loch at low water and tidal flushing time calculated using a tidal prism method (Gillibrand and Turrell 1997; Gillibrand *et al.* 2002).

The presence of sites previously used for fish farming may make an area more sensitive to the establishment of further farms than a pristine location would be (Rydberg *et al.* 2003). The leakage of inorganic nutrients from deposits on the sea floor, as well as from

the farm itself, results in long term ecological effects, which are not considered within ECE methodology. In most cases, it will take several years before a balance between input and output is established at the sediment-water interface. A proportion of the inorganic nutrient waste from the farms will be stored in the sediments**,** but the rest is returned to the water column, eventually showing up as larger internal nutrient recirculation and potentially as excess winter nutrient concentrations (Beveridge 1996; Stigebrandt 1999; Rydberg *et al.* 2003; Islam 2005).

In lochs with restricted exchange (i.e. flushing time of more than two days) and larger fish farms, nutrients originating from fish farms may dominate the plankton production. If one assumes a net phytoplankton production of 30 gC m^{-2} y^{-1}, a 1,000 t y^{-1} (40 t N y^{-1}) fish farm may cause a wide-spread sedimentation equal to the plankton production over an area of 10 km^2, assuming that no nutrient export to the waters outside the loch takes place. This type of comparison may work as a conservative tool for estimation of the long-term environmental effects of fish farms (Rydberg *et al.* 2003). After many years, regeneration of nutrients might be near 100%, presuming that denitrification is not efficient and burial losses are not large, which could be the case.

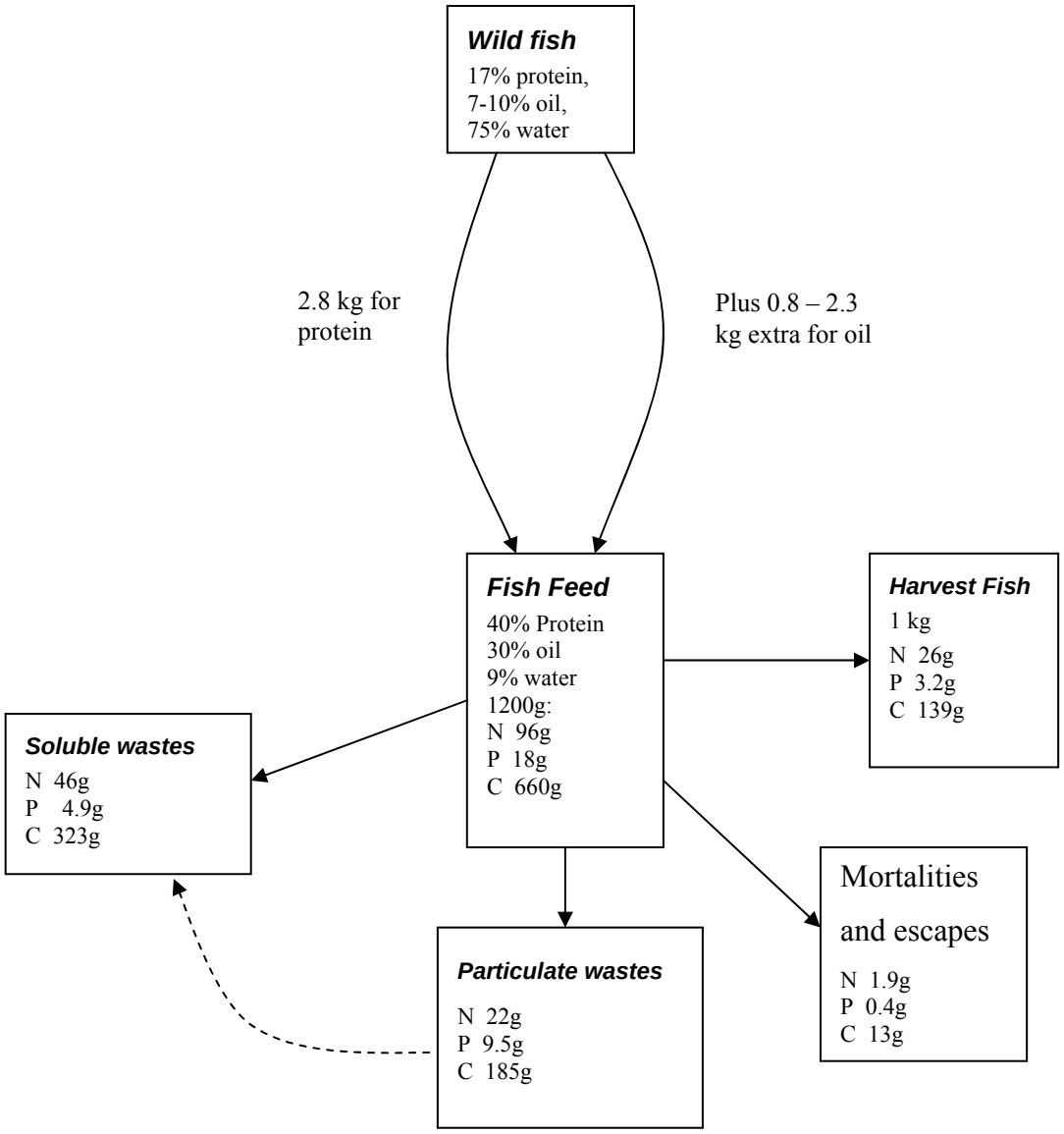

Figure 1.3 A budget for the flow of nutrients from oceanic wild caught fish to the coastal environment for a harvest of 1 kg of farmed salmon assuming no substitution with vegetable protein or oil and a ratio of fish feed to product of 1.2:1 (Black 2001).

Macroalgae can take up nitrogen from sea water at high rates resulting in growth of up to 9% in biomass d^{-1} (Subandar *et al.* 1993). Fucoid primary production can be 700 g C $m^{-2}yr^{-1}$ (Hawkins *et al.* 1992) compared to 95 g C $m^{-2}yr^{-1}$ for phytoplankton (Burrell 1988). Dring (1991) gives typical production values of 50 – 100 g C $m^{-2}yr^{-1}$ for phytoplankton and from 300 – 900 g C $m^{-2}yr^{-1}$ for macroalgae. Waste nutrients emanating from fish farms present a means of increasing production of seaweed crops and, by harvesting the macroalgae, local nutrient pollution would be alleviated and

result in further net losses of nutrients from coastal ecosystems. By using macroalgae that are also of commercial value, as human or animal food, their cultivation will generate a secondary income for the fish farmer and an economic as well as an environmental incentive.

The ability of many species of macroalgae to effectively utilise nutrients from effluent in sea water, resulting in their rapid growth, is well documented both for tank and sea-based fish cultivation systems (Chopin *et al.* 1999; Neori *et al.* 2000; Neori *et al.* 2004). The green alga *Ulva lactuca* can remove 90% of ammoniacal nitrogen and the red macroalga, *Gracilaria chilensis* can remove up to 95 % of dissolved ammonium from fish effluent in integrated tank cultivation systems (Cohen and Neori 1991; Buschmann *et al.* 1996). Using effluent from cultivated abalone (edible marine gastropods) as an ammonium source, Evans and Langdon (2000) showed *Palmaria mollis* had a total ammoniacal nitrogen (TAN) uptake of 1306 μmol kg^{-1} h^{-1}. *Laminaria saccharina* has been found to remove 170 to 339 μmol l^{-1} h^{-1} (26 – 40%) of the incoming dissolved inorganic nitrogen (DIN) when cultivated in salmon farm effluent (1993). *Gracilaria chilensis* was found to have a 40% higher growth rate when cultivated at a distance of 10m from salmon sea-cages in Chile (1997). Similarly, Petrell *et al.* (1993) demonstrated that 1.5μM ammonia levels found adjacent to salmon sea-cages enhanced the growth of brown macroalgae. Petrell and Alie (1996) subsequently showed the commercial viability of kelp cultivation adjacent to salmon farms.

This project is the first to address the potential bioremediation attainable by cultivation of macroalgae adjacent to sea-based fish farms in the UK. Estimates of the effectiveness of macroalgae at removing excess N will be calculated by comparing the growth rates and nitrogen content of macroalgae cultivated at fish farm and pristine sites. The aim,

therefore, is to demonstrate an environmental advantage to cultivating macroalgae on fish cultivation sites and to produce a model for their capacity to reduce nutrient flux through potentially eutrophic surface coastal waters.

Specific objectives

1) to determine the distribution of soluble compounds of ammonia, nitrogen and phosphorus in the immediate vicinity of selected fish cage sites and to compare these levels with those of pristine sites.
2) to contrast the rates of macroalgal growth on fish farm sites with those on sites away from the influence of fish farming.
3) To investigate, through the use of stable isotope signatures, if the nitrogen absorbed by the seaweeds is of fish farm origin.
4) To investigate the relationship between ammonium and nitrate as nitrogen sources for the cultured seaweeds.
5) to assess the ability of commercially important seaweeds, cultivated in the immediate vicinity of caged fish, to reduce the nutrient loading diffusing to the surrounding waters.
6) to produce an estimate of the total nitrogen removal potential of the seaweeds and preliminary cost: value analyses.

Cultured Seaweeds

The edible red alga *Palmaria palmata* (Linnaeus) Kuntze was the main species studied. The project followed on from EU-funded research at Queens University Belfast demonstrating the alga's capacity for rapid and sustained growth on long-lines in the sea (Browne 2001). The potential for using the kelp *Laminaria saccharina* was also investigated during the project.

Palmaria palmata, commonly known as Dulse, Dillisk (English), Dilleasc or Creathnach (Irish: shell dulse) is an edible intertidal or shallow subtidal red alga found throughout the temperate zone of the northern hemisphere. It is distributed throughout the British Isles, but seems to be absent from some parts of the east coast of England. In the Atlantic, it is found from arctic Russia to northern Portugal in the east and from arctic Canada to USA (New Jersey) in the west. It is a native and abundant species in Britain and Ireland where it grows as an epiphyte on kelp stipes (especially on *Laminaria hyperborea*) and mussel beds and on rock surfaces (Guiry 1977; Irvine and Guiry 1983).

Palmaria palmata was widely used for food by the maritime Irish and Scots. It was also dried and eaten uncooked in Iceland, Norway, France and eastern Canada (Seaweed website, www.seaweed.ie). Although it has been utilised over centuries (regulations for the gathering of dulse are mentioned in the Icelandic sagas of the 10th century), increasing economic affluence resulted in a decline in dulse harvesting during the 20th century. Recent interest in naturally derived foods has created increased pressure on natural populations and has highlighted the need for alternative methods of growing good quality, high value plants to supplement those harvested from natural populations.

Laminaria saccharina was chosen as it is a large, fast-growing alga and thus has good bioremediation potential. The alga also has potential, although unproven, commercial value. *Laminaria saccharina* is edible and is very similar to *L. japonica* or Kombu which constitutes the largest aquaculture crop, by weight, in the world. *Laminaria saccharina* is also currently being tested as a potential source of novel polysaccharides with biomedical applications (Cumashi *et al.* unpubl.) as an adjunct to this project.

Loch Duart Ltd

The principal industrial partner in this project is Loch Duart Ltd. The company is based at Badcall Bay (Figure 1.4). Various companies have been processing salmon at this site since the 19th century. It was one of the first companies to enter into salmon culture in Scotland (starting 1975) and currently one of the few Scottish owned salmon farming businesses amongst the larger multinationals that dominate salmon production in Scotland. In order to maintain market share in this increasingly competitive area, Loch Duart Ltd. has been a leader in the area of quality salmon, utilising farming practices that are not always the most economical but aimed at growing fish of the best quality under best environmental practice (www.lochduart.com).

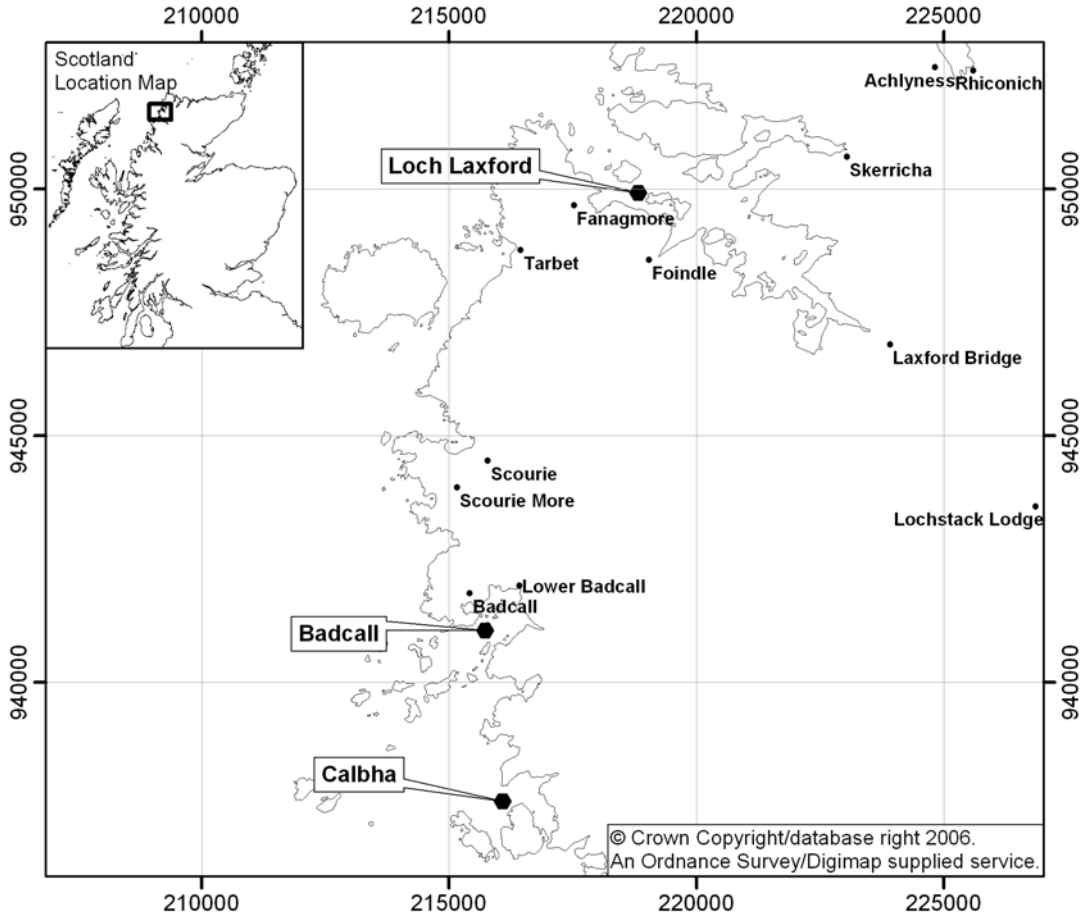

Figure 1.4 Location of salmon farm lease sites for Loch Duart Ltd: Loch Laxford, Badcall and Calbha in north west Scotland.

Farming practices include lower stocking densities and feeding regimes such that only the dominant fish are fed to satiation resulting in leaner, fitter salmon. Nets on salmon cages are not treated with antifoulant, rather the fish are transferred around pens and the nets are regularly pulled out of the water to restrict the development of fouling organisms. Environmental recognition includes recently gaining ISO 14000 accreditation and animal welfare certification from the RSPCA (Freedom Foods). Loch Duart's interest in this project is to alleviate any possible impact that the farms may be having on the nutrient status of surrounding waters by promoting the culture of seaweeds. At the same time they hope to develop a product which may provide a second income.

In 2003, Loch Duart had three marine farm sites, one at Badcall Bay (also the site of head office); Calbha approximately 3 km south of Badcall and Loch Laxford 8 km north of Badcall (Figure 1.4) and these are the sites where cultivation trials were conducted for the years 2003-2005.

In addition to other farm practices aimed at reducing the impact of farms on the environment, Loch Duart rotate farming between their sites so that one farm site is fallow every year. The salmon are grown for two years at each site from introduction as smolt (mean size approximately 200 g) to harvest (mean size approximately 5 kg). In 2003, salmon were introduced as smolts to the *Calbha* site, salmon reached harvest size at Loch Laxford and Badcall Bay was left fallow. As the aims of this project concerned farms as a source of nutrients, experiments each year were conducted at the Loch Duart sites with the maximal biomass of salmon and thus maximal feed input. In 2003 this was Loch Laxford; 2004: *Calbha* and in 2005: Badcall Bay (Figures 1.5 & 1.6).

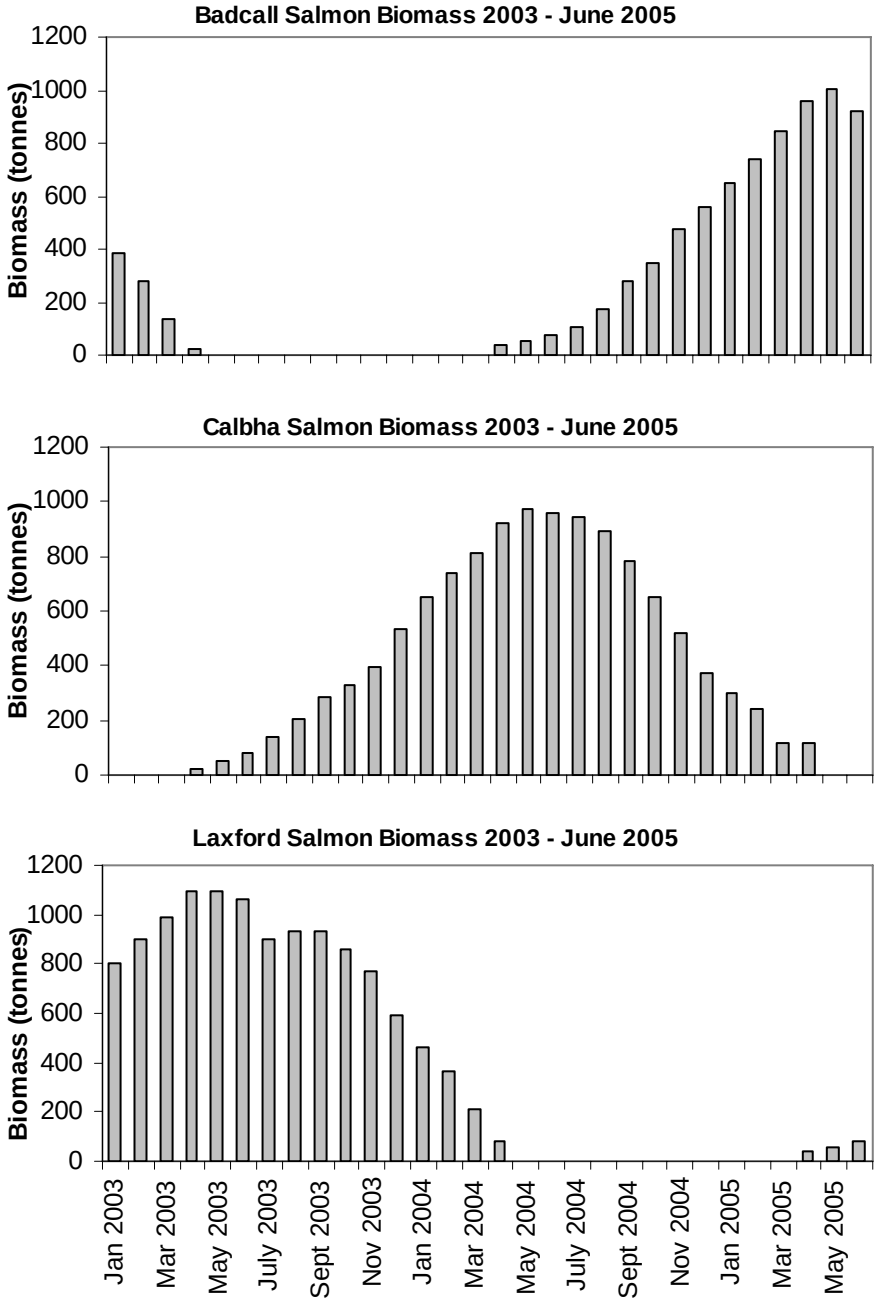

Figure 1.5 Salmon biomass at each of the three farm lease sites; Badcall, Calbha and Laxford for the period: 2003 – 2005 (source: Loch Duart Ltd, 2005).

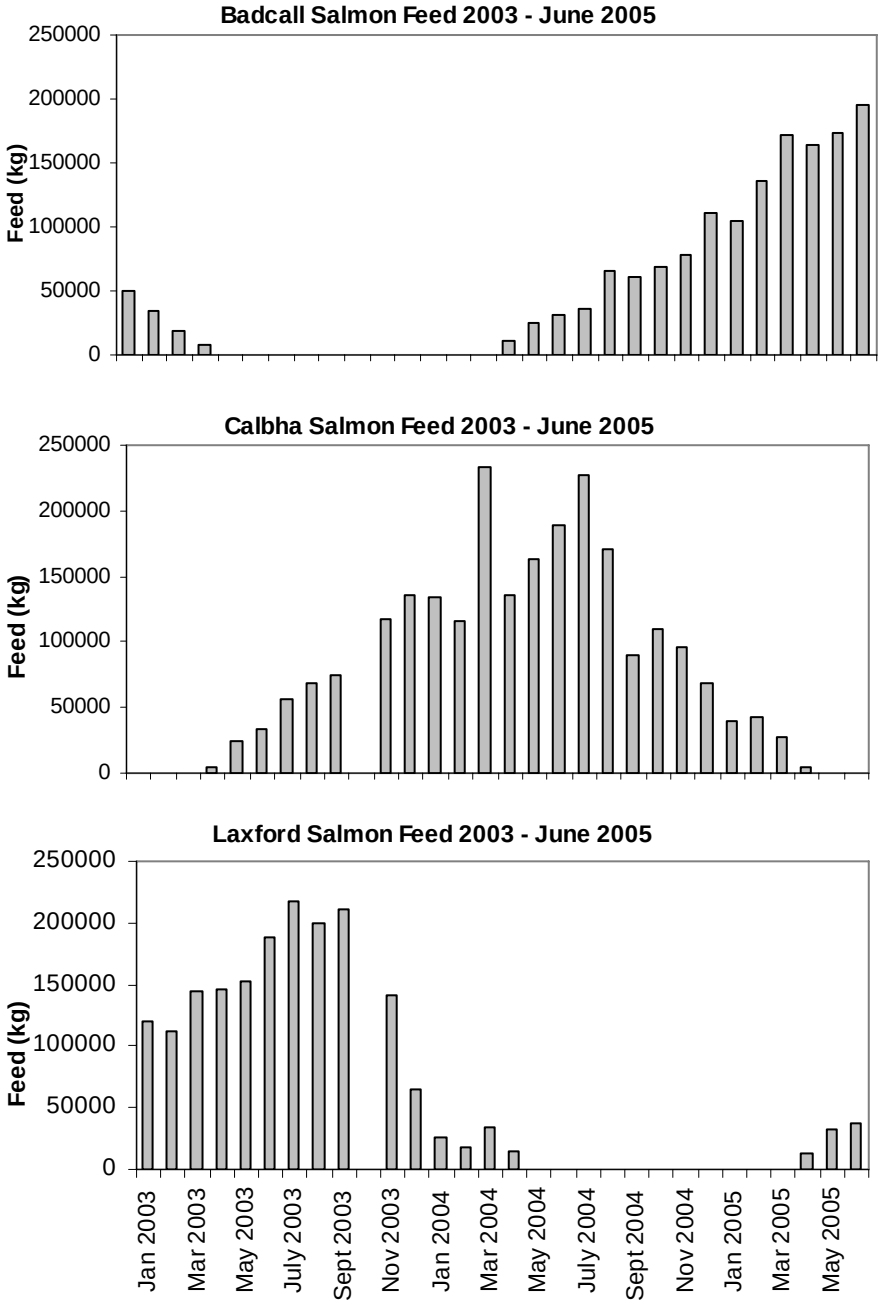

Figure 1.6 Amount of salmon feed added to fish farm cages at each of the three farm lease sites; Badcall, Calbha and Laxford by month for the period: 2003 – 2005.

CHAPTER 2

HYDROGRAPHIC DATA IN THE VICINITY OF THE FISH FARM CAGES

Introduction

The main premise of this project is that macroalgae cultured in the vicinity of the salmon cages will utilise the excess nutrients generated. The most frequently limiting nutrient for seaweeds in coastal waters is nitrogen. In temperate areas, nitrogen is available to the algae as nitrates for much of the year (Lobban and Harrison 1996). On the Scottish north west coast, nitrates become limiting in summer (Gillibrand *et al.* 2003) which can result in a dieback of macroalgae (Rhyther and Dunstan 1971). Macroalgae have also been shown to utilise ammonium as a nitrogen source and some algae have demonstrated differential uptakes of ammonium and nitrate with a preference for ammonium (Lobban and Harrison 1996).

Ammonium concentrations in the vicinity of fish farms are elevated as a result of excretion by the fish (Handy and Poxton 1993a). Growth of macroalgae may be enhanced for much of the year by growing them adjacent to salmon farms. In summer, ammonium could be the principal source of nitrogen for macroalgae close to fish farm cages and enable an extension of the growing season.

Current knowledge suggests peaks in the concentration of ammonium adjacent to salmon cages relates to the timing of feeding the salmon (Ahn *et al.* 1998; Pitta *et al.* 1998; Karakassis *et al.* 2001; Karakassis *et al.* 2005), however, there is little information available on the distribution of the ammonium around the cages and how

far and for what period of time the plume extends from the cages. This information is necessary for determining placement of seaweeds for culture in order to maximise any benefit.

The aims of research detailed in this chapter were to determine:

- ambient levels of nutrients in the vicinity of Loch Duart Ltd. fish farms (section 2.1)
- ammonium concentrations in the immediate vicinity of Loch Duart Ltd. fish farms (section 2.2)
- patterns of distribution of and distance that enhanced nutrients extend from cages (section 2.3)
- temporal variation in ammonium concentration around the fish farms (section 2.4).

Background

Approximately 8% of fish feed is nitrogen and nearly 50 % of this nitrogen finishes up as dissolved in the water column. For every 1200 g of fish feed, at least 46 g of nitrogen is lost to the environment in the dissolved form (see chapter 1).

The greater proportion of nitrogen excreted by teleost fish such as Atlantic salmon (*Salmo salar*) is as ammonia (90%) and the remainder consists principally of urea. The excretion of ammonia is energetically less expensive than converting ammonia to urea via the ornithine urea cycle (five high-energy phosphate bonds consumed per one urea molecule produced (Dosdat et al. 1996; Wright and Land 1998)).

The equilibrium between un-ionized ammonia (NH_3) and ionized ammonium (NH_4^+), that has a pK of 9.24 at 25^oC (as described by the Henderson-Hasselbalch equation), is affected strongly by pH and much less by temperature. Alkaline pH and higher temperature favour the un-ionized form. As an approximation, at pH 9.3, about 50% of ammonia is un-ionized; at pH 8.3, about 10% is un-ionized; and, at pH 7.3, about 1% is un-ionized. Volatilization is thus enhanced at elevated pH due to equilibrium relationships and the resultant increase in the partial pressure of ammonia gas (as described by Henry's Law). The pH of seawater is considered to be very stable with small fluctuations between 7.5 and 8.5. Ammonia volatilization is not important at pH <7.5. (Hargreaves 1998). Modelling of a temperate wastewater pond showed that ammonia volatilization is inconsequential as a mechanism of nitrogen removal (Ferrara and Avci 1982). For the purposes of this project, the ammonia excreted by the fish is assumed to be converted to ammonium and is the main nutrient tested.

The post-prandial pulse of ammonia excretion in salmonids is well documented. Excretion rates may vary according to unimodal or polymodal rhythms depending on the frequency of feeding (Kaushik and Cowey 1991). The amplitude and timing of the peak rate is dependent on the amount of nitrogen intake by the fish. The maximum or peak ammonia excretion rates of salmonids occurs within 3-5 hours after a meal and is usually 30-60% higher than daily mean rates (Brett and Zala 1975; Kaushik and Cowey 1991; Forsberg 1997).

At Loch Duart Ltd. the daily timing of fish feeding is dependent on the size and age of the fish. At each feeding session, fish are fed until there is a marked reduction in surface activity and then they are not fed again for at least two hours. When fish are first introduced to the sea between February and May (first year fish) they are fed four times a day with the farm operating a split shift so that fish can begin feeding early in the morning soon after daybreak. After three months, the feeding is reduced to three meals a day and for the final seven months (second year fish) it is reduced to two meals a day. Feeding is restricted to daylight hours.

Pilot Study

A pilot study (Kelly, unpubl.) was conducted on nutrient availability in May 2001 prior to the start of this project to confirm the presence of elevated nutrient concentrations around fish farms that would support macroalgal growth. This was conducted adjacent to one of Loch Duart Ltd.'s cage-farm sites (Badcall Bay) which was stocked with year one sea winter salmon, a total biomass of 350 tonnes. The sample sites were: A, the centre of the raft of cages; B, 25 m north east of the cage group; C, 25 m south west of the cage group and D, an offshore site, considered to be out of the zone of influence of the farm. The analysis was restricted to ammonium as most of the dissolved inorganic

nitrogen (DIN) originating from salmon sea-cage farms is found in this form (Petrell *et al.* 1993).

The data showed elevated ammonium levels at sites A, B and C that were typical of those at a sea-cage (Petrell *et al.* 1993). The levels at site A were significantly greater than those at B, levels at site B and C were equivalent (Table 2.1). Ammonium concentrations ranged from 2.7 – 3.9 μM at a depth of 3 m at sites A, B and C. At 10 m depth the ammonium levels were an order of magnitude lower and equivalent to those at the offshore site (mean 0.46 μM).

Table 2.1 Mean ammonium (μM) levels for replicate 0.2 μm filtered seawater samples taken in the vicinity of salmon cages.

μM ammonium	**Surface**	**3 m**	**10 m**
A on cage raft	3.2	3.9	0.4
B 25 m N-E	-	2.8	0.7
C 25 m S-W	-	2.8	0.3
Doffshore site	-	0.3	0.6

Site Selection

At Loch Duart Ltd. farms, young salmon are transferred to sea between February and April when they are approximately 200 g. They are held in pens for 18 months and graded according to size until they reach approximately 5 kg. Sites for conducting seaweed culture and monitoring of seawater for nutrients were chosen on the basis of having maximal biomass of cultured salmon for each season. These were usually sites where fish were coming into their final six months starting in late winter-early spring. Addition of feed to the pens and excretion of wastes are at a maximum for a site during this period, thus maximising the impact and increasing the likelihood of detecting differences in ammonium concentrations. In 2003 this was at Laxford, in 2004, at Calbha, and in 2005 at Badcall (see Figures 2.1 a, b and c and chapter 1).

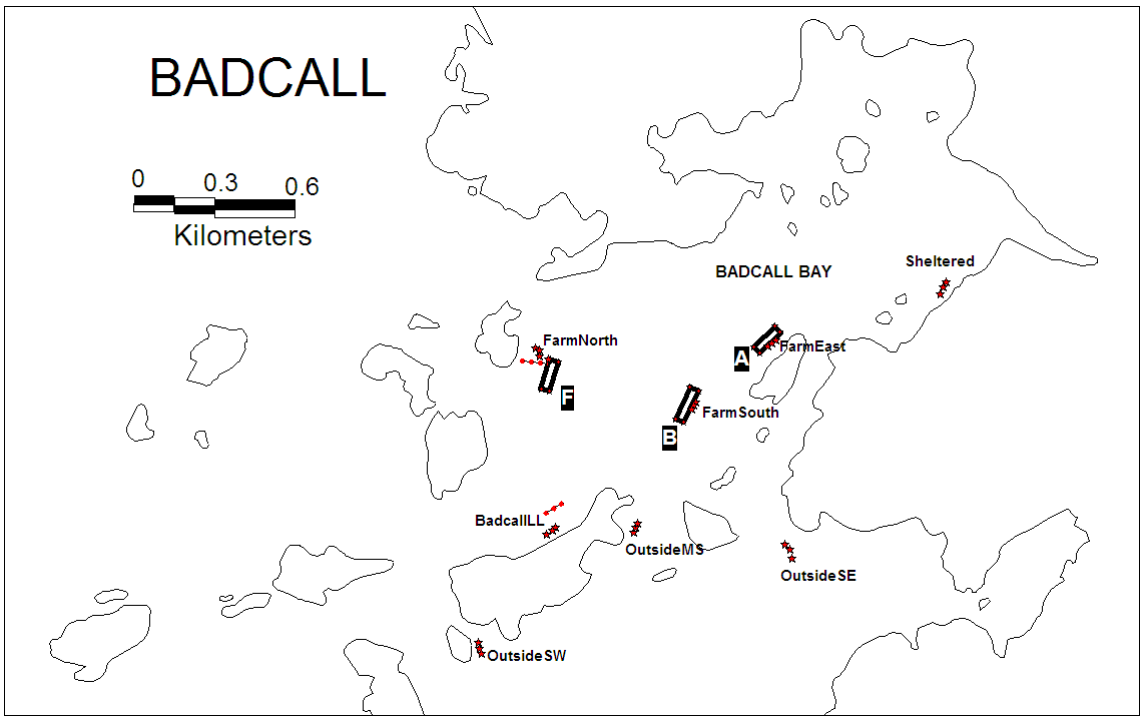

Figure 2.1 a) Location of salmon cages (with associated walkways) and nutrient sampling stations at Badcall Bay. Letters are walkway identification labels. 'F' walkway had a seaweed longline attached in 2005. Nutrient sampling stations in 2005 were sited adjacent to buoys as noted by small stars and site names in this figure ('LL' = longline).

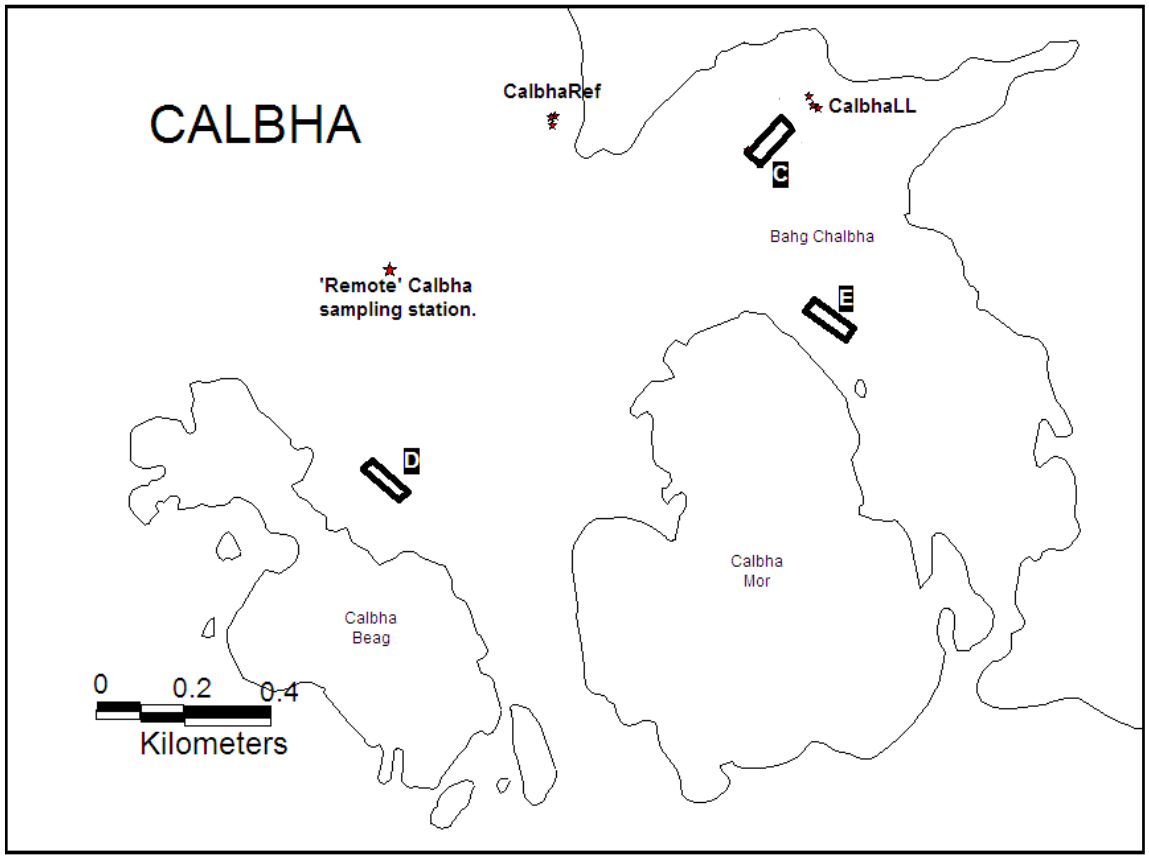

Figure 2.1 b) Location of salmon cages and nutrient sampling stations at Calbha. The larger star indicates a reference sampling station remote from cages. 'D' walkway had longlines attached in 2004.

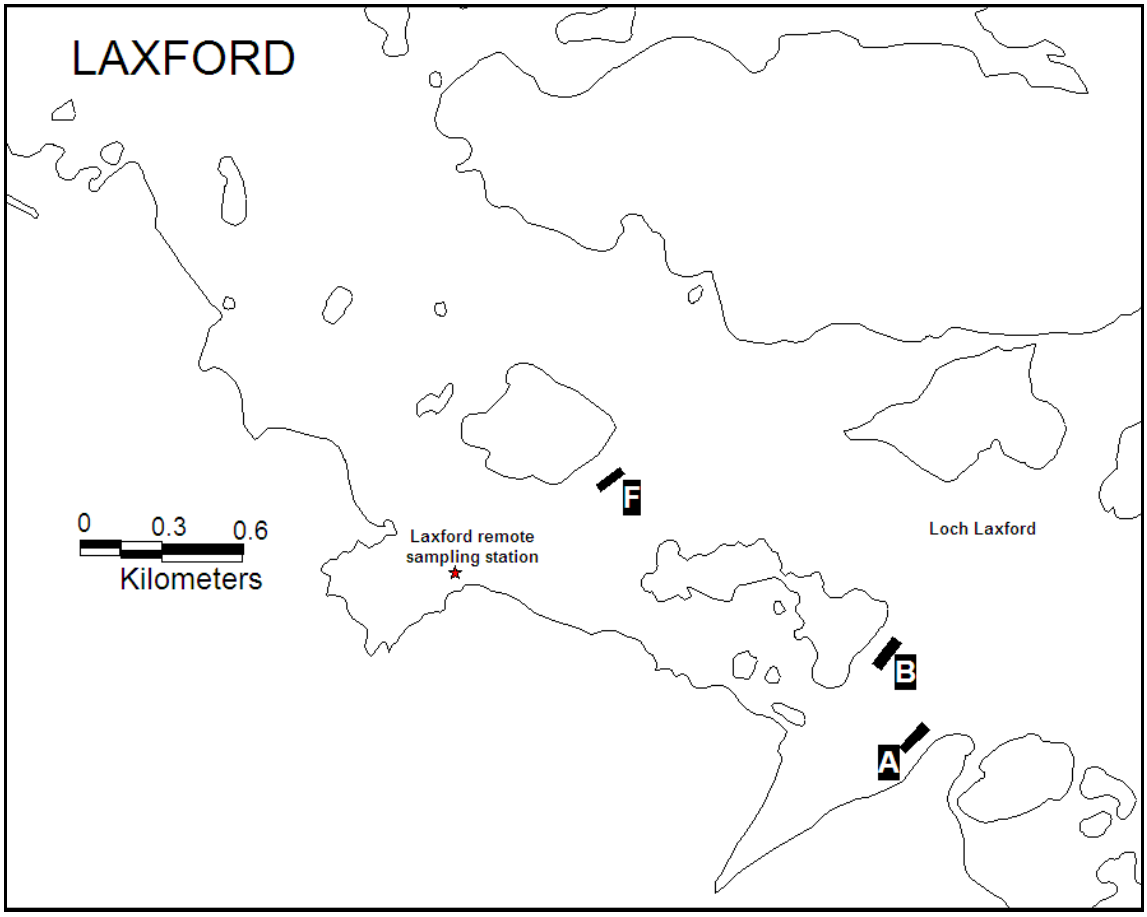

Figure 2.1 c) Map showing location of salmon cages and nutrient sampling station at Loch Laxford. The star indicates a reference sampling station remote from cages. 'F' walkway had longlines attached in 2003.

As a result of the increasing biomass of fish cultured on European coasts each year, nutrient inputs to coastal waters from fish farms have been under increasing levels of scrutiny internationally. There is concern that fish farming may contribute to eutrophication of coastal waters. In general, under eutrophic conditions nutrient concentrations are increased, primary production (possibly including nuisance species) is increased, and organic enrichment of the bottom sediments leads to increases in oxygen consumption as the excess material decomposes. Under exceptional conditions, serious depletion of oxygen levels in the water can occur, especially in areas where water mixing is restricted, such as in deeper waters below the halocline, or in shallower waters affected by thermal stratification during the summer. Depletions in oxygen

concentration have been associated with kills of benthic fauna and fish (www.helcom.fi).

Under 'Convention for the Protection of the Marine Environment of the North east Atlantic' (OSPAR), five Ecological Quality Objectives related to Eutrophication (EcoQOs) have been defined to reflect the eutrophic state of coastal waters. These are more specific with regard to physico-chemical criteria for assessing water quality than the EC Water Framework Directive (OSPAR 2004).

The EcoQOs agreed at the 5th North Sea Conference (Bergen-Declaration 2002) are:

A. Winter DIN and/or DIP (dissolved inorganic phosphate) should remain below a justified salinity-related and/or area-specific percentage deviation from background not exceeding 50%;

B. Maximum and mean chlorophyll *a* concentrations during the growing season should remain below a justified area-specific percentage deviation from background not exceeding 50%;

C. Region/area-specific phytoplankton eutrophication indicator species should remain below respective nuisance and/or toxic elevated levels (and there should be no increase in the duration of blooms);

D. Oxygen concentration, decreased as an indirect effect of nutrient enrichment, should remain above area specific oxygen assessment levels, ranging from 4-6 mg oxygen per litre;

E. There should be no kills in benthic animal species as a result of oxygen deficiency and/or toxic phytoplankton species.

These were developed in parallel to, and derived from, the "Comprehensive Procedure", that was established to assess and classify the eutrophication status of the OSPAR maritime area into problem areas, potential problem areas, and non-problem areas (OSPAR 2005).

The assessment parameters which relate to nutrient enrichment and thus are relevant to this project are:

1. **Riverine total N and total P inputs and direct discharges (RID)**

   Elevated inputs and/or increased trends

   (compared with previous years)

2. **Winter DIN and/or DIP concentrations**

   Elevated level(s) (defined as concentration >50 % above salinity related and/or region specific background concentration)

3. **Increased winter N/P ratio (Redfield N/P = 16)**

   Elevated ratio is > 25.

Increased winter N/P ratios (compared to the Redfield ratio = 16) and absolute excess of nitrate may increase the risk of nuisance and toxic algal species, while increased ratios of N/Si (> 2) and P/Si (> 0.125) may cause shifts in species composition (from diatoms to flagellates, some of which are toxic).

2.1 Ambient Nutrient Concentrations

The aim of this section was to determine the ambient concentrations of nutrients, particularly nitrogen based nutrients, in waters surrounding the Loch Duart Ltd. salmon farms.

Methods

Seasonal samples

From June 2003 until June 2005, at 4-6 week intervals, 'ambient' seawater samples were taken from sites at least 500 m distant from the principal farm site cages where seaweed culturing and monitoring was taking place for that year (Laxford in 2003, Calbha in 2004 and Badcall in 2005). Samples were taken using a Nisken bottle set to collect seawater at a depth of 4 m. Samples were collected at this depth because it is the depth at which the caged salmon commonly swim (farm personnel, pers. com.) so excreted products such as ammonium are likely to be concentrated. It is also the mid-depth for photosynthesis for the seaweeds to be cultured and is not as affected as the surface by stratification of the water column as a result of freshwater inputs or thermoclines. Kelly's (unpubl.) original pilot study showed very low levels of ammonium at 10 m depth and this has also been noted by others e.g. Karakassis *et al.* (2001) and SAMS based studies: Biofaqs (2003) and Pete (pers com).

Seawater samples were poured from the Nisken bottle into a 100 ml syringe and filtered through Whatman 0.45 μm filters into acid washed bottles. The sample bottles were kept cool and within hours transferred to a deep freezer (-20^{o}C). Ammonia is known to be a volatile chemical and cooling the samples limits dispersal of this compound. The samples remained in the deep freeze until analysed. All samples were analysed for nitrate, phosphate and ammonium. As silicate is not required for growth of macroalgae

and, early in the sampling programme, nitrite concentrations were found to be very low in contrast to nitrate and ammonium, not all samples were analysed for this ion. Analyses were conducted for ammonium, nitrate, nitrite, silicate and phosphate using a Lachat flow injection autoanalyser (QuickChem 8000).

Results

Ambient concentrations of nutrients

Ambient nitrate concentrations varied seasonally across all sites ranging from 0 to approximately 7 μM, with a peak in December – January and lowest levels from late May to early August (see Fig. 2.3). Ammonium concentrations were variable and up to 2 μM but mostly approximately 1 μM with more elevated levels in June to July and lows from November to February (see Fig. 2.3). Phosphate concentrations followed a similar pattern to the nitrate (see Fig. 2.3 and 2.4) with peaks of 0.4 to 0.8 μM but commonly approximately 0.4 μM. When measured, nitrite levels were low (278 readings, max: 0.5 μM, average: 0.09 μM, se: 0.006 μM) and not considered a significant source of nitrogen at the sites. Silicate was only sampled in June and July 1993 at Loch Laxford and values varied from 0.1 to 1.0 μM (50 readings).

The OSPAR harmonised Eutrophication Assessment criteria (OSPAR 2004) recommended upper assessment concentrations for the UK for winter DIN and DIP of 10 μM and 0.8 μM which would correspond to 50% above ambient winter concentrations of approximately 6.7 and 0.53 μM respectively. These ambient values are less than the maximum ambient winter values for nitrate and phosphate recorded as part of this project (7.4 and 0.8 μM respectively). However, only one value of all 'away from farm' or ambient seawater samples analysed (105 samples) exceeds the upper assessment concentrations and was not measured in winter (Figure 2.3 a). Critical

values for the N:P ratio relative to critical values of the Redfield ratio used for determining eutrophication status are also plotted. They show that the majority of the winter values are close to the Redfield ratio of 16:1 and very few are above the 25:1 cut off mark for distinguishing problem areas. We might expect some elevated values due to the relative proximity of the samples taken to fish farms. Most of the elevated values are for Badcall Bay which is a more enclosed site and thus likely to have less exchange with coastal waters. Ammonium in this bay is also likely to have a higher residence time and there will be some build up of nitrogen in the bay.

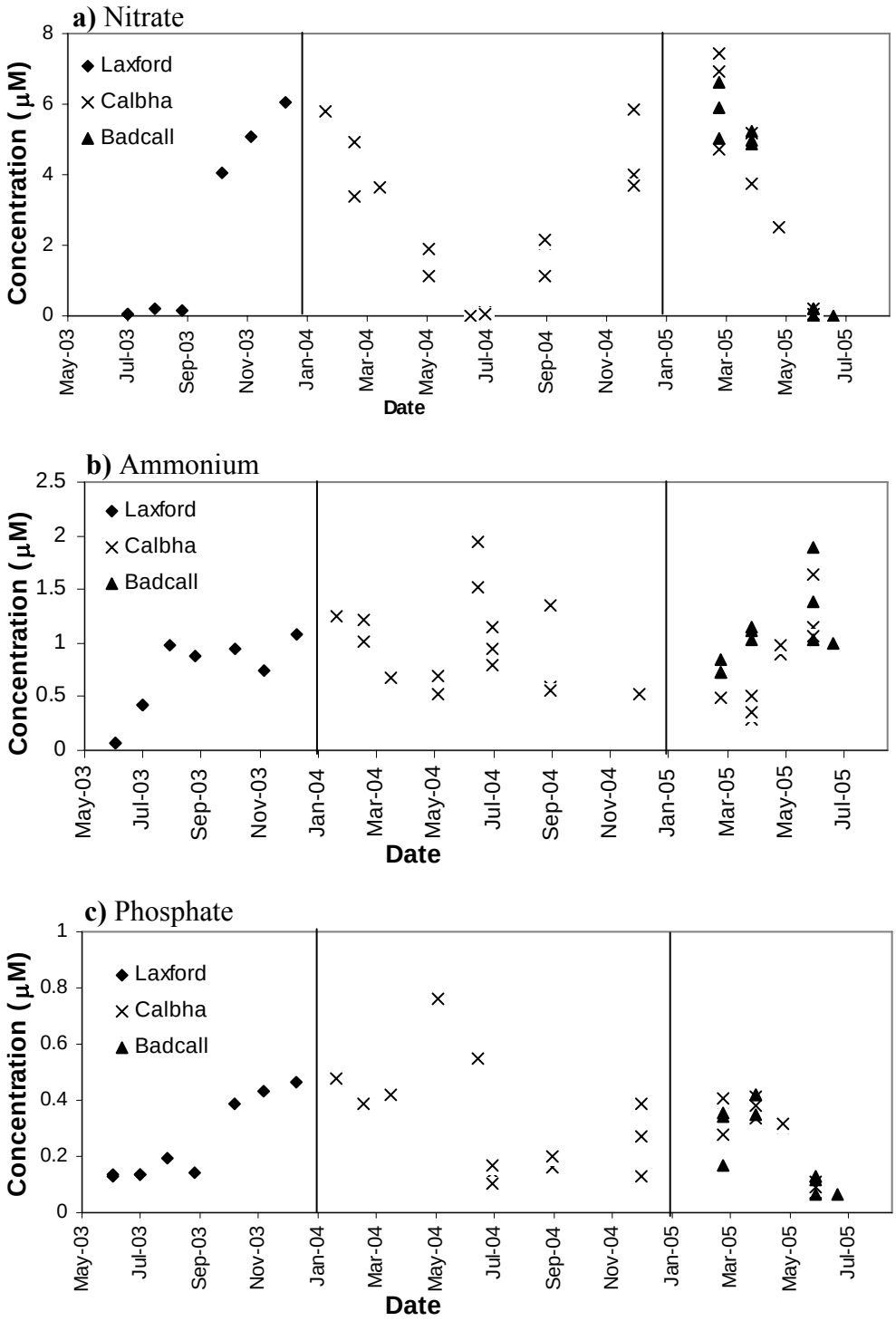

Figure 2.2 Variation in **a)** nitrate, **b)** ammonium and **c)** phosphate concentration for ambient waters in the vicinity of the fish farm cages, Loch Duart Ltd.

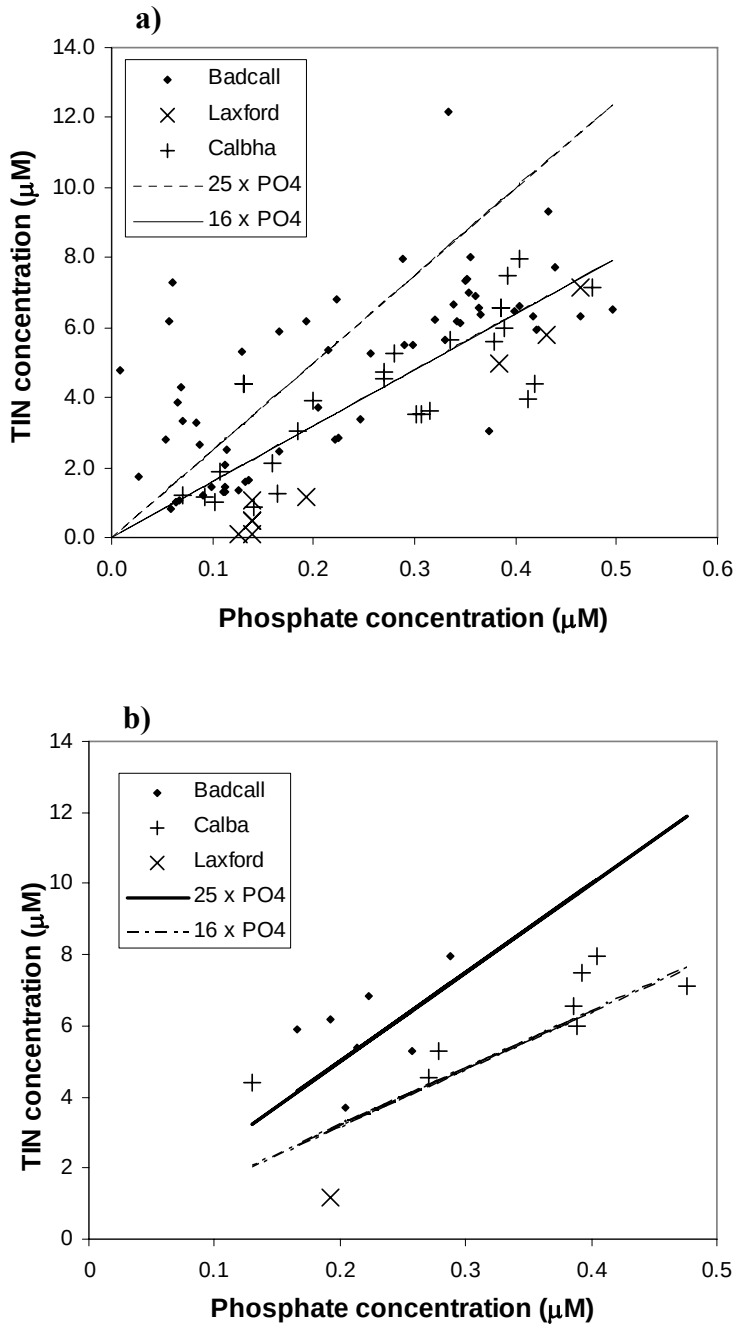

Figure 2.3 Total inorganic nitrogen (TIN) and corresponding phosphate concentrations determined for Loch Duart Ltd. sites 2003-2005 for a) all samples and b) winter samples only. **a)** The two lines are the N:P ratio lines 25:1 (solid) and 16:1 (dashed) for comparison. **b)** The winter TIN:P values exceeding 25:1 are primarily Badcall Bay samples taken in February 2004.

2.2 Nutrient Enhancement around Salmon Farms

The aim of this section was to confirm the enhancement of nutrients in the vicinity of salmon farms, especially ammonium and seasonal differences. Sampling was confined principally to the peak growth season of the algae concerned (*Palmaria palmata* and *Laminaria saccharina)*: winter, spring and early summer.

Methods

In conjunction with the Atlantic Arc Aqua Group (AAAG) project, also based within the Invertebrate Marine Biology section of SAMS, seawater samples were collected on a regular basis in Calbha and Badcall Bays, close to and at distance from salmon cages (Calbha was a fallow site in 2005 i.e. no fish cages during this sampling). The AAAG project was investigating protein levels in wild *Palmaria palmata* in relation to proximity to salmon farms. Samples were taken as part of this thesis to correlate with growth and production of cultured *Palmaria palmata* and *Laminaria saccharina* at Calbha and Badcall (see Chapter 6).

Samples were collected at 4 m depth monthly from February 2005 to June 2005. At each sampling session, three samples were collected from sites adjacent to the three salmon cage sets in Badcall Bay (three sites: FarmEast, FarmSouth and FarmNorth) and at distance (seven sites: Sheltered, BadcallLL, OutsideSW, OutsideMS, OutsideSE, CalbhaRef and CalbhaLL; see maps in Figures 2.1 a) & b). The samples collected at the salmon farm cages ('Farm') were collected either from the walkway on the sides of the cages or from a small dinghy tied up to the cages, i.e. 2-10 m from the nets containing the salmon. The samples were collected at the same locations on each sampling date

without particular reference to currents, tides or stage in the salmon feeding process. The water samples were collected, treated and analysed as described in the previous section.

Within the bay at Calbha, during the sampling period, salmon biomass was decreasing as fish were being successively harvested from the cage groups (Chapter 1: Figure 1.5). Fish were emptied from walkway C in January, E in February and D in May (Figure 2.1b). Badcall had increasing levels of salmon over the same period (Chapter 1: Figure 1.5).

Statistical analysis

Sites were compared for each sampling session using one way analysis of variance. Data were checked for normality (Anderson-Darling) and homogeneity of variance (Bartletts and Levenes tests). Transformations of the data were done where necessary (natural log) and post hoc tests were conducted using Tukey tests. Where present, error bars presented on graphs are 95% confidence intervals. Statistics packages used were Excel, JMP IN (SAS Inst Inc) and Minitab.

Results

Ammonium

The results for ammonium only are considered here as the other nutrients (nitrate and phosphate) showed no difference with proximity to salmon farm cages. On the five occasions that nutrients were sampled (not all sites were sampled on 28/4/2005 due to weather and time restrictions), there were significant differences between the farm sites and all other reference sites (sites pooled, Figure 2.4 and Table 2.2) for ammonium. Mean ammonium concentrations adjacent to the salmon farm cages ranged from 1.5 to 6.8 μM with most occurring in the 1.5 to 3 μM range. These values were 0.6 to 4.2 μM

greater than mean ammonium values measured away from the farm sites. Of the reference sites, the ‘sheltered’ site and the mid south (OutsideMS) site were consistently higher than the other reference sites and the Badcall Bay sites were consistently higher than the Calbha Bay sites.

Concentrations of ammonium in Badcall Bay sites may be higher than Calbha sites due to the more enclosed nature of Badcall Bay. There is less opportunity for the free exchange of seawater and this may lead to some entrapment of ammonium within this system. This would also explain why the ‘sheltered’ site in particular is consistently higher than the rest. The ‘Mid South’ site may be higher as this may be the ‘escape route’ for ammonium from Badcall Bay.

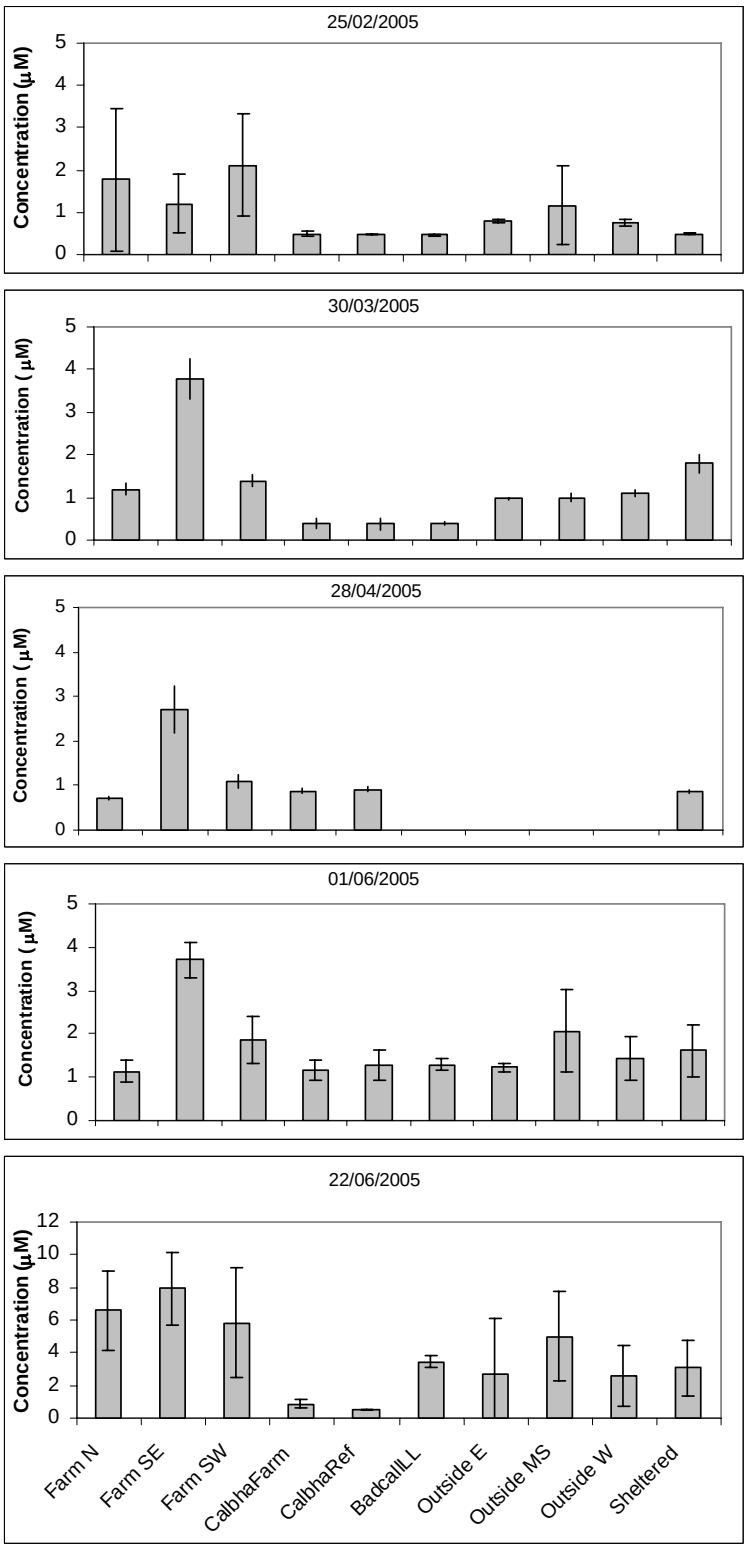

Figure 2.4 Mean ammonium concentrations at 10 sites in Badcall and Calbha Bays (three Badcall farm sites on the left) for the dates noted. See the lowest axis for site names (vertical bars indicate 95% CI).

Table 2.2 Summary of ANOVA results for comparison of farm sites versus non- farm sites for ammonium concentration (Site nested within Farm); df - degrees for freedom for F ratio, F ratio and significance level (*p*) for farm versus non-farm and site analyses.

Date	Farm F: df	F	*p*	Farm Conc. Mean (μM)	Non-Farm Conc. Mean (μM)	Site F: df	F	*p*
25/02/2005	$F_{(1,20)}$	15.4	<.0001	1.7	0.7	$F_{(8,20)}$	0.7	ns
30/03/2005	$F_{(1,20)}$	235.9	<.0001	2.1	0.9	$F_{(8,20)}$	52	<.0001
28/04/2005	$F_{(1,12)}$	62.4	<.0001	1.5	0.9	$F_{(4,12)}$	73.4	<.0001
01/06/2005	$F_{(1,20)}$	30.6	<.0001	2.2	1.4	$F_{(8,20)}$	1.8	ns
22/06/2005	$F_{(1,20)}$	34	<.0001	6.8	2.6	$F_{(8,20)}$	4.8	<.01

2.3 Extent of the nutrient plume around the cages.

The aim was to determine the extent of the plume of ammonium about the fish farm cages. This information would assist in predicting the location of enhanced ammonium concentrations and the optimal positions for any seaweed cultures to maximise nutrient interception.

Methods

Three intensive nutrient monitoring programs ('snapshots') were conducted to determine dispersive patterns of the effluent plume, two of these were at Laxford (3/7/2003 and 31/7/2003) and one at Calbha (5/5/2004). These nutrient sampling sessions were conducted at the same time as drogues; current meters and weather stations were deployed adjacent to the farm.

Intensive 'snapshot' sampling

At Laxford, on each of 03/07/2003 (12:00 to 15:00GMT) and 31/7/2003 (9:30 to 13:30GMT) a total of 48 samples were collected from the waters in the immediate vicinity of the Laxford sea cages at 'F' walkway to 50 m. The samples were taken in a grid pattern about the cages. Distances were chosen on the basis of two previous pilot sampling sessions that indicated elevated ammonium to at least 25 m from the cage group (Kelly unpubl., Sanderson unpubl.). On each transect, six samples were taken at 4 m depth and 10 m apart, 0, 10, 20, 30, 40 & 50 m, starting closest to the cages. The dinghy was maintained at each station, when sampling, using a rope marked at designated distances attached to the cages. Transects were run from the corners and the mid points of the sides of the farm cages (i.e. eight transects, see Figure 2.6). On the first session at Laxford, transects were sampled sequentially. Due to the time lapse

between the first and last transect, and the possibility of pulses of nutrients influencing determined nutrient plumes, transects were sampled in no particular order around the cages on the second and third sampling snapshots.

The third sampling session was conducted at the Calbha 'D' walkway on 5/5/2004 (9:00 to 14:30 GMT). To maximise results for sampling effort, only four samples were collected on each transect at distances of 0, 15, 30 and 45 m, all at 4 m depth. Transects were run from each corner and the mid-point of the ends of the cage group and two from the sides (i.e. 10, see Figure 2.6). Further samples were taken in the direction of drogue movements and randomly at distances of up to 200 m from the cages. The water samples were collected, treated and analysed as described previously.

Nutrient snapshots were conducted at the same time as current meters and drogues were deployed adjacent to the seacages. The results from these aided in interpretation of nutrient distribution data.

Current meters

Type S4 (InterOcean Systems Inc. San Diego CA USA, S4 electromagnetic current meter: Sampling rate 2Hz, averaging for 1 minute in every 10) and ADCP (RD Instruments, San Diego, CA, USA Workhorse Sentinel 600kHz Acoustic Doppler Current Profiler Ensemble length 10 minutes, 200 pings per ensemble, standard dev 0.2 cm/s) current meters were used to determine current speed and direction at points close to the cages. Type S4 current meters determine current directions through variations in an electromagnetic field caused by the water flow. The ADCP (Acoustic Doppler Current Profiler) measures current speed and direction for a number of depth bands (= 'bins') in the water column through the use of acoustic technology. Current meters log

direction and speed of the seawater for user defined intervals and lengths of time. The number of readings is determined by the battery life and the amount of memory the machine has which, in turn, determines the amount of time the current meter can be deployed for.

At Loch Laxford 'F' walkway two S4 current meters were deployed, one near the surface (1 m depth at spring low) and one close to the bottom (3 m) in ca. 18 m depth (MSL) at 949,969 N 218,866 E (Brit OS Grid) at a distance of 130 m SE of the cages from 3/7/2003 to 27/8/2003 with readings recorded every ten minutes.

At Calbha 'D' walkway two sets of current measuring apparatus were deployed to get a better perspective of water movement around the cages. An ADCP was deployed at 937,273 N, 215,571 E approximately 60 m north of the north east corner of the cages in ca. 35 m of water (MSL). This recorded current speed and direction in 2 m 'bins' throughout the water column i.e. approximately 15 bins. Two S4s were deployed approximately 20 m south of the south east corner of the cage at 937,094 N 215,643 E in ca. 21 m of water (MSL) also from 3/7/2004 to 27/8/2004 with readings being recorded every ten minutes. One of the S4s was at 2m depth at low tide and the second was situated 5 m below the first.

Current meters are used to measure water speeds at their location. Ideally, a grid of current meters deployed at varying depths would give the most comprehensive picture of current patterns about a salmon farm cage group. This, however, is not practicable due to the cost of current meters and the logistics involved in placing multiple current meters on a working salmon farm. Also, often real time knowledge of currents is required and this is not possible with current meters that log data that subsequently

needs to be downloaded and analysed. Knowledge of current patterns is augmented through the use of drogues that track currents in real time. Data from meteorological stations in conjunction with known tidal movements were also used as an aid in the interpretation of current meter and drogue results.

Drogue methodology

Drogues with Differential Global Positioning System (DGPS) technology were used to track water movements about the farm cages throughout the time of intensive seawater sampling sessions (second two snapshots only). The drogues consisted of a surface buoy containing a GPS and drifter sock in the water column. The depth of the middle of the drifter sock can be set at the depth of interest which, in this case, was 4 m (the depth at which seawater samples were to be taken). Each of the drogues communicated their position to a base station every 30 seconds. A laptop computer at the base station calculated their position to the nearest meter and recorded these values (accuracy as follows determined from bench tests: 57 % +/-1 m, 83 % +/- 2 m, 99 % +/-3 m, Cromey pers com). Two different strategies were used in drifter deployment: a group deployment for calculation of a dispersion coefficient, and a widely spaced deployment to determine current patterns about the cages.

Grouped arrangement for dispersion coefficient

A group of DGPS drifting buoys were released near the cages to assess dispersion potential of the site. This allowed tracking of water movements and calculation of dispersion coefficients for use in modelling and general site investigation. The dispersion coefficient is a factor taken into account by SEPA when assessing the loading levels of fish for a site. Buoys were released as close as possible to one another without affecting each other's movement. Their positions were recorded after release

and plotted. Their position relative to each other and their rate of movement is used to calculate a dispersion coefficient for the site (see Figure 2.5, Cromey pers com after Yanagi *et al.* (1982).

The dispersion coefficient relates to the change in area taken up by drifting buoys over time and is presented for two axes as 'k_x' and 'k_y'. The table below (Table 2.3) presents coefficients for a range of water bodies. Land-locked water bodies and those that depend on the wind for mixing generally have lower coefficients. High coefficients are commonly reflective of tidal systems.

Table 2.3 A summary of measurements of horizontal dispersion coefficients made by Cromey (SAMS) during DGPS drifting buoy surveys (BF = Beaufort Scale). All sites were tidal with the exception of the inland freshwater loch and eastern Mediterranean sites which were primarily wind–driven sites (Cromey, pers. com). k_x and k_y are length in two axes perpendicular to each other of area taken up the drifting buoys.

General site description	Run length (hrs)	k_x ($m^2 s^{-1}$)	k_y ($m^2 s^{-1}$)
Loch Sunart (BF 5)	2.7	0.68	0.01
Loch Diabaig	3.3	0.10	0.31
Loch Kishorn	4.2	0.28	0.11
Loch Craignish	2.9	0.03	0.09
Sea loch narrows above sill	1.6	0.02	0.69
Sound of Mull (strait)	1.7	0.25	0.19
Sound of Mull (inshore eddy)	2.1	0.79	0.32
Sound of Mull (main channel)	1.6	14.80	0.46
Inland fresh water loch[a] - calm (BF 2)	1.1	0.02	0.00
- windy (BF 4)	1.0	0.07	0.12
Eastern Mediterranean - Simi	2.5	0.00	0.03
Eastern Mediterranean - Chios	2.9	0.17	0.00
Eastern Mediterranean - Korinthiakos	1.4	0.42	0.15
SEPA management model value (theoretical)	NA	0.10	0.10

[a] Primarily wind driven or non-tidal

Widely spaced deployment for determination of current patterns

Up to 6 drogues were placed regularly around the cage system, at least 25-50 m from the cages to limit the chances of snagging on mooring chains and to limit the chance sof them stalling against the cages. Recording of position on a regular basis and later

plotting enabled direction and speed of currents with respect to the cage system to be determined.

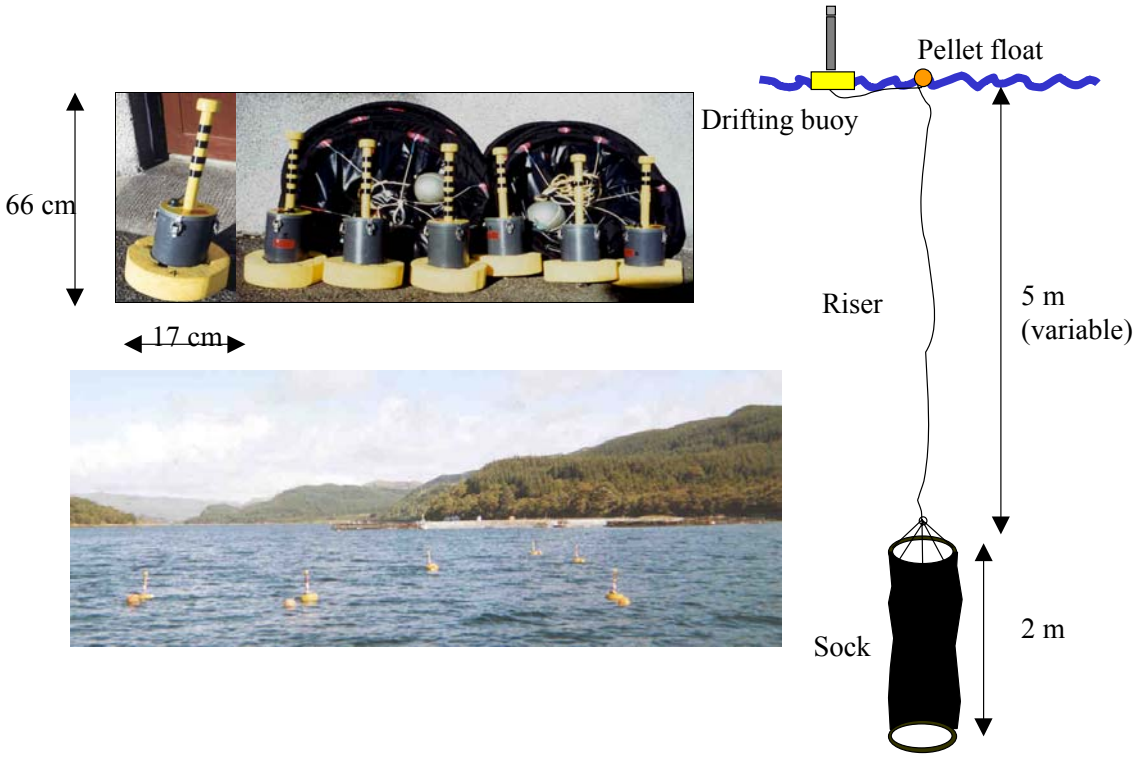

Figure 2.5 Diagram showing drogues consisting of DGPS drifters and sock and typical deployment near a fish farm (Acknowledgement: Chris Cromey).

Meteorological station

To enable an assessment of the influence of the wind on currents if required, a Davis Meteorological station was deployed on site. This machine records temperature, wind speed and direction and pressure. This was placed on the salmon cages with a wind vane erected on a pole so that the wind was measured above the level of the salmon cages and therefore not influenced by factors at the level of the cages.

Results

Intensive nutrient sampling sessions

The colour figures (Figures 2.6 – 2.8) following indicate the distribution of ammonium around the fish cages for the July 2003 and May 2004 intensive sampling occasions. They indicate elevated ammonium levels up to 3.5 μM, 50 m from the cage groups, with enhanced concentrations extending 200 – 300 m from the cages. Levels of phosphate, nitrate, nitrite and silicate were close to ambient. All three sessions indicate a pooling of ammonium on the inside of the cages towards the adjacent islands. At Laxford (Figures 2.6 & 2.7), both snapshots show directional biases in ammonium concentration, with a sharp decline in concentrations in the north east direction from the cages and enhanced concentrations extending in a southerly easterly and north westerly direction. At Calbha (Figure 2.8) there are reduced concentrations of ammonium in the north with enhanced concentrations in a southerly and easterly direction.

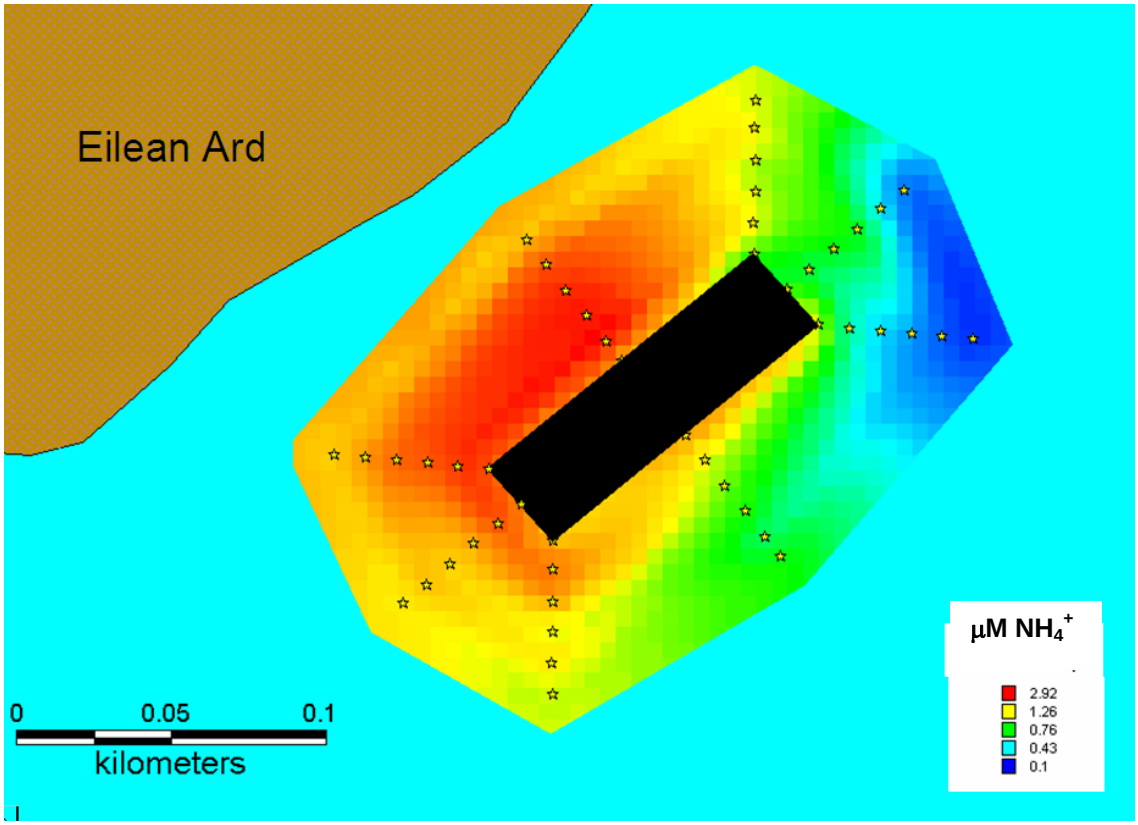

Figure 2.6 a) Nutrient maps generated from nutrient data collected in a grid pattern around the cages adjacent to Eilean Ard in Loch Laxford**,** determined 12.00 – 15:00 GMT, 3/7/2003. ☆ denotes sampling point. Depth: 20-30 m.

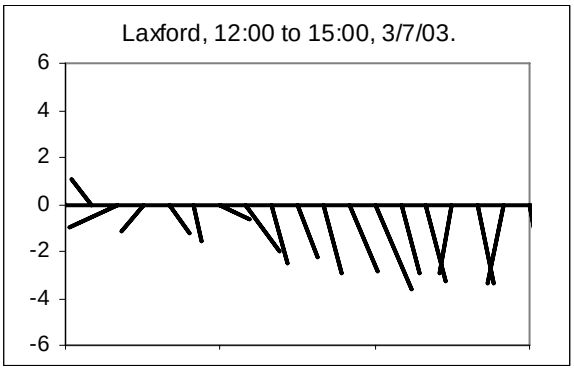

Figure 2.6 b) Current directions during 3/7/2003 'snapshot' at Loch Laxford. Readings were taken every ten minutes (horizontal axis), units are cm s^{-1}. Data are from a surface current meter approximately 100 m south east of the cages.

Tides on 3/7/2003:

Low Tide 10:30 GMT, 4.52 m
High Tide: 4:34 GMT, 1.34 m

Fish biomass at walkway: 287 tonnes.

Table 2.4 Fish feed supplied to F walkway salmon stock, Loch Laxford, prior to survey on 3/7/2003.

DATE	Kgs of feed
30/06/2003	3331
01/07/2003	1564
02/07/2003	2931
03/07/2003	2256

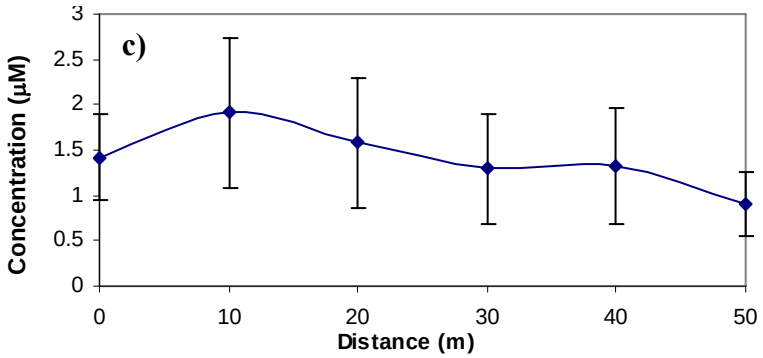

Figure 2.6 c) Mean ammonium concentrations for the eight transects about 'F' walkway**,** Loch Laxford, 3/7/2003 (vertical bars indicate 95% CI).

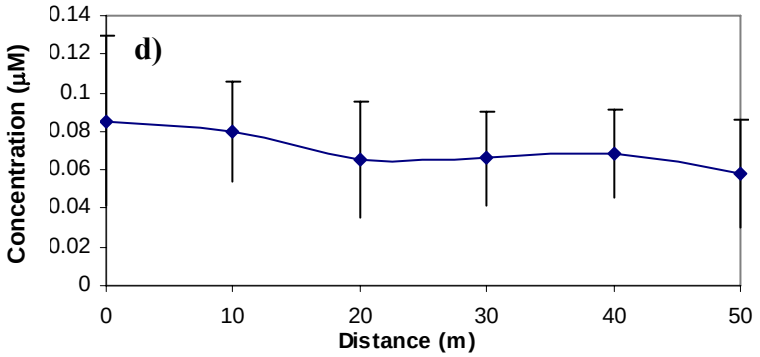

Figure 2.6 d) Mean nitrate concentrations for the eight transects about 'F' walkway**,** Loch Laxford, 3/7/2003 (vertical bars indicate 95% CI).

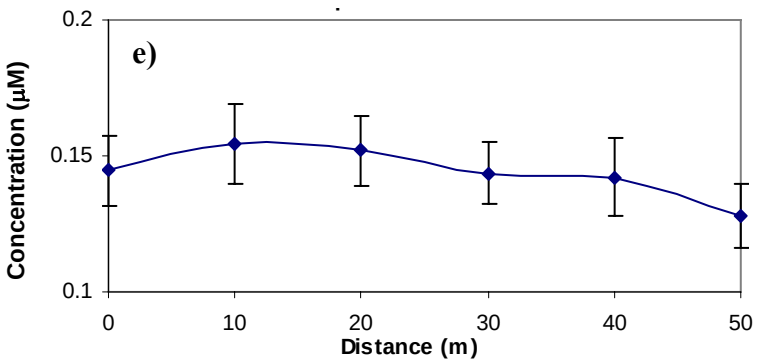

Figure 2.6 e) Mean phosphate concentrations for the eight transects about 'F' walkway**,** Loch Laxford, 3/7/2003 (vertical bars indicate 95% CI).

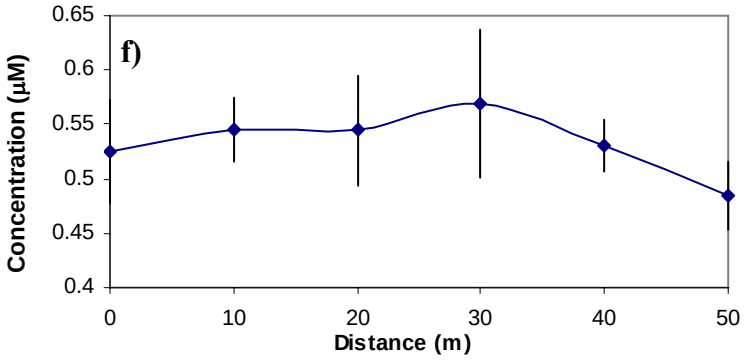

Figure 2.6 f) Mean silicate concentrations for the eight transects about 'F' walkway**,** Loch Laxford, 3/7/2003 (vertical bars indicate 95% CI).

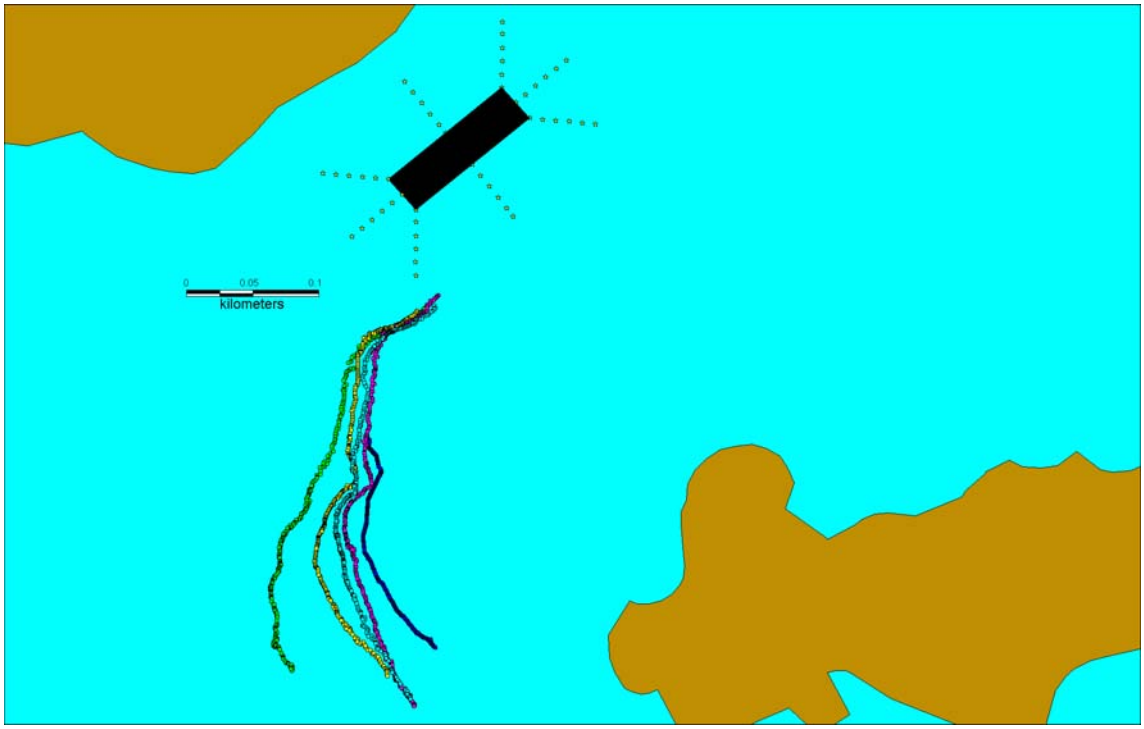

Figure 2.7 a) Map showing movement of drifters for calculating the dispersion coefficient F walkway, Loch Laxford (30/7/2003). Drifters were released 11.30 GMT and retrieved at 3.30 GMT.

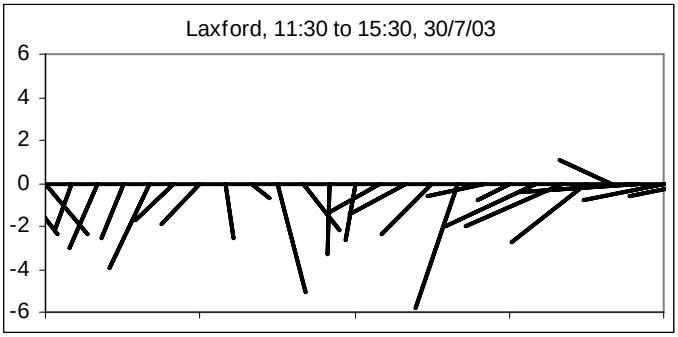

Figure 2.7 b) Current directions during dispersal event on 30/7/2003 at Loch Laxford, F walkway. Readings were taken every ten minutes (horizontal axis); units are cm s^{-1}. Data are from a surface current meter approximately 100 m south east of the cages.

Tides on 30/7/2003:

High Tide: 8:43 GMT, 4.78 m.
Low Tide: 14:53 GMT, 1.15 m.

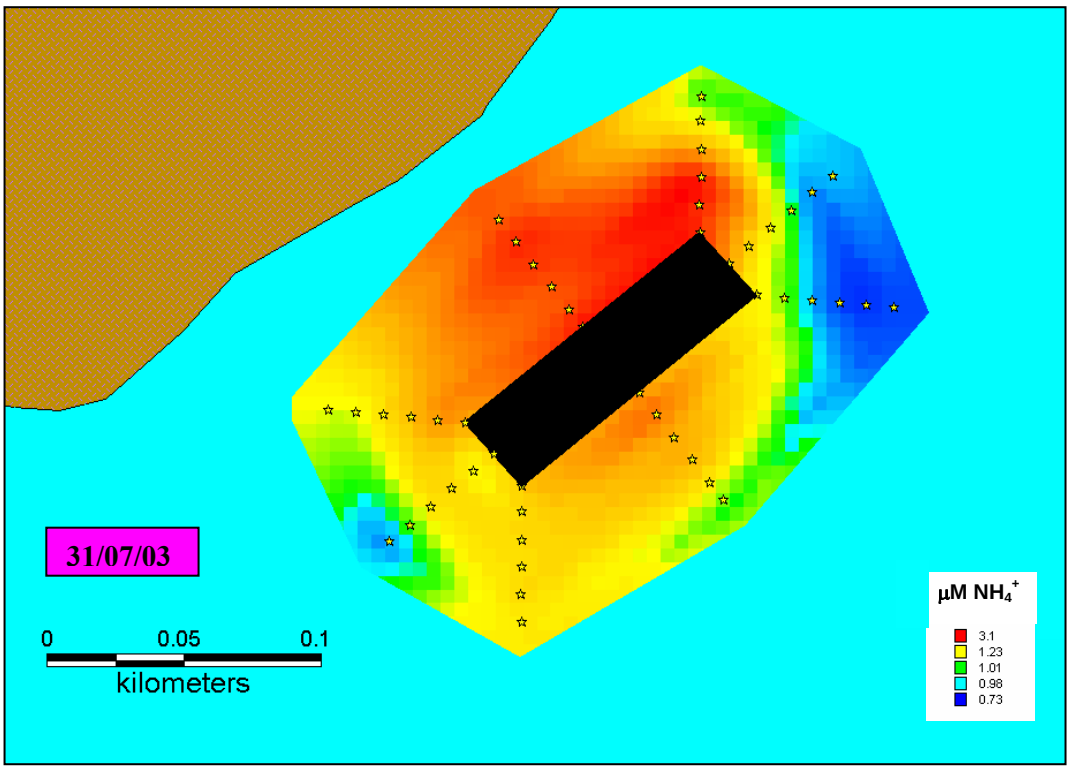

Figure 2.7 c) Ammonium distribution around cages adjacent to Eilean Ard in Loch Laxford determined 9.30– 13:30 GMT, 31/7/2003. '☆' denotes sampling points.

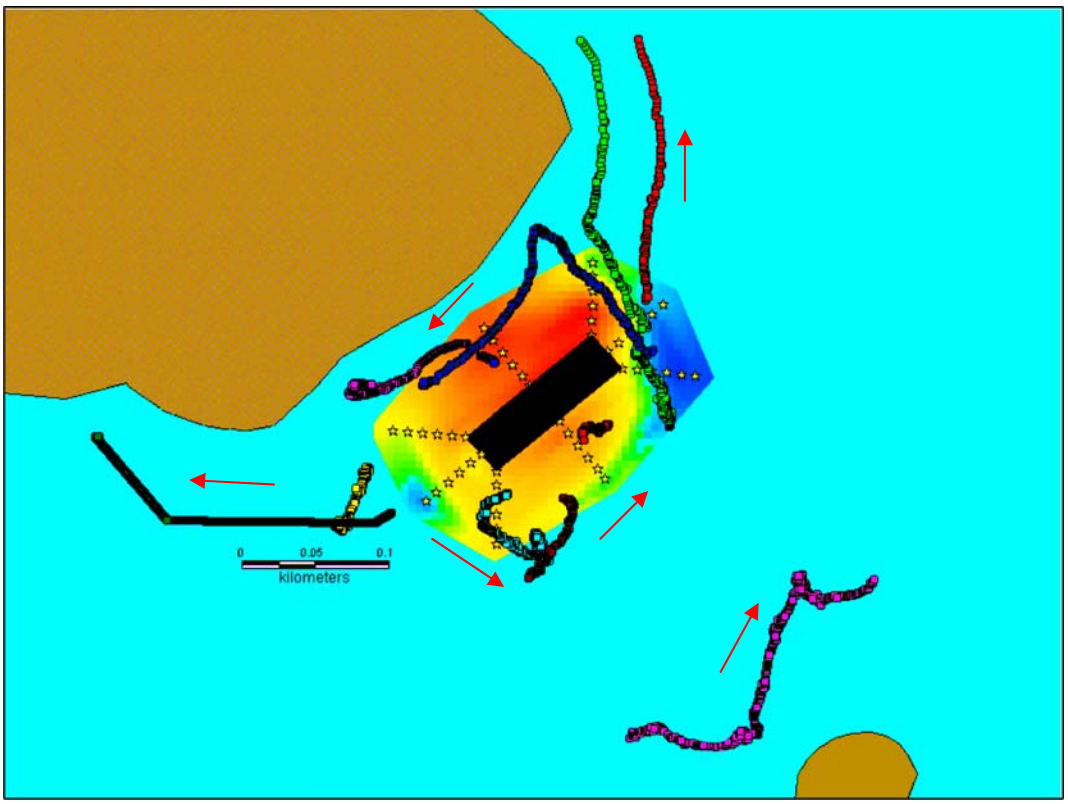

Figure 2.7 d) Nutrient map with drogue movements during sampling time**.** Drogues released within 25 to 50 m from F walkway, Loch Laxford, (31/7/2003).

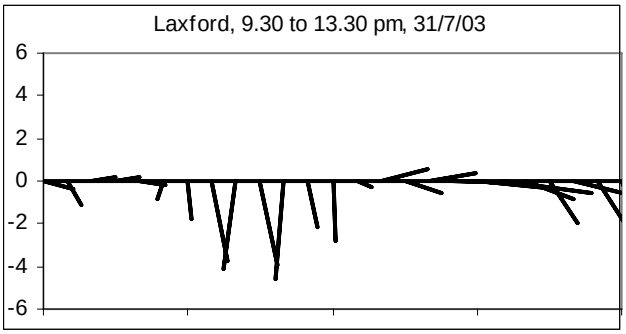

Figure 2.7 e) Current directions during dispersal event at Loch Laxford on 31/7/2003. Readings were taken every ten minutes (horizontal axis); units are cm s^{-1}. Data are from a surface current meter approximately 100 m south east of the cages.

Tides on 31/7/2003

High Tide: 9:30 GMT, 4.84 m.
Low Tide: 15:41 GMT, 1.05 m.

Fish biomass at walkway: 294 tonnes.

Feed to Laxford 'F' walkway prior to survey on 31/7/2003:

DATE	Kgs of feed
28/07/2003	1733
29/07/2003	2249
30/07/2003	1858
31/07/2003	1908

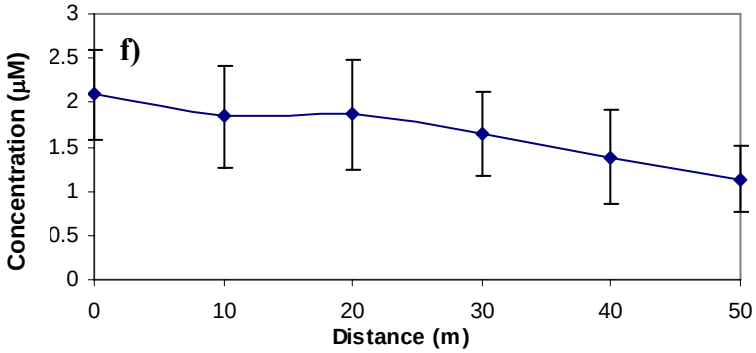

Figure 2.7 f) Mean ammonium concentrations for eight transects about 'F' walkway, 31/7/2003 (vertical bars indicate 95% CI).

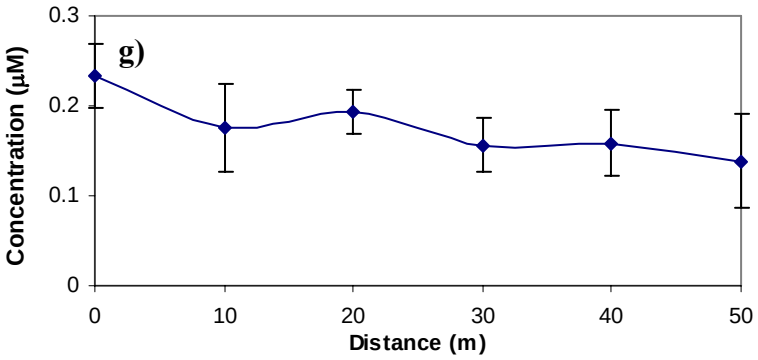

Figure 2.7 g) Mean nitrate concentrations for eight transects about 'F' walkway, 31/7/2003 (vertical bars indicate 95% CI).

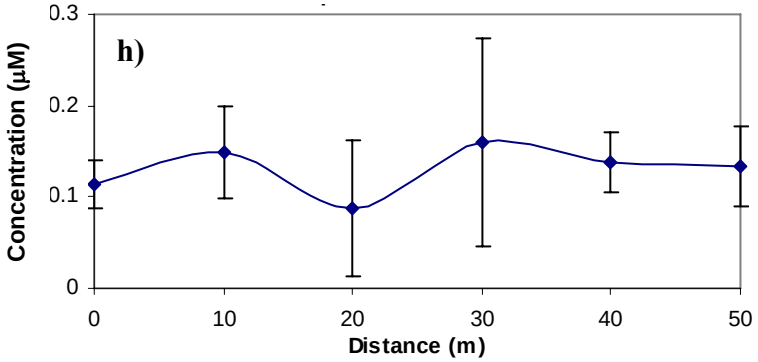

Figure 2.7 h) Mean phosphate concentrations for eight transects about 'F' walkway, 31/7/2003 (vertical bars indicate 95% CI).

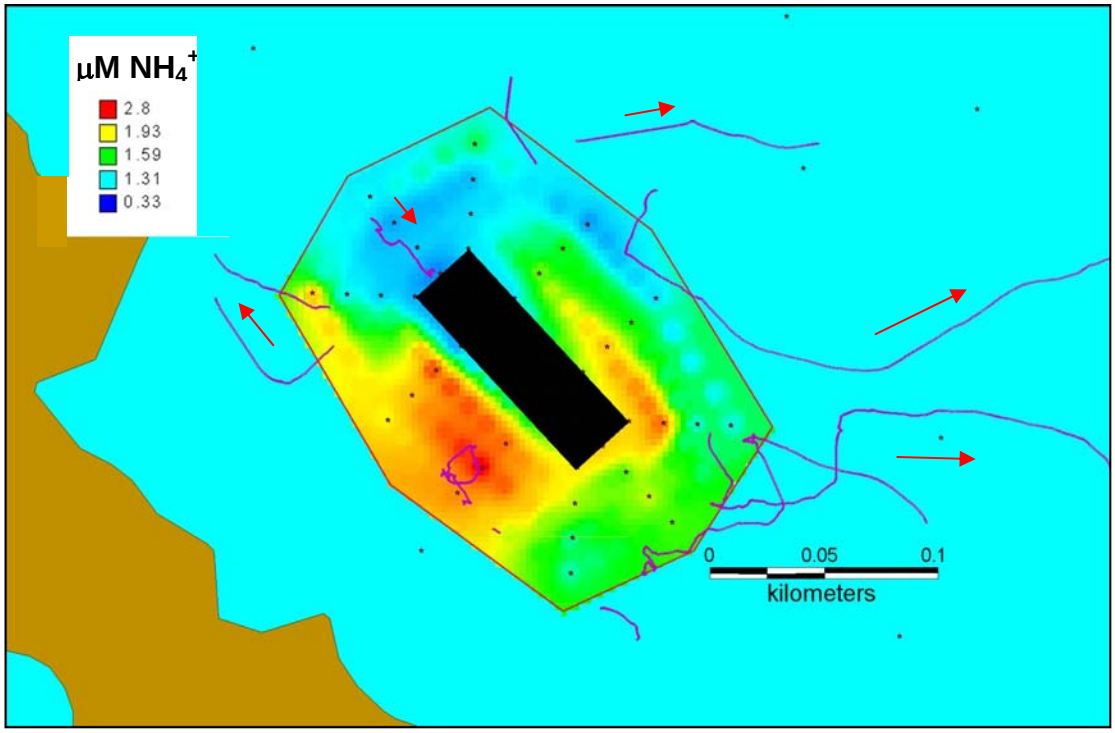

Figure 2.8 a) Ammonium concentrations to 45 m from the cages interpolated from samples collected adjacent to Calbha 'D' walkway, 9.00 to 14.30 GMT on 5/5/2004. '☆' denotes sampling point. Drogue movements are superimposed on the nutrient sampling results. Drogues were released 30-50 m from the cages. Arrows show their direction of movement. Ammonium concentrations **a)** to 45 m; **b)** greater than 45 m from the cages.

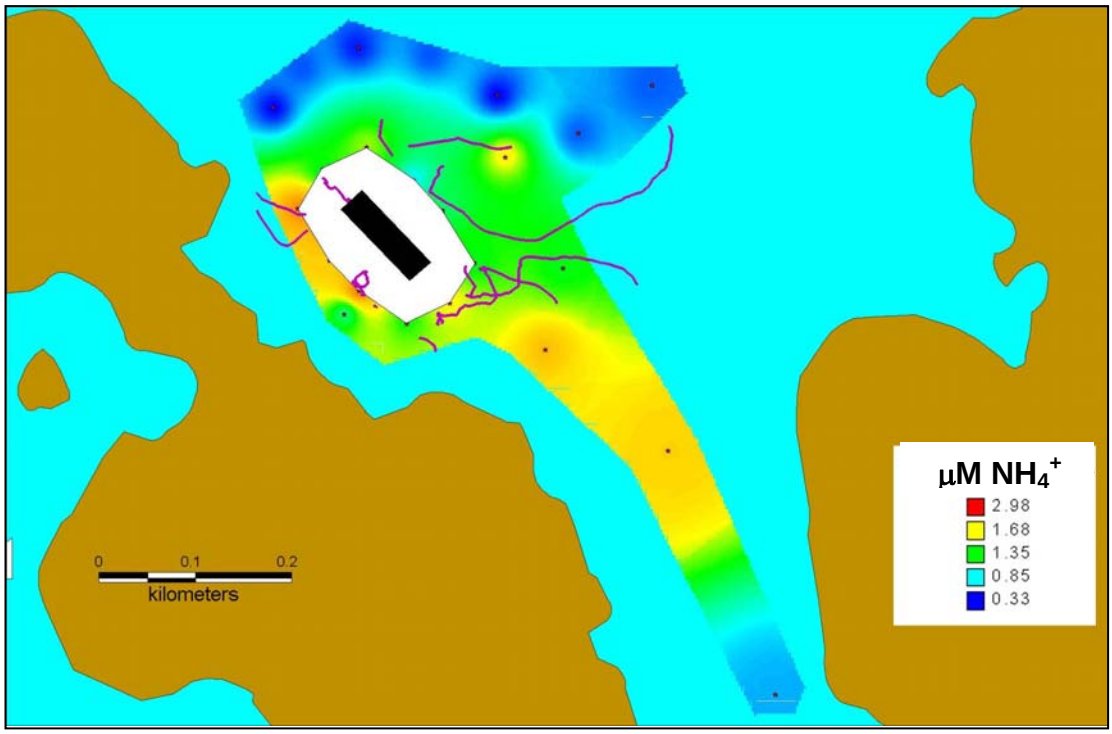

Figure 2.8 b) Ammonium concentrations greater than 45 m from the cages

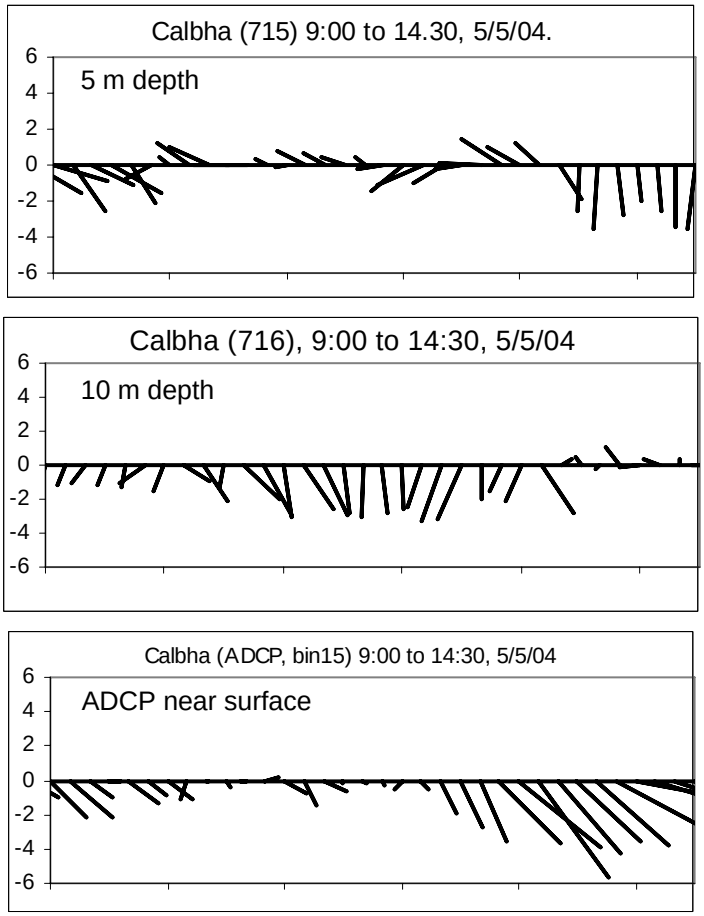

Figure 2.8 c) Currents measured during the nutrient sampling on 5/5/2006. Current directions at 5 m and 10 m depth as measured by current meters located 20 m from the south west corner of cages. The bottom diagram shows surface currents measured by an ADCP current meter approximately 70 m north of the northern end of the cages. Readings taken every ten minutes, units are cm sec^{-1}.

Tides on 5/5/2004

High Tide: 7:52 GMT 5.24 m
Low Tide: 14:27 GMT 0.37 m

Fish biomass at walkway: 280.5 tonnes

Feed to Calbha 'D' walkway prior to survey on 5/5/2004:

DATE	kgs of feed
02/05/2004	0
03/05/2004	0
04/05/2004	1651
05/05/2004	1592

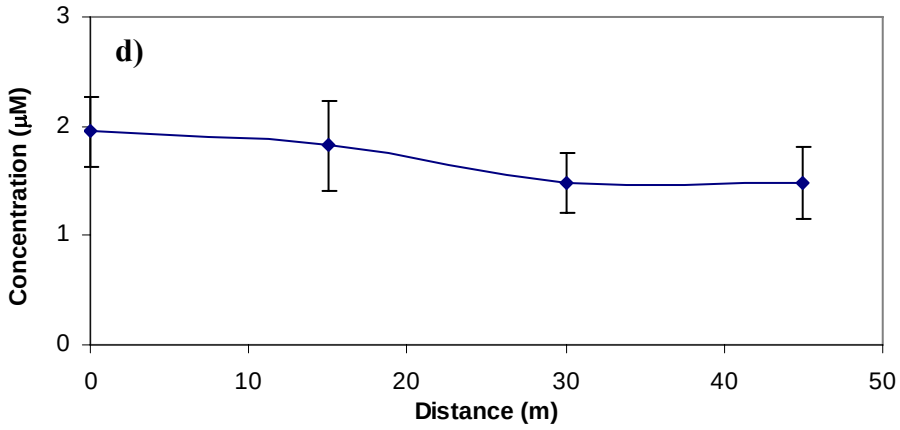

Figure 2.8 d) Mean ammonium concentrations for the eight transects about 'D' walkway, Calbha, 5/5/2005 (vertical bars indicate 95% CI).

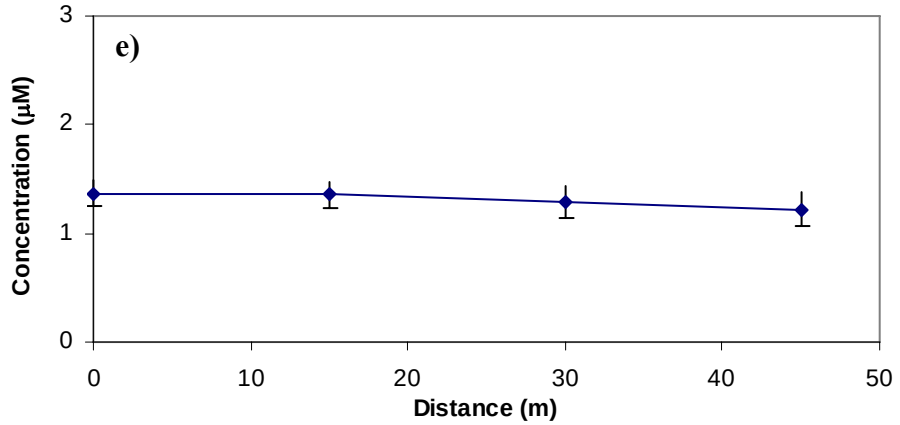

Figure 2.8 e) Mean nitrate concentrations for the eight transects about 'D' walkway, Calbha, 5/5/2005 (vertical bars indicate 95% CI).

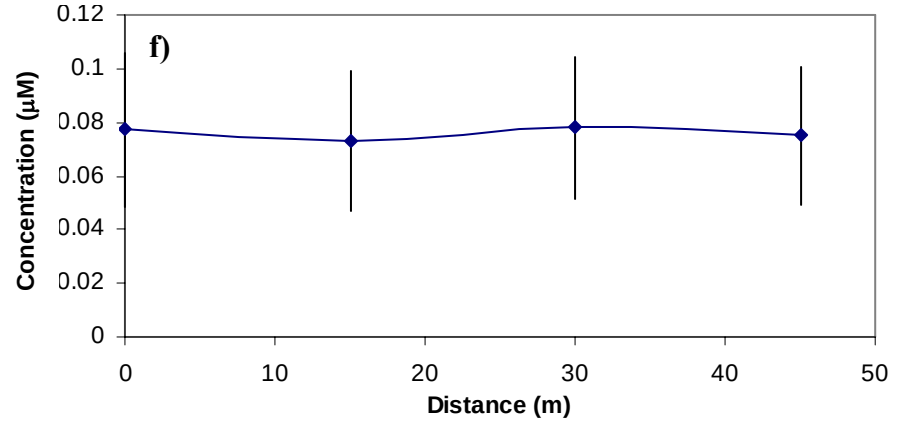

Figure 2.8 f) Mean nitrite concentrations for the eight transects about 'D' walkway, Calbha, 5/5/2005 (vertical bars indicate 95% CI).

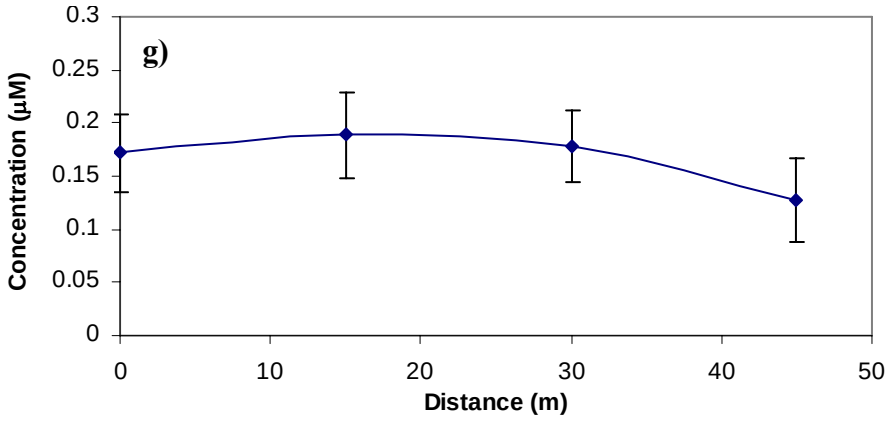

Figure 2.8 g) Mean phosphate concentrations for the eight transects about 'D' walkway, Calbha, 5/5/2005 (vertical bars indicate 95% CI).

Drogues

Drogues were released in two different patterns with differing aims:

1. to determine the dispersion coefficient and

2. to monitor the fate of currents in the vicinity of the cages while intensive nutrient sampling was being conducted.

Grouped arrangement for dispersion coefficient

At Laxford, cage group 'F', on 30/7/2003, for the period from 12.00 to 15.20 GMT, the drogues dispersed away from the salmon farm to a distance of 300 m (Figure 2.7a, b). The maximum recorded speed of any one drifter over this period was 5.5 cm sec^{-1} with a mean speed of 2.7 cm sec^{-1}. Wind was from a northerly direction with a mean speed of 1.7 m sec^{-1}. This trial was during an outgoing tide and gave calculated dispersion coefficients of 0.073 $m^2 s^{-1}$ (k_x) and 0.006 $m^2 s^{-1}$ (k_y).

At Calbha, cage group 'D', on 6/5/2004, for the period from 9.00 to 11.07 GMT, the drogues dispersed away from the salmon farm in a north westerly direction to a distance of 280 m with a mean speed of 4.2 cm sec^{-1}. Wind was from an easterly direction with

a mean speed of 5.5 m sec^{-1}. This trial was performed during an outgoing tide and gave calculated dispersion coefficients of 0.070 m2 s^{-1} (k_x) and 0.026 m2 s^{-1} (k_y).

Widely spaced deployment

At Loch Laxford on 31/7/2003, drogues were deployed about the farm on an outgoing tide. Mean speed of the drogues was 3.1 cm s^{-1} and the maximum for any given time was 14.4 cm s^{-1}. Their dispersal pattern indicated that there were eddying currents about the farm in a counter clockwise direction (Figure 2.7d). They also indicated a strong ebbing current (speeds up to 14.4 cm s^{-1}) that flowed out the main channel of Loch Laxford at the northern end of the cage system. This current partly impinged on the northern end of the cages resulting in a lowering of ammonium levels in this area. One drogue went out with the outgoing tide and stopped in behind Eilean Ard suggesting that there may be some pooling of effluent products in this area which has implications for the later nitrogen isotope analysis (see chapter 7).

Calbha

On 5/5/2004, drogues were deployed at the time of an outgoing tide (Figs 2.9 a, b). Mean speed of the drogues was 1.5 cm s^{-1} and the maximum for any given drogue was 3.9 cm s^{-1}. Their dispersal pattern indicated currents coming into this part of the bay from the north impinging on the northern end of the cages. The current continues parallel to the coast, round and then into the main channel in the centre of the bay where it is likely to flow out of the bay with the outgoing tide. There was some eddying of currents on the western and south western sides of the cages.

All three snapshots indicate ammonium distributions consistent with determined current patterns. Where the currents impact on the cages, the ammonium plume extends for

little to no distance in the direction from which current comes. On the side extending in the direction of the current, ammonium concentrations are higher and appear to continue for distances of up to at least 2-300 m from the cages. All snapshots also showed elevated and highest concentrations on the side of the cages towards adjacent islands, on the downstream side of the cages with respect to currents. Water depths become shallower in these regions, further concentrating available ammonium.

2.4 Diel ammonium distributions about the salmon cages.

The aim of this section was to establish the daily duration of ammonium enhancement in the vicinity of the cages.

Methods

Sampling was conducted at regular intervals and sites throughout the day at 'F' walkway at Calbha on 23 August 2004 (session 1) and 1 December 2004 (session 2).

***Session 1**: 23 August 2004*

Seven sampling stations were established (Figure 2.9). A drogue with the sock set at 4 m depth was used to determine current directions at this depth during sampling. The drogue was returned repeatedly to the north east corner of the cages and currents were determined to be moving in a northerly direction. Four of the sampling stations were placed at regular distances from the northern end of the fish cages as the movement of the tide was determined flow in a northerly direction. Water sampling was conducted at 4 m depth as detailed previously (Section 1). One water sample was taken from each station per sampling round. Seven rounds were made between 10.00 and 16.00 GMT. The term 'round' refers to sampling all seven stations once.

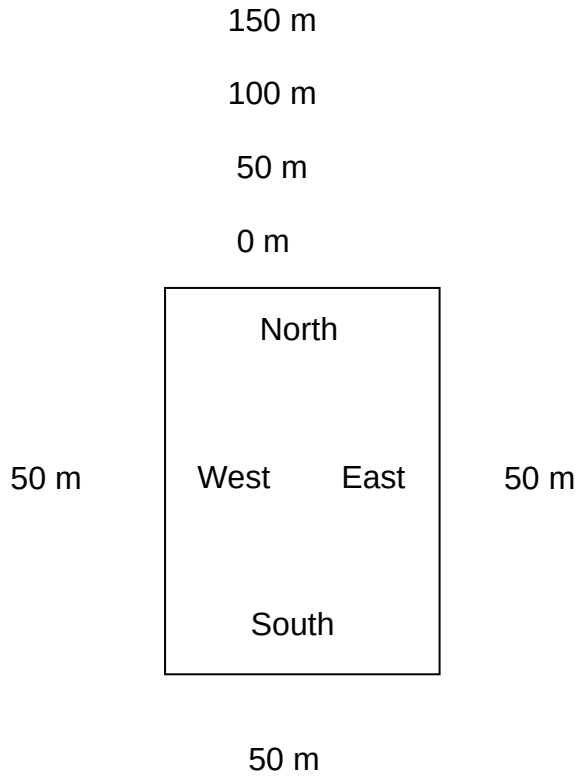

Figure 2.9 Positions of seawater sampling stations with respect to cages at Calbha 23/8/2005.

Table 2.5 Start and finish times (GMT) of each sampling round at Calbha 23/8/2004.

Round	Start	Finish
1	09:30	10:38
2	10:43	11:01
3	11:39	12:07
4	12:41	13:04
5	13:59	14:29
6	14:58	15:22
7	15:05	16:12

Statistical analysis

A two way ANOVA was conducted on the data to test for differences with factors of time (round) and station. As there was only one replicate of each sample, Friedmans non-parametric test (Dytham 2003) was selected as the appropriate test for differences between sites and times.

***Session 2**: 1 December 2004*

Five sampling stations were established (see Figure 2.10). Movement of the tide was northwards from the north from the cages at the time of sampling as indicated using a drogue with the sock/vane set. Water sampling was conducted at 4 m depth as detailed previously (section 1). One sampling station was at the walkway edge at the northern end of the walkway (N0) and four of the sampling stations were 50 m from the mid points of each of the sides and ends of the fish farm cages. On this occasion, in order to take into account within site variation, three samples were taken at each sampling station per round. Four rounds were made between 10 and 16.00 GMT. For this session, 'round' refers to sampling each of the five stations (three samples taken at each).

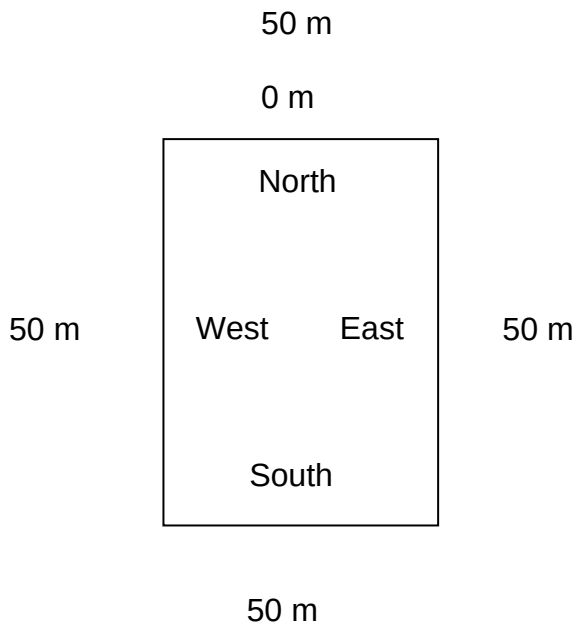

Figure 2.10 Positions of seawater sampling stations with respect to cages at Calbha 1/12/2004.

Statistical Analysis

Stations and rounds were compared using a two way analysis of variance. Data were checked for normality (Anderson-Darling) and homogeneity of variance (Bartletts and Levenes tests). Post hoc tests were conducted using Tukey tests.

Table 2.6 Start and finish times (GMT) of each sampling round at Calbha 1/12/2004.

Round	Start	Finish
1	09:03	10:55
2	11:27	12:26
3	13:18	14:05
4	15:03	15:46

Results

***Session 1**: 23 August 2004*

ANOVA using Friedman's non parametric test indicated that the ammonium concentrations at the 50 m south station (S50) were significantly lower than the 0 m north (N0) station (Figure 2.11 a & b). This is consistent with the direction of currents determined at the time. There were elevated levels of 1.5-2.0 µM of ammonium throughout the sampling period for the stations at 50 m east, west and north of the cage group.

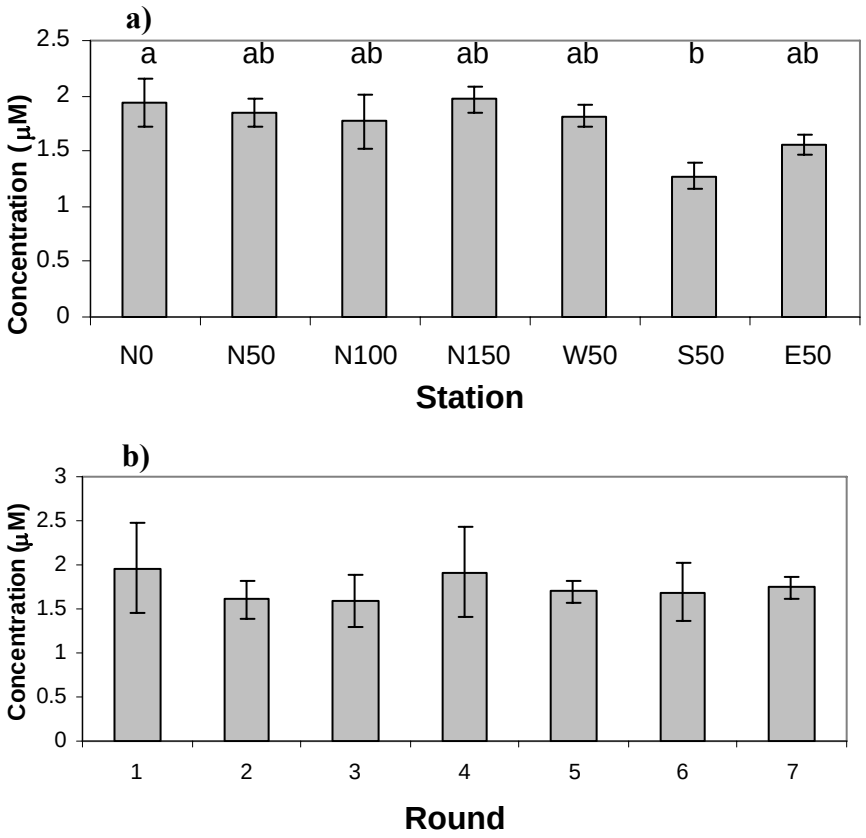

Figure 2.11 Overall ammonium concentrations for (a) station and (b) round, Calbha 23/8/2004 (vertical bars indicate 95% CI). Values which share at least one letter are not

significantly different at $p = 0.05$.

***Session 2**: 1 December 2004*

Ammonium concentrations in sampling round 1 were significantly lower than those in later rounds and N0 was lower than S50 (Figure 2.12 a, b). In a 2-way analysis of variance, there was a significant interaction between time and distance. For round 1, N0 was higher than the four other sampling stations; in round 2, N0 was significantly higher than N50 only and in round 3 N0 was significantly higher than E50, S50 and W50. In round 4 there was no significant difference between any sites (2 WAY ANOVA session: $F(3,35) = 8.4$, Site: $F(4,35) = 12.1$, Session X site: $F(12,35) = 2.3$).

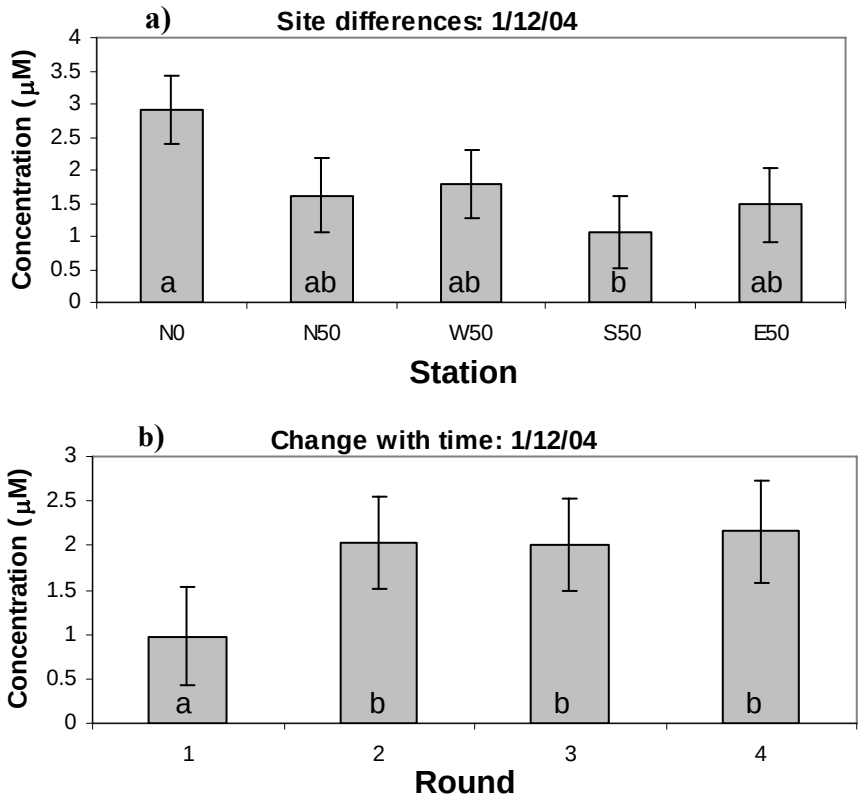

Figure 2.12 ammonium concentrations for (a) station and (b) round, Calbha 1/12/2004 (vertical bars indicate 95% CI). Values which share at least one letter are not significantly different at $p = 0.05$.

Discussion

Ambient nutrient levels at the Loch Duart Ltd. sites fall within the range recorded for Scottish west coast waters (Slesser and Turrell 2005). Ammonium concentrations

measured close to the cages (<50 m) were enhanced when compared to sites away from the cages with a high of 8 μM but most ranged from 2 to 4 μM. This compares with measurements of 3-4 μM found by Pitta *et al.* (1998), 3-8 μM by Karakassis *et al.* (2001), 2-10 μM by Merceron *et al.* (2002) and 2-7 μM by Petrell and Allie (Petrell and Alie 1996). Enhancements of 1 μM ammonium were detected at distances of 200 m believed to be derived from the salmon farm.

In winter when ambient plant-available nitrogen levels in the form of nitrate are high (7 μM), the ammonium enhancements originating from the farm are relatively low. However, in summer, when ambient nitrate and ammonium levels are low, farm-derived nitrogen is likely to be the principal source for plant growth. Exposure to farm-derived ammonium is dependent on the distance from the cages. The larger the distance from the cages, the lower the ammonium concentrations and the shorter and more variable the exposure time.

Seawater phosphate and nitrate concentrations close to the farm cages (<30 m) were consistently elevated suggesting a small degree of enhancement of these nutrients arising from the cages. Enhanced nitrate concentrations are likely to be a product of microbial action on ammonium. High DIN:P values near to farm cages are also reflective of the high nitrogen input.

Currents in the vicinity of the cages have a significant effect on the distribution of ammonium. Currents disperse the ammonium in the direction of flow, although this study has shown that predictions of nutrient regimes in the vicinity of the farm, that are often based on current measurements at one point, can be very misleading. For example, the intensive sampling sessions or ‘snapshots’ revealed significant pooling of

ammonium on the western sides of the Loch Laxford and Calbha cages between the cages and adjacent land masses. This is brought about by protection provided by the cages from currents and the consequent eddying on the lee side. The water is also shallower on this side and may be concentrating the ammonium.

At Laxford, it is likely that ammonium levels are close to ambient most of the time at the north eastern end of the cage group, due to the currents impacting there as a result of the ebb and flow of the tide in the main channel of Loch Laxford. If cultured algae were sited off the northern end of the cage group, it is possible they may not be exposed to significant concentrations of farm-derived nutrients. This factor is thus an important consideration when selecting a site to maximise interception of nutrients emanating from the cages.

The ammonium concentration values for the Badcall Bay sites were high when compared to the Calbha sites and the seasonal ambient samples. The measurements were also higher than those recorded for the northern Minch (Gillibrand *et al.* 2003; Slesser and Turrell 2003) including similar loch areas on the Scottish coast (Gubbins *et al.* 2003). Badcall Bay is a relatively enclosed Bay and water movement in and out is likely to be limited and leading to enhanced values of ammonium.

The measurements for Badcall Bay in June 2005 were particularly high. This sampling session was conducted in mid-summer when spring bloom organisms were senescing and generating ammonium (Dugdale and Goering 1967; Glibert *et al.* 1988). There was also a moderate to strong south westerly breeze noted at the time of sampling which may have mixed the benthic-generated ammonium through the water column within the bay.

Significant elevation of ammonium concentrations in the vicinity of the fish farms were detected within three hours after the initiation of daily feeding. This corresponds with predicted post prandial peaks in the output of wastes (mainly ammonium) from the fish after feeding. Elevated levels continued for at least 4 hours indicating availability for extended periods of time during the day for plant uptake.

The results presented in this chapter demonstrate that there are elevated levels of ammonium at the appropriate depths for potential utilization by marine macroalgae (i.e. the photic zone), for an extended period of time at distances from the fish farm cages that may exceed 200 m. Careful assessment of current patterns around farm cages should be made if the objective is to maximise exposure of cultured algae to farm-derived nutrients.

CHAPTER 3

GROWTH OF *PALMARIA PALMATA* IN THE VICINITY OF FISH FARM CAGES.

Introduction

The main aim of this chapter was to determine if seaweeds will grow in the vicinity of salmon cages at Loch Duart Ltd sites in north western Scotland and how this might affect their growth. *Palmaria palmata* was trialled as there is a market for this alga in Northern Ireland and demand cannot currently be met by supply (Browne 2001, Dolphin Sea Vegatables 2005). The possibility of culturing *P. palmata* and the potential for its use as a 'nutrient sponge' adjacent to salmon farms assumes that *P. palmata* will grow in this environment and that the nutrients will have a positive effect on the growth of the alga. The aims of this part of the thesis were to use tethered *P. palmata* to determine:

- if position relative to the salmon cages had an effect on growth of the alga
- the effect of depth on the growth of *P. palmata* adjacent to the farm cages.

Cultivation of seaweeds from fragments is a method used commercially for *Eucheuma* spp. In south east Asia (Ask and Azanza 2002) and for *Gracilaria* spp. in Chile (Buschmann *et al.* 2001a). Large plants are divided into small pieces, which are then tied individually onto horizontal nets or lines in the lower intertidal zone. After cultivation, the small plants are sold and the largest plants are used to provide fragments for the next crop. Browne (2001) tested the potential for cultivating *P. palmata* using

tethered plants in Strangford Lough in Northern Ireland. Findings from Browne's study included:

- Growth of *P. palmata* on site-specific culture rigs over a 4 week cultivation period was greater at 1-3 m depth than at 0-1m depth at two sites in Strangford Lough and was thought to be related to higher light intensities or lower nutrient levels.
- When *P. palmata* was cultured on a longline in Strangford Lough for periods of 1, 2, 3 and 4 weeks, relative growth rate (RGR) was approximately 0.1 g g^{-1} d^{-1} for all culture periods over 6 months of the year, from April to September/October. This equates to nearly a doubling in wet weight in one week.
- *Palmaria palmata* plants from a longline and a site-specific culture rig showed no significant difference in RGR from the surface to 7 m depth over a two week period.
- Relative growth rate (RGR) of *P. palmata* was similar at a range of sites from a high energy site with high current speeds and wave action, to a low energy site with almost no current or wave action in Strangford Lough.

In 2003 at Loch Laxford, Loch Duart Ltd. had fish of near harvestable size, thus the largest biomass and maximal nutrient input, so growth trials were carried out at that site. To improve the chances of determining an effect by maximising interception of nutrients, current meter data were consulted for location of cultured plants with respect to fish farm cages. As part of their environmental monitoring obligations to the Scottish Environmental Protection Agency (SEPA), Loch Duart Ltd. had collected two weeks of current meter data for the Loch Laxford site and this was used to determine the location for the tethered plants most likely to maximise their interception of nutrients from the salmon cages. The data indicated changes in current directions from minutes to weeks

(Figure 3.1). The tidal influence in the data appeared to be fairly strong and the overall current direction was south south east. The longlines with tethered algae were thus located on the south eastern corner of the cages to maximise the chances of nutrient interception.

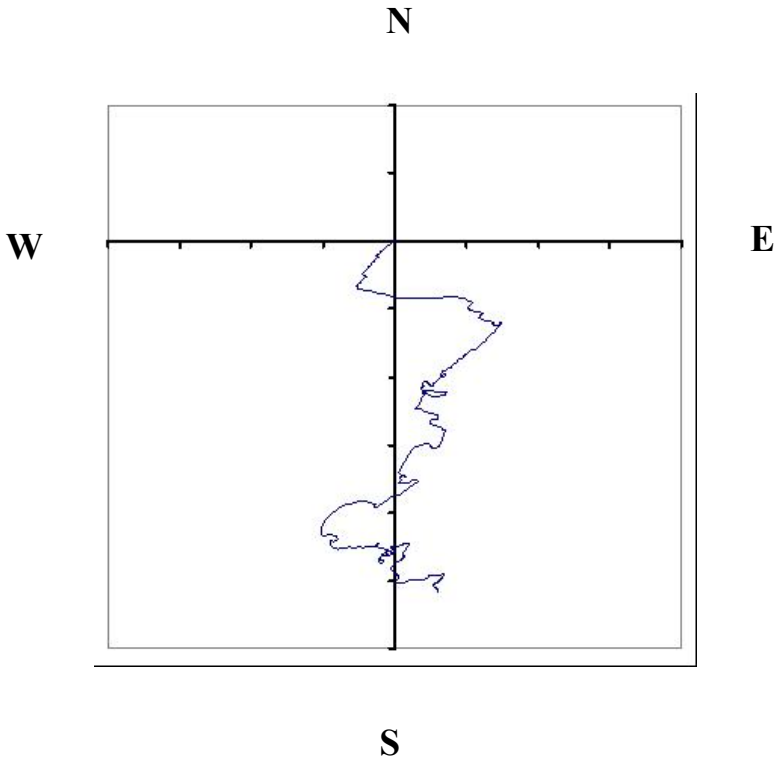

Figure 3.1 Cumulative vector plot for surface current data, Loch Laxford Eilean Ard 'F' cage group, 23 February to 10 March 1999, indicating overall southerly current movement. Axis units are 2 km: taken from report to SEPA for Loch Laxford (exact location not specified), by Stirling University.

Methods

In May 2003, a trial 50-m longline (3-cm diameter polypropylene rope) was attached to the south east corner of salmon cages at Loch Laxford ('F' cage group) in alignment with residual current flow in order to test the methodology and establish durability of the design (see Figure 3.2). In June, another two longlines and a reference station were established from the same corner. Weighted lines or droppers (10-12 mm polypropylene rope) with attached plants were hung from the longlines at distances of 0, 5, 10, 25 and 50 m from the salmon cages (Figure 3.3).

Two plants were attached to each dropper at 1 m intervals from 1-7 m depth. The plants were attached by inserting the stipe of the plant in the lay of the rope. Only a minimal amount of the stipe was inserted to avoid damaging the fronds. In total there were six plants for each depth x distance combination.

The reference station was set up approximately 500 m in a southerly direction from the salmon cages. This consisted of three anchored buoyed lines to which algae could be attached in the same manner as those on the droppers adjacent to the salmon cages. Each line had a mooring weight on one end and a buoy at the other. A 7-m section of line could be interchanged on these buoyed lines at each sampling session. A smaller weight (1-2 kg) was attached to the line mid-water to ensure that the line hung vertically.

After the initial trial in May that established tethered plants would survive, 180 plants were attached to all the lines (i.e. 3 lines x 6 distances x 5 depths x 2 replicates) and were replaced monthly thereafter.

***Palmaria palmata* transplants**

Plants used for transplanting were harvested at sites where *P. palmata* was abundant. Medium sized (8-12 cm in length) clean plants of simple morphology with a minimum of epiphytes were used. Plants of simple morphology had broader blades, up to 3 cm in breadth, with few branches or marginal proliferations (see www.algaebase.org for *Palmaria palmata* description).

In May and June 2003, plants to be outplanted at Loch Laxford were harvested at Isle of Seil, 20 km south of Oban, and also from a site adjacent to SAMS Marine Laboratories

at Dunstaffnage. These plants were held in flow-through tanks at SAMS until taken to Loch Laxford for deployment. The holding time of the plants was kept to a minimum and was never more than 6 days. Due to the possibility that plants deteriorate whilst being held, plants were later sourced locally to Loch Duart Ltd. at Oldshoremore (30 km north of the Loch Duart Ltd. Badcall base). Plants sourced local to the salmon farm could be put out on site either the same day or the day after. When plants were held overnight they were maintained in aerated cool seawater (5-15^{o}C).

At each of the source sites, *P. palmata* plants were found either on the stipes of *Laminaria hyperborea* in the upper subtidal or from lower intertidal rocky reef areas. Plants from the intertidal reef areas appeared to differ in their life history and appearance from stipe-attached plants. The bulk of the intertidal rocky reef plants were annual, appearing in spring, growing until mid-summer and then dying back in late summer. In mid-late summer, they had a very 'battered' appearance, being more subject to turbulence in the surf zone and having been exposed to longer and more frequent alternating periods of desiccation and submersion. Subtidal plants from kelp stipes appeared to flourish in the spring and to grow through the summer with many surviving to late summer and growth being reinitiated from basal fronds in the following spring. There were also differences in the morphology of the plants with those collected from kelp having a longer stipe perhaps, making them more suitable for longline deployment.

There was some concern that plants sourced from differing geographic and/or intertidal versus subtidal locations may give rise to unexplained variation when comparing between months. To address this, growth rate comparisons were made on three occasions, however there was no discernable difference in growth response between

plants regardless of their source. After July, all plants were sourced from intertidal sites at Oldshoremore.

Plant measurement

Plants were photographed before and after their time in the water on the longlines. In order to minimise any harmful affects on the plants through being handled and being out of the water, plants were photographed on site at the salmon cages. Plants could be taken from holding buckets, attached to lines, photographed and deployed into the water adjacent to the salmon cages within minutes. The camera was mounted on a PVC frame and plants were placed on a white specimen tray adjacent to a ruler for size reference. Initially (May-June) a Sony Hi8 video camera was used for image capture. A frame grabber was used to obtain images of individual seaweeds on return to the laboratory. After the first two sampling sessions, an Olympus digital camera was used (Model No. C-5050, 5 megapixel) allowing greater resolution. This camera was kept in an underwater housing (Olympus) allowing increased protection for the camera from the weather and sea.

In the laboratory, photographs were downloaded on to computer and area and length of the seaweeds was calculated from photographs taken before and after deployment using ImageJ software (public domain Java image processing program: http://rsb.info.nih.gov/ij/). The length of each plant was measured, as it has been shown that length is strongly correlated with biomass (Martinez and Rico 2002).

A synopsis of the sampling regime is contained in Table 3.1. This shows a mean sampling time interval of 30 days and that the monitoring period covered the time of year when ambient nutrient levels, particularly nitrogen and phosphate may be low

(June to September). When ambient levels are low, any enhancement as a result of being in proximity to the fish cages can be expected to be at its greatest.

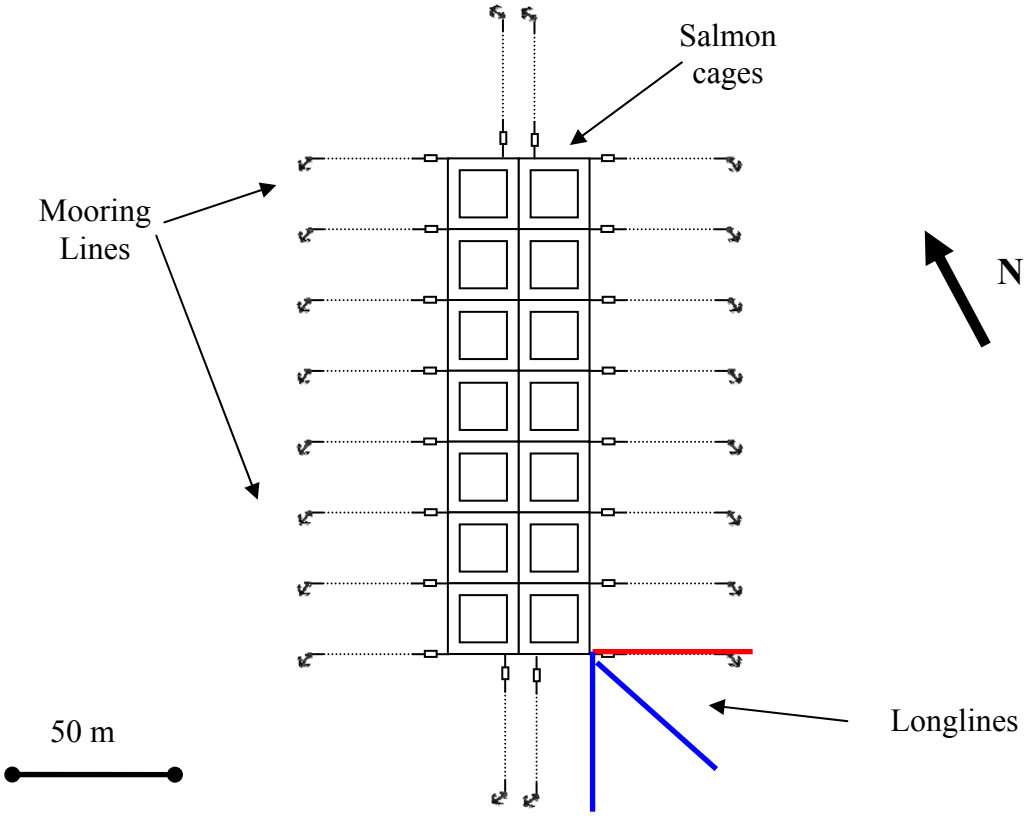

Figure 3.2 Position of the longlines relative to the fish farm cages at Loch Laxford. The red longline is the original trial longline put in place in May 2003.

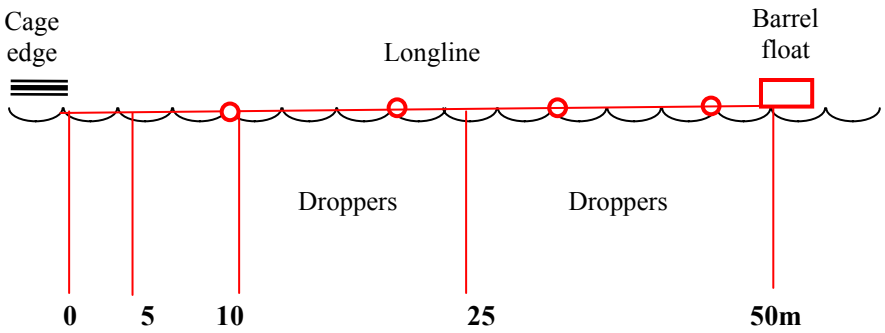

Figure 3.3 Arrangement of droppers and longlines relative to the salmon cages.

Epiphyte and colour measurement

Epiphyte cover and colour of the *P. palmata* plants were also noted and changes determined from the photographs. Epiphyte cover of the plants was recorded on a scale of 1-5 where 1 was equivalent to 0-10% cover, 2; 21-40%, 3; 41-60%, 4; 61-80% and 5; 81-100%.

The colour of the plants from the photographs was noted as being black, brown, green, green-brown, pink or red / purple. For colour analysis, plants were judged as being either acceptable (pink or red / purple) or not (green or black/brown).

Analyses

Growth was calculated as area-based relative growth rate (RGR) using the formula:

$$RGR = \ln (Area_{t1} / Area_{t0}) / \Delta t$$

Where '$Area_{t0}$' is the area of the plant at deployment time '0', '$Area_{t1}$' is the area of the plant on recovery and Δt is the period of time (in days) that the plant was tethered in the sea in days (Deboer *et al.* 1978; Lobban and Harrison 1996).

Differences in growth rate and epiphyte cover were assessed using analysis of variance, and Tukey pairwise comparisons of means. Two-way ANOVA were conducted across all the data and separately for the months of May, June and July using distance and depth as the variables. There were insufficient data for two-way analyses in August, September and November, and variation with depth and distance was analysed separately using one-way ANOVA. Data were examined for major deviations from

normality and homogeneity of variance, and transformed if required. Statistical packages used included Minitab, JMP IN (SAS Inst.) and Excel (Microsoft).

Table 3.1 Details of *Palmaria palmata* transplants at Loch Laxford 2003. 'Kelp' (K) refers to kelp stipe sourced plants and 'Intertidal' (I) to plants from rocky reefs.

Sample period	Plants obtained from	Kelp/ Inter tidal	Date in	Date out	Days
May	Isle of Seil	K	07-May-03	03-Jun-03	27
June	Isle of Seil	K	03-Jun-03	02-Jul-03	29
Comparison:	Oldshoremore	K	03-Jun-03	02-Jul-03	29
July	Oldshoremore	I	02-Jul-03	31-July-03	29
Comparison:	Dunstaffnage	I	02-Jul-03	31-July-03	29
August	Oldshoremore	I	31 July-03	27-Aug-03	27
September	Oldshoremore	I	27-Aug-03	01-Oct-03	35
November	Oldshoremore	I	06-Nov-03	11-Dec-03	35

Results

Tethered plants of *Palmaria palmata*

Plant loss

Plant loss was expressed as a percentage of the 180 plants deployed each month. The greatest recovery of plants (96%) was obtained for May with progressively higher losses for following months with a maximum of 94% in August, see Table 3.2, Figure 3.4).

Of the plants recovered, some were noted as being black, brown, or green which I have termed: 'discoloured'. In May and June the greatest proportion of discoloured plants occurred. In May the number of discoloured plants decreased with depth and distance from the cages and there were no obvious patterns for June (Table 3.2, Figure 3.4).

Table 3.2 Plant loss and mortality for tethered *Palmaria palmata* (expressed as a percentage of 180 plants deployed).

Month Name	DISTANCE	Original Number plants	% Remaining of original	% of Remaining discoloured	DEPTH	Original Number plants	% Remaining of original	% of Remaining discoloured
May	**0**	10	100	80	**1**	10	100	50
	5	10	80	38	**2**	10	100	30
	10	10	100	50	**3**	10	90	50
	25	10	100	10	**5**	10	100	40
	50	10	100	0	**6**	10	90	0
May Total		50	96	35		50	96	34
June	**0**	36	75	41	**1**	36	72	25
	5	36	58	14	**2**	36	61	28
	10	36	83	47	**2.5**	36	75	44
	25	36	81	35	**3**	36	67	17
	50	36	64	39	**5**	36	67	23
	Reference	36	50	50	**7**	36	69	19
June Total		216	69	38		216	69	26
July	**0**	36	89	0	**1**	36	83	0
	5	36	86	6	**2**	36	89	0
	10	36	75	0	**2.5**	36	75	6
	25	36	72	0	**3**	36	64	3
	50	36	61	0	**5**	36	50	3
	Reference	36	47	0	**7**	36	75	0
July Total		216	72	1		216	72	1
August	**0**	30	10	30	**1**	36	3	0
	5	30	3	0	**2**	36	3	0
	10	30	3	0	**3**	36	11	3
	25	30	7	0	**5**	36	6	0
	50	30	0	0	**7**	36	6	0
	Reference	30	10	0				0
August Total		180	6	17		180	6	1
September	**0**	30	43	0	**1**	36	25	0
	5	30	20	15	**2**	36	42	0
	10	30	13	0	**3**	36	36	3
	25	30	27	0	**5**	36	19	0
	50	30	47	0	**7**	36	19	0
	Reference	30	20	0				0
September Total		180	28	0		180	28	0
November	**0**	30	43	0	**1**	36	61	0
	5	30	20	0	**2**	36	50	0
	10	30	50	0	**3**	36	39	0
	25	30	33	0	**5**	36	25	0
	50	30	63	0	**7**	36	33	0
	Reference	30	40	0				0
November Total		180	42	0	0	180	42	0
Grand Total		1022	48	17		1022	48	8

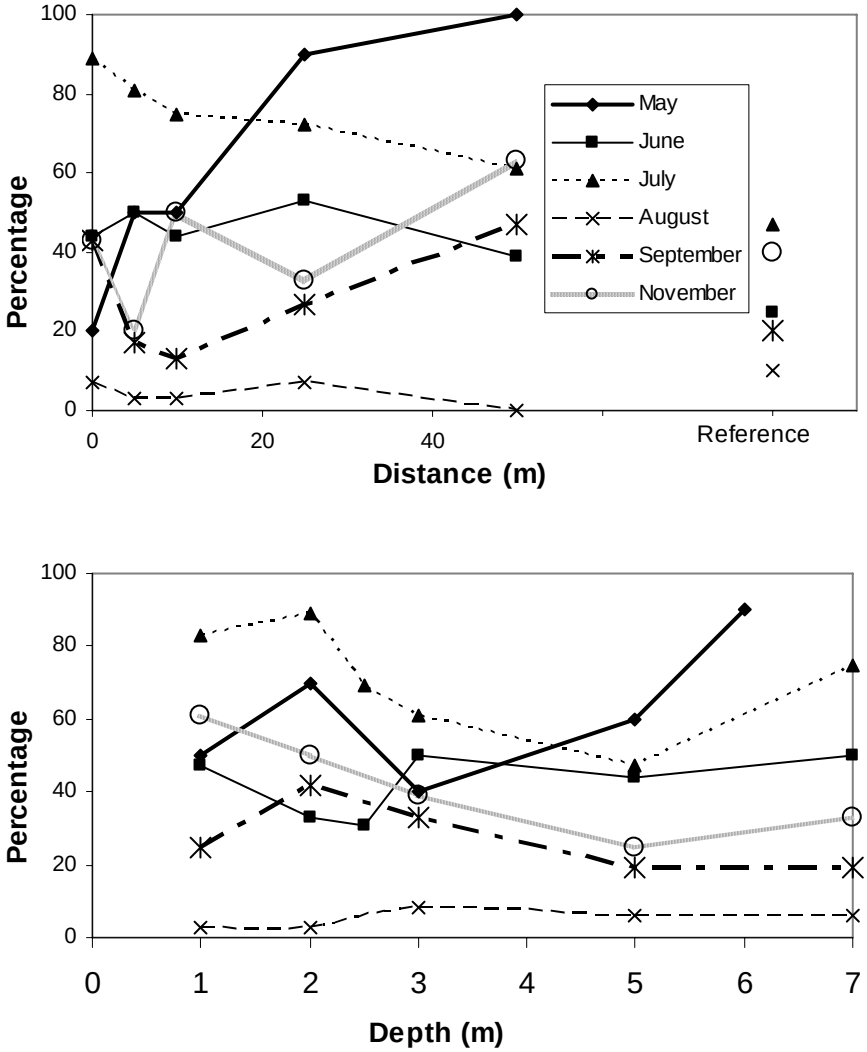

Figure 3.4 Remaining, living, tethered *Palmaria palmata* plants as a percentage of original numbers for each month for a) distance for farm cages and b) depth.

Plant growth

Mean measured parameters for plants with percentage change for each month from May to September 2003 at Loch Laxford are shown in table 3.3. Growth, expressed as percentage change in length and area (as distinct from RGR) across all distances and depths showed that the highest change in both length and area occurred in May, and that changes in length and area decreased through to November. A similar pattern was observed in the growth rates (RGRs, Figure 3.5). Table 3.3 also shows that the initial plant lengths were similar and the time intervals between sampling sessions were

comparable. RGRs for individual plants demonstrated a wide variation in values ranging up to 0.1 cm^2 cm^{-2} day^{-1} (median 0.02 cm^2 cm^{-2} day^{-1}) over the month long sampling periods.

Table 3.3 Mean change in length and area of *Palmaria palmata* at Loch Laxford in 2003 for months from May to September.

MONTH	Mean initial area (cm^2)	% Area increase	Mean initial length (cm)	% Length increase	Growth Interval (Days)
May	20.9	196.1	9.0	67.1	27
June	17.6	95.9	8.8	31.5	29
July	34.4	67.7	10.6	31.5	29
August	38.4	43.1	11.1	27.9	27
September	30.2	26.6	10.7	14.4	35
November	27.0	31.6	10.4	16.5	35

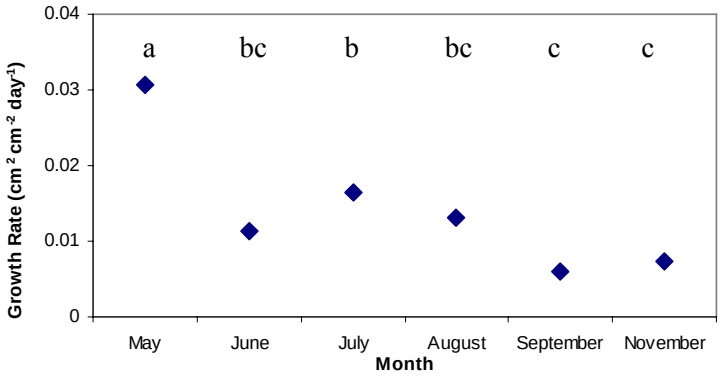

Figure 3.5 Variation in RGR with month, May to November 2003 for tethered *Palmaria palmata* at Loch Laxford. Values which share at least one letter are not significantly different at $p = 0.05$.

Two-way ANOVA of data across depth and distance for RGR showed a significant effect for depth: ($F_{(5,457)}$ = 2.75, p = 0.02) and for distance: ($F_{(4,457)}$ = 3.64, p = 0.006) but no significant interaction (Figures 3.6 and 3.7). Growth rates of plants at 10 m and 25 m from the farm cages were significantly higher than at the reference site, and plants at 2 m depth had higher growth rates than those at 1 m and 7 m depth (Figure 3.7).

Analysis of the results by month (Figure 3.8) showed a trend for lower growth close to the cages in May. In the summer months, this trend was reversed with greater growth closer to the cages, except for very close (0 and 5 m). Growth decreased with increasing depth in most months although growth at 1 m depth was often less than at greater depths. Growth at 2 m depth was significantly greater than at 7m depth for July and November. The August results were not included due to the low return of plants for that month.

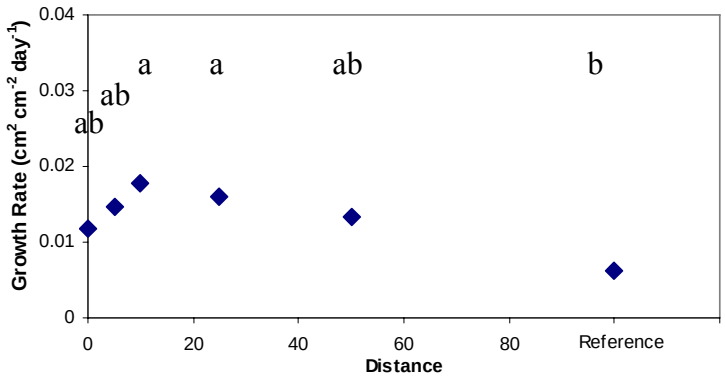

Figure 3.6 Mean RGRs of tethered *Palmaria palmata* plants at different distances from the Laxford cage group (cm^2 cm^{-2} day^{-1}) for all combined data. Values which share at least one letter are not significantly different at p = 0.05.

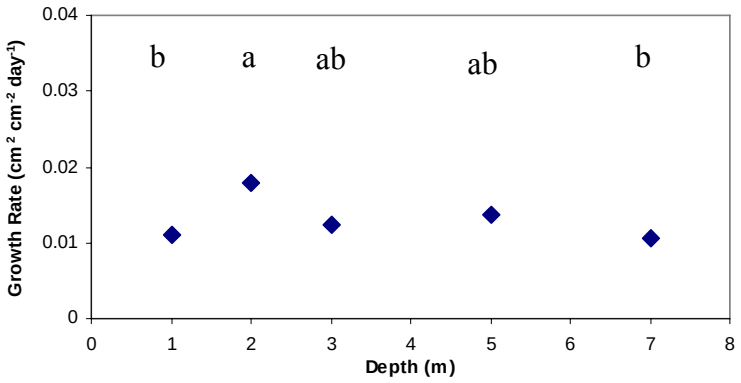

Figure 3.7 Mean RGRs of tethered *Palmaria palmata* at different depths for all data combined. Values which share at least one letter are not significantly different at p = 0.05.

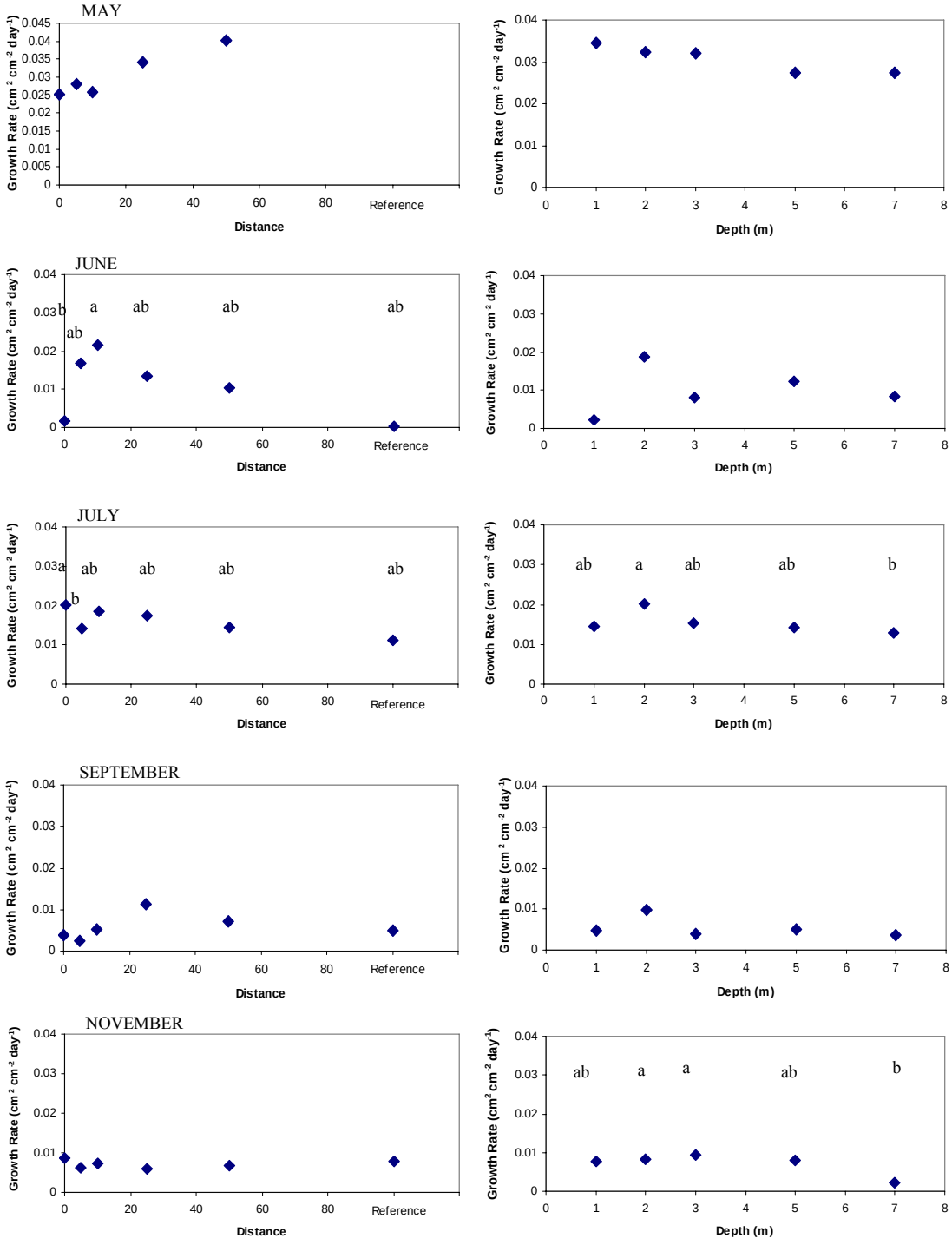

Figure 3.8 Variation in RGR with distance and depth by month for tethered *Palmaria palmata*. Values which share at least one letter are not significantly different at p = 0.05. ANOVA details are presented in Table 3.4. Graphs without letters have no significant differences between measurements.

Table 3.4 ANOVA results for RGR comparisons for depth and distance for month for *Palmaria palmata* tethered algae measurements as depicted in Figure 3.8.

Month	Distance			Depth		
	F df	F	p	F df	F	p
May	F(4,23)	1.18	ns	F(4,23)	0.38	ns
June	F(5,118)	2.58	0.03	F(4,118)	1.26	ns
July	F(5,125)	3.95	0.002	F(4,125)	4.49	0.002
September	F(5,45)	2.11	ns	F(4,46)	2.35	ns
November	F(5,69)	0.3	ns	F(4,70)	3.16	0.019

Epiphytes

Epiphyte growth on the tethered plants was greatest in June, and virtually absent in November (Figure 3.9). Epiphyte growth was most noticeable at distances of 5-10 m from the cages with the least epiphyte growth at the reference site (500 m distant; Figure 3.10). Across all months, there was significantly higher epiphyte growth at 2 m than at either 1 or 7 m depth (Figure 3.11).

Epiphytes to the plants were mainly filamentous brown algae, probably ectocarpoids or chain diatoms. There were many unattached epiphytes caught up with the tethered *P. palmata,* especially close to the salmon cages. The unattached algae included filamentous brown algae and diatom chains, but often included *Enteromorpha* spp (now *Ulva* spp. see Hayden et. al. 2003) spp. and *Ulva* spp. Often the epiphytes appeared to have detritus trapped amongst them which may have included salmon feed and faecal particles.

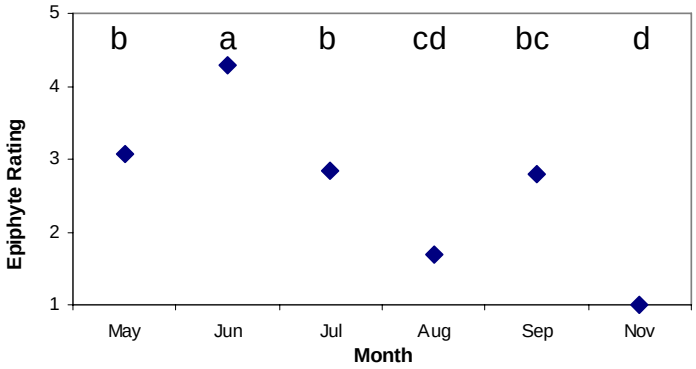

Figure 3.9 Variation in epiphyte cover between May and November 2003. Values that share at least one letter are not significantly different at $p = 0.05$. ANOVA $F_{(5, 481)} = 86.9$, $p < 0.0001$

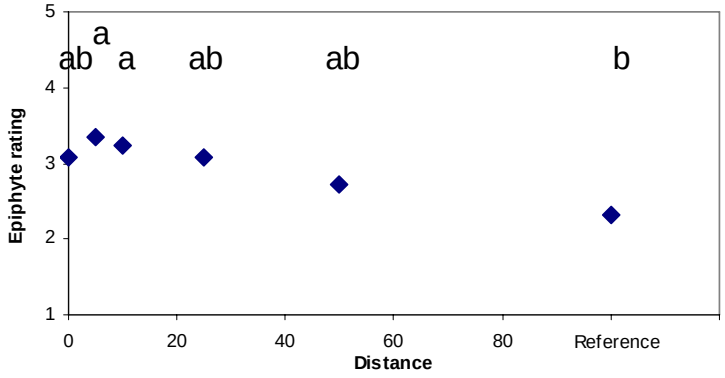

Figure 3.10 Epiphyte cover across all months at different distances from the cage groups. Values which share at least one letter are not significantly different at $p = 0.05$. ANOVA $F_{(5, 457)} = 2.88$, $p = .014$

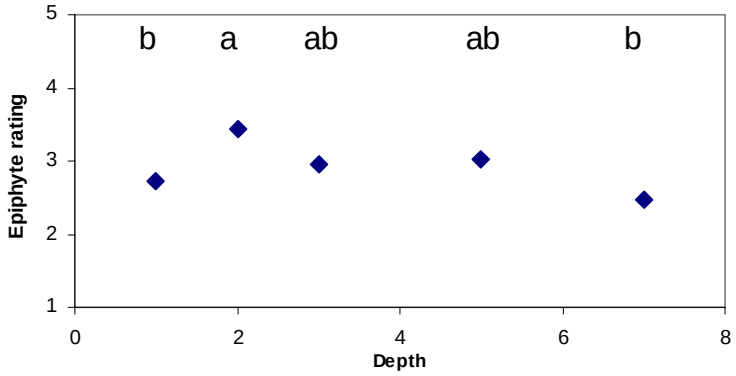

Figure 3.11 Epiphyte cover across all months at different depths from the cage groups. Values which share at least one letter are not significantly different at $p = 0.05$. ANOVA $F_{(5, 457)} = 5.53$, $p < .0001$

Variation in epiphyte rating with depth and distance from the farm cages for each of the

months is depicted in Figure 3.12 and ANOVA results are summarized in Table 3.5. The monthly change in the amount of epiphyte cover over distance (from the cages) closely followed tethered *P. palmata* growth rate changes, except in May. In May, there was a greater incidence of epiphytes closer to the farm, decreasing steadily with distance to 50 m. For June, there was a high incidence of epiphytes at all distances, decreasing slightly with distance from the cages to 25 m. From July to September, there were peaks between 5 and 50 m. In November, epiphyte rating was very low across all distances.

In May, there was a moderate cover of epiphytes at all depths and a high coverage at all depths in June. In July and September, there was a decrease in cover with increasing depth from 2 to 7m.

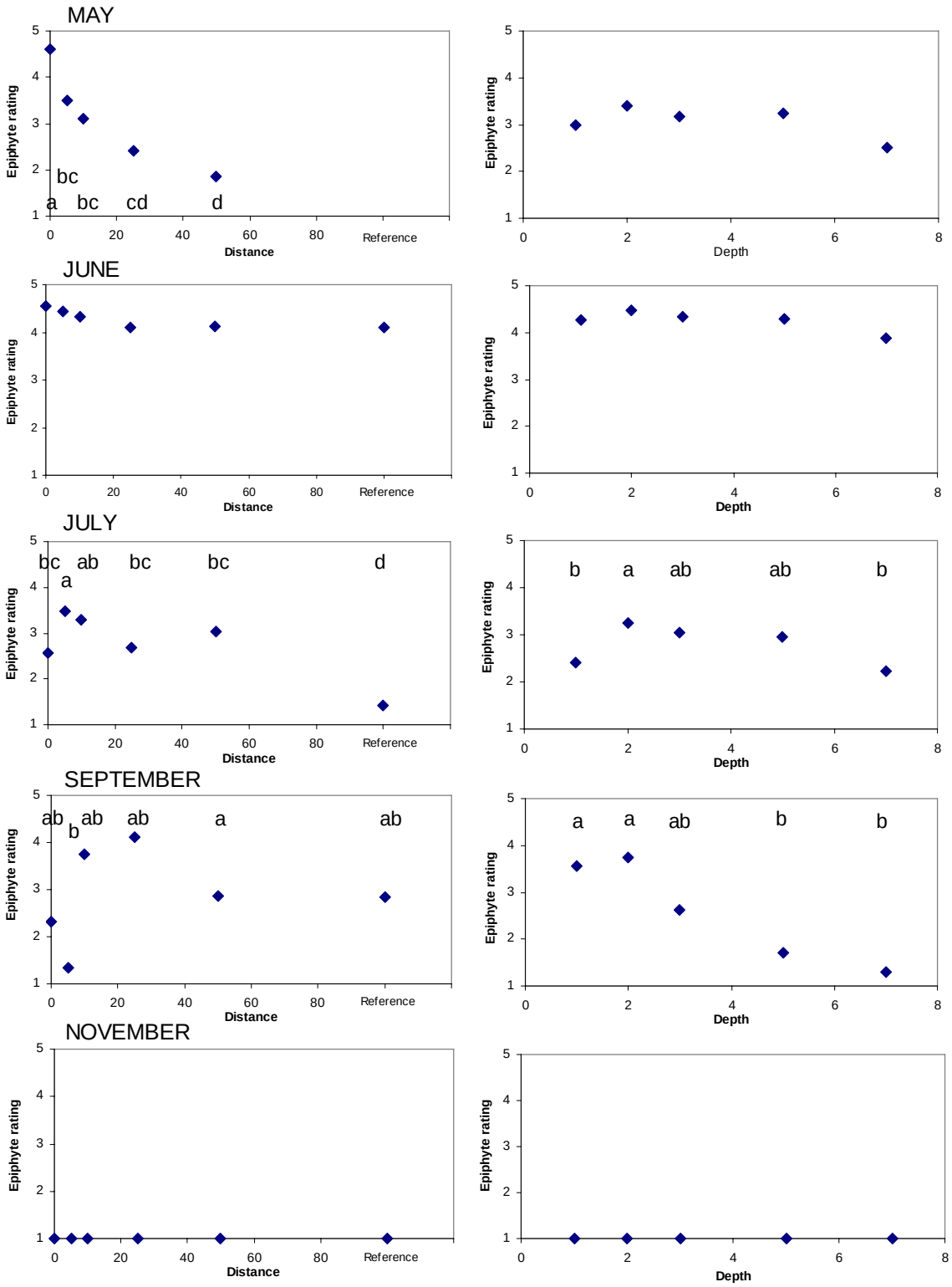

Figure 3.12 Epiphyte rating variation for distance and depth. Values which share at least one letter are not significantly different at $p = 0.05$. No letters indicates no significant differences.

Table 3.5 ANOVA results for epiphyte cover comparisons for depth and distance for each month for tethered *Palmaria palmata* (as depicted in Figure 3.12); F ratio, degrees of freedom and significance level.

Month	Distance			Depth		
	F df	F	*p*	F df	F	*p*
May	F(4,43)	11.27	<0.0001	F(4,43)	0.59	ns
June	F(5,142)	1.02	ns	F(4,143)	1.68	ns
July	F(5,149)	7.67	<0.0001	F(4,150)	3.96	0.004
September	F(5,45)	3.69	0.007	F(4,46)	6.76	<.0001
November						

Colour

Plant colour was deemed as acceptable when it was pink or red / purple. The lowest percentage of plants of acceptable colour was in June, colour acceptability then increased until November (Figure 3.13).

Overall, there are more plants of acceptable colour closer to the farm than at distance and colour improved with depth. (Figures 3.14 a-d).

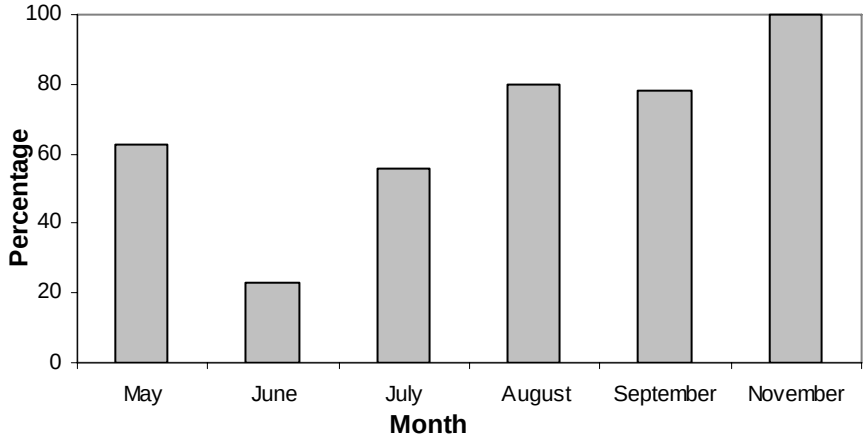

Figure 3.13 Percentage of *Palmaria palmata* of acceptable colour for each month. Total plant numbers for each month: May, 48; June, 148; July, 155; August, 10; September, 51; November, 75.

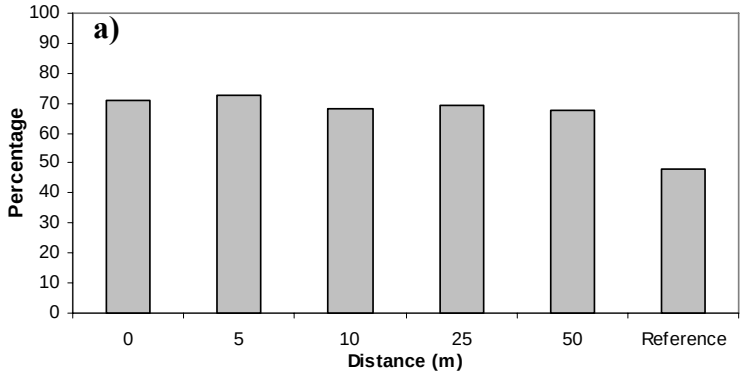

Figure 3.14 a) Occurrence of plants of acceptable colour in relation to distance from salmon cages. Plant numbers across all months: 0 m: 71, 5 m: 52, 10 m: 57, 25 m: 56, 50 m: 65, Reference: 86.

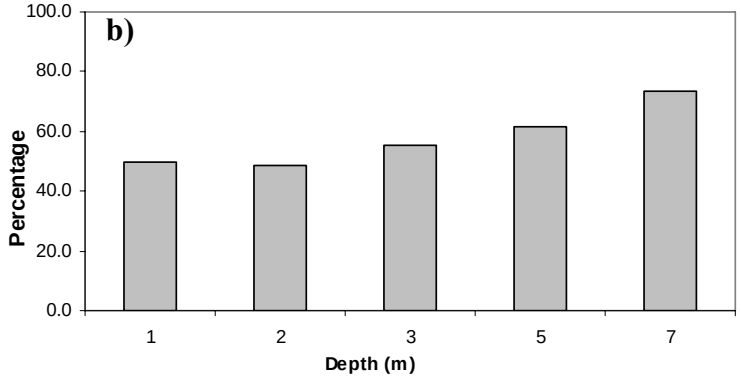

Figure 3.14 b) Occurrence of plants of acceptable colour in relation to depth. Plant numbers across all months: 1 m: 98, 2 m: 150, 3 m: 87, 5 m: 70, 7 m: 82.
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Discussion

Plant losses and discoloured plants

The smallest proportion of tethered plants were lost in May when growth was highest. The number of highly discoloured plants, as a proportion of remaining plants, peaked in May and June. Over these two months bleaching of *P. palmata* can occur (see also Chapters 5 and 6). This is believed to result from UV damage and/or nutrient depletion and coincides with extended periods of clear skies, bright sunshine and low water movement. A greater proportion of acceptably coloured plants was found in deeper waters for May. UV damage and nutrient depletion are less likely to impact at depth.

Plotting colour index against nitrogen content of *P. palmata* plants harvested in 2005 (Chapter 5) showed a good correlation (see Figure 3.15) supporting the theory that pigments (including phycoerythrin) could be a storage product of *P. palmata* for nitrogen (Morgan and Simpson 1981b; Morgan and Simpson 1981c; Martinez and Rico 2002). Low nitrogen leads to pigment breakdown. Phycoerythrin is likely to be one of a number of catalytic and storage proteins e.g. Rubisco that will also be reduced in nitrogen limiting conditions (Raven pers. com.).

However, two different kinds of photoinhibition have been defined, dynamic and chronic photoinhibition (Osmund 1994). In general, sun-adapted algae exhibit dynamic photoinhibition, i.e., a reversible photoprotective mechanism consisting in a down-regulation of the photosystem II (PSII) in order to handle excess energy increasing thermal energy dissipation. In contrast, when shade-adapted algae are transferred to high irradiance environment, e.g. shallow water, chronic photoinhibition becomes evident. This phenomenon is characterized by photodamage of PSII reaction centres and subsequent proteolysis (Critchley and Russell 1994). Thus, expression of photodamage occurs when the rate of degradation of D1 proteins exceeds the rate of repair (Aro *et al.* 1993; Figueroa and Gomez 2001).

For *Palmaria palmata*, Sagert and Schubert (1995; 2000) found decreasing amounts of phycoerythrin with increasing irradiance in *P. palmata* and Martinez and Rico (Martinez and Rico 2002) have postulated that phycoerythrin act as a storage product for nitrogen in *P. palmata*. Thus in May there thus was either increased degradation of proteins as a result of UV damage or nitrogen depletion with decreasing depth. In June, there was no consistent pattern to colour with depth over the range investigated, probably relating to low nitrogen availability throughout the water column.

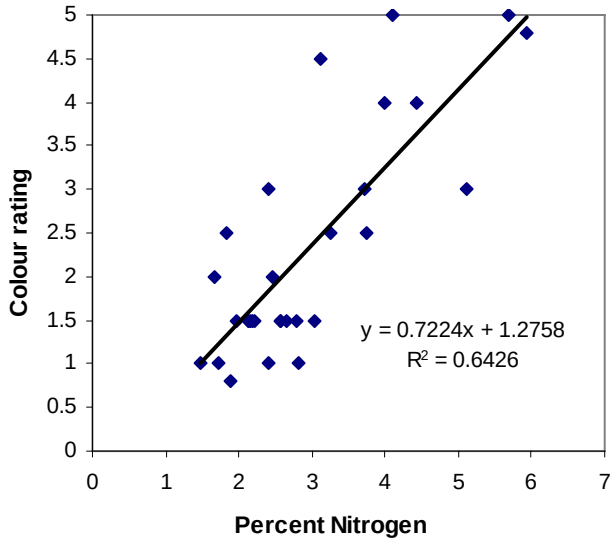


Figure 3.15 Percentage nitrogen (dry weight) for colour-rated *Palmaria palmata* plants harvested from frames as detailed in chapter 6.

Tethered plants of Palmaria palmata – growth rates

Maximal mean growth rates for individual plants for the trial months ranged up to 0.1 $cm^2cm^{-2}day^{-1}$. These rates compare well with those recorded by Browne (2001) for field grown plants and as part of this project in tank culture (Chapter 6) of more than 0.1 $g\ g^{-1}day^{-1}$ and an SGR by Morgan and Simpson (1981) for *P. palmata* of 7.8% although the growth measurements were based on weight rather than area. RGRs using length, area and weight are unlikely to be directly comparable due to non-linear relationships between these three parameters. In fact, a linear determined RGR is likely to be less than area and area less than weight.

In May when growth rates were highest, the mean rate for all *P. palmaria* plants was 0.03 $cm^2\ cm^{-2}day^{-1}$. In May, ambient nutrients were more readily available (see chapter 2) compared to later summer months and incident daily light was increasing. Overall growth rates closer to 0.1 $cm^2cm^{-2}day^{-1}$ may have been achieved if plants had been deployed for March – April when nutrients are high and light levels are believed to be sufficient for *P. palmata* growth but too low for other species, in particular for

competing epiphytes. *Palmaria palmata* grows well on low incident light levels of less than 100 µmol photons m^{-2} s^{-1}consistent with the alga's habitat attached to the stipes of *Laminaria hypeborea* (see chapter 5).

In May, when ambient concentrations of nutrients were higher than in succeeding months (Chapter 2), growth was highest further away from the farm and epiphytes were most abundant in the vicinity of the cages. Epiphytes appeared to make better use of excess nutrients in this month and may have inhibited the growth of *P. palmata* close to the cages. However, particulate matter in the water column such as fish feed detritus and fish faeces that lands on the algal surface may also be limiting plant growth. During this period, plants that were brought to the surface close to the farm, to 15 m from the cages, often had a coating of brownish slime. In later summer months when ambient nutrients were low, better growth occurred closer to the farm, suggesting utilization of farm-derived nutrients despite the slime. The extra light available in summer may enhance growth through any covering layers. It is also possible that the plants may be sourcing nutrients from the slime as has been recorded for *Sargassum* spp. sourcing nutrients from particulate matter on the Great Barrier Reef by Schaffelke (1999).

Epiphytic algae consist primarily of filamentous algae which often are able to utilise nutrients very quickly due to their high surface to volume ratio (Wallentinus 1984; Lobban and Harrison 1996; Pedersen and Borum 1996; Karez *et al.* 2004). Close to the cages, where there is greater nutrient availability, the filamentous algae and *Ulva* spp (and *Enteromorpha* spp.) responded quickly to the increasing availability of light with increased daylength in early spring. At greater distances from the farm cages, intermittent exposure to nutrients may suit *P. palmata* better than the epiphytic algae

due to its capacity for N storage (Lapointe 1985; Pickering *et al.* 1993; Martinez and Rico 2002).

Optimal growth for the tethered *P. palmata* was found between 10 and 25 m from the cages. Care was taken when laying the longlines that their orientation would not result in a decrease in available light as a result of shading from the cages. As they were on the south eastern side of the cage group, light interception was maximised. The decrease in growth of *P. palmata* close (< 10 m) to the cages was not due to shading by the cages. When lines were brought to the surface from close to the cages, not only was there a brown slime, but there were also masses of unattached algae tangled around the droppers. These species included *Enteromorpha* (*Ulva*) spp. and filamentous brown algae, possibly ectocarpoid species and long-chain diatoms. While in the water, under conditions of neutral buoyancy, these algae were caught up on the lines and when bought to the surface, they came away from the lines, sometimes taking some of the tethered seaweeds with them. This solid mass of epiphytes would have shaded the tethered plants and contributed to lower growth very close to the cages (< 10 m distance) and higher losses of plants. The unattached epiphytes were not recorded in the epiphyte ratings of the *P. palmata* as they were not attached and their presence would have contributed to the lower epiphyte ratings recorded close (< 10 m) to the cages for July to September. In addition to shading, the loosely entangled epiphytes may have reduced water exchange and nutrient supply to the *P. palmata* and other attached epiphytes.

Browne (2001) found high RGRs for growth of *P. palmata* from April to September/October in Strangford Lough. Results of the present study in north west Scotland, supported by the plants from the reference site, seem to indicate a high growth

rate for May followed by decreasing rates for subsequent months. This could be explained by nutrient level differences between Strangford Lough and Loch Laxford. Browne (2001) admits that the Lough is a repository for effluents from the surrounding populace and this may result in higher concentrations of nutrients. On the sustainable Mariculture in northern Irish Lough Ecosystems website (SMILE; www.ecowin.org/smile/strangfordlough), they claim a mean nutrient concentration for Strangford Lough for ammonium of 2.8 μM, for nitrate of 13.5 μM and phosphorus of 2 μM which seem high relative to ambient levels in the vicinity of the Loch Duart fish farm sites. They also claim that the Irish Sea is the largest source of nitrogen and silicate loading to Strangford Lough. Of the anthropogenic sources a greater proportion of its nitrogen loading from catchment-derived activities (primarily agriculture), than from sewage or industry.

Another influencing factor may be water motion. Strangford Lough is subject to tidally generated currents. While the Loch Laxford plants are also in a loch with a large tidal range, the farm cages are situated in the lee of an island potentially limiting flow around the cages and plants. Water currents would serve to minimise epiphytes on plant surfaces through abrading the surfaces as a result of the movement of the plants and enhance nutrient exchange through decreasing the diffusion boundary layer (Lobban and Harrison 1996; Hurd 2000; Harrison and Hurd 2001).

In this study, growth rates of tethered *P. palmata* were greatest at 2 m depth. This agrees with the findings of Browne (2001) who found lower growth rates for algae on site specific rigs in depths of 0-1 m than at 1-3 m at two sites in Strangford Lough. Browne believed that the slower growth in the shallower waters may have been due to high light or nutrient deprivation. At Loch Laxford, these two factors may also be

influencing growth of *P. palmata* in shallower waters. Temperature and salinity profiles (Calbha and Badcall bays only, Appendix 1, Figures 1.20 & 1.21) show some stratification of the water column indicating varying water chemistry in shallower waters (2-10 m) which may account for lower growth rates.

Unlike Browne (2001) who found no difference in RGR between 0 and 7 m depth for tethered plants grown on a longline and a site specific rig, overall growth here was found to be less at 7 m than at 2 m depth across all sites and was probably caused by lower light at this depth.

The occurrence of epiphytes on the plants follows closely the seasonal availability of light. In November, when the days are quite short, there were few or no epiphytes on the seaweeds. In May when the days were lengthening, the epiphytes became more abundant especially close to the cages (higher nutrients). In September when the days were shortening, there were again maximal amounts closer to the cages (higher nutrients). In the summer months, attached epiphytes were common everywhere except very close to the cages. However, as mentioned earlier, the quality of unattached algae and particulate matter found on the lines very close to the cages may have prevented development of an epiphytic community as found at greater distances.

The *P. palmata* on the lines for these experiments were at low densities, meaning they were potentially a greater risk of colonisation by epiphytes. Higher densities of plants can result in self-cleaning through rubbing of adjacent plants and they also minimise establishment of other algae through dominating space on the lines similer to the situation in tanks (Fletcher 1995, Ask and Azanza 2002). Culturing, as opposed to tethering, of the algae on the lines should result in a lower occurrence of epiphytes due

to higher densities of plants. This would result in cleaner more acceptable plants with lower mortalities and increased yield. The problem of epiphytes is common to cultured seaweed operations and strategies would have to be developed to minimise their impact.

Colour

Seasonal patterns in colour of the plants followed growth rates. Generally the plants closer to the farm had better colour than those further away. This is likely to be a reflection of availability of nutrients. The colour of *P. palmata* plants has been related to nitrogen content of the plant and thus to nitrogen availability (Figure 3.15). The better colour close to the cages however also may have been enhanced as a result of the shading by the unattached epiphytic seaweeds. In November, colour was good at all sites, in agreement with high ambient nutrient concentrations and low light. The observed trend of darker plants with depth may be as a result of some photodegradation from high light levels in the shallower waters but also may be related to some stratification of the water column with lower nutrient levels in shallower waters.

The results from the use of transplants for determining the potential for culture of *P. palmata* are not all directly translatable. Transplanting the seaweeds is likely to have stressed them and led to greater levels of mortality than would have been the situation for cultured plants deployed on site. Plants taken from intertidal locations also may have suffered growth changes as a result of a change in habitat. Plants from the intertidal zone may also have microscopic stages of epiphytic algae attached that only bloom once in their new environment and are, thus, not reflective of plants that might be cultured on site (e.g. Ask and Azanza 2002). The assumption is that all the plants are equally affected within a month and trends observed within the month are representative. It should also be noted that the findings determined here at Loch Laxford apply in the

direction of the determined main currents which are believed to enhance nutrient availability and may not apply in all directions around the cages.

SUMMARY

Growth of *P. palmata* was significantly higher at 10-25 m from the fish farm cages than immediately adjacent or at greater distances, particularly during the summer.

The best growth during summer, when it might be assumed the fish farm cages are the primary source of nutrients, was not as high as during periods of high light availability and high ambient nutrient availability in spring.

In comparison to the reference station, higher growth and better colour of plants was found in the vicinity of fish farm cages (<10 m) extending to at least 50 m distance. Very close to the cages (<10 m) growth appears to be inhibited by unattached epiphytes and/or farm-derived water-borne matter likely to consist of fish feed and fish faeces.

Growth was best at 2 m depth and significantly greater than at either 1 or 7 m depth.

A large percentage of the plants suffer discoloration in May and June perhaps as a result of UV damage or nutrient deprivation. If plants are to be grown close to the cages, strategies to mitigate this might include putting the plants deeper at times when there are conditions of high light and low water movement. This may also assist in minimising epiphytes as epiphytes appear to do well under conditions of high light in the shallower waters.

CHAPTER 4

CULTURE OF *PALMARIA PALMATA* AND *LAMINARIA SACCHARINA* IN THE VICINITY OF THE FISH FARM CAGES

Introduction

Macroalgal culture in coastal waters has been targeted as a means of utilising excess nutrients that may otherwise contribute to eutrophic conditions. Their potential utility has been documented not only for tank and sea-based fish cultivation systems (e.g. Evans and Langdon 2000; e.g. Chopin *et al.* 2001; Fei 2004; Neori *et al.* 2004; Zhou *et al.* 2006) but for sewage and industrial waste water as well (Anderson *et al.* 1999; Aravindhan *et al.* 2004; Torres *et al.* 2004; Mehta and Gaur 2005).

Successful cultivation of macroalgal species adjacent to salmon cages has been documented and there is further ongoing research. Troell *et al.* (1997) found *Gracilaria chilensis* had a 40% higher growth rate when cultivated at a distance of 10 m from salmon sea-cages in Chile, and Petrell and Alie (1996) demonstrated how 1.5 μM ammonia concentrations found adjacent to salmon sea-cages enhanced the growth of brown macroalgae. In Canada, culture of *Porphyra* spp. is being trialled adjacent to salmon cages (Chopin *et al.* 2000; Carmona *et al.* 2006) and in China, *Gracilaria lemaneiformis* (Bory) Dawson is being co-cultured with the fish *Sebastodes fuscescens* (Zhou *et al.* 2006). In this project, *Palmaria palmata* and *Laminaria saccharina* were chosen for culture trials adjacent to fish farm cages in north west Scotland.

Culture of *P. palmata* has received much attention in recent times throughout Europe because of its perceived high value for use either as a food or as a fodder to animals

such as abalone and sea urchins. It is also a hardy alga with high growth rates, qualities that make it attractive as an aquaculture species. *Laminaria saccharina* was chosen as it is a large, fast-growing alga and thus has good bioremediation potential. The alga also has potential although unproven, commercial value. *Laminaria saccharina* is edible and is very similar to *L. japonica* or Kombu which constitutes the largest aquaculture crop, by weight, in the world. *Laminaria saccharina* is also currently being tested as a potential source of novel polysaccharides with biomedical applications (Cumashi *et al.* unpubl.) as an adjunct to this project. The alga is also a potential source of alginates .

The methodology for the mass culture of brown algae from spores is well developed and has been conducted since 1955 for the brown algae Wakame (*Undaria pinnatifida*) and 1970 for Kombu (*Laminaria japonica*) in Japan (Ohno and Largo 2006). Commercial cultivation of red algae has been restricted mostly to Nori (*Porphyra* spp) but recently cultivation from isolated spores has been successfully developed for the carrageenophytes *Kappaphycus alvarezii* (de Paula *et al.* 1999), *Sarcothalia crispata* (Avila *et al.* 1999), *Gigartina skottsbergii* (Buschmann *et al.* 2001b), and agarophytes such as *Gracilaria verrucosa* (Oza *et al.* 1994), *G. chilensis* (Alveal *et al.* 1997; Halling *et al.* 2005), *G. parvispora* (Glenn *et al.* 1998), *Gelidium rex* (Rojas *et al.* 1996), and *Gracilariopsis bailinae* (Rabanal and Azanza 1999).

Current development of *P. palmata* mass culture technology involves scaling up algal production techniques from the laboratory as well as further refining optimal conditions for spore release and culture. Mass culture of *L. saccharina* is well progressed and this project involved adapting existing techniques to the field situation at Loch Duart Ltd. sites.

The culturing methods for *P. palmata* used here were developed from the work of Browne (2001), Le Gall (2004) and Pang and Lüning (2004; 2006). The culture methods used for *Laminaria saccharina* were modified from those of Dawes (1987), Holt (1984) and as outlined in Kawashima (1993).

Palmaria palmata

Palmaria palmata has an unusual life history. Van der Meer and Todd (1980) were the first to complete the life cycle in culture. The foliose plants observed on the shore are either male gametophytes or tetrasporophytes which are isomorphic. Female and male gametophytes are very dissimilar in appearance, the female being a microscopic crust-like plant which produce carpogonia borne directly by the vegetative cells. The male gametophyte produces spermatia that can fertilise the carpogonia of the female crusts. Once fertilised, the carpogonium develops into a tetrasporophyte. Initially, the diploid tetrasporophyte grows attached to the female, but it eventually overgrows it. Adult tetrasporophytes produce haploid tetraspores by meiosis and these develop into male or female gametophytes. Sexual maturity is reached when male plants are 9-12 months old and more than 20 cm long and when female plants are only a few days old and microscopic. Therefore, it is believed that females are fertilised by males from a previous generation.

Browne (2001) refined the culture of *P. palmata* in attempting to scale up culture. Her methodology for culturing *P. palmata* included surface cleaning of mature tetrasporic plants, then introducing them to tanks containing sterilized seawater at the rate of 100 g wet weight per 0.05 m^2 of tank surface area. Her recommended substrate for growing spores was Kuralon string, which is the same string used for the culture of *Undaria pinnatifida* in Korea. The string was placed under the free floating *P. palmata* fronds on suitable frames. To initiate spore release, the tanks with free floating algae were

exposed to bright light and then placed into darkness. Spore release occurred over a period of 48 hours. The best results for spore growth were obtained at a temperature of 10^{o}C, irradiance of 10 µmol photons m^{-2} s^{-1} and a long day light: dark (16:8 h) cycle. Aeration was provided to the cultures during and after spore release.

The method employed by Le Gall *et al.* (2004) for spore release shocks plants through desiccation followed by immersion. Generally, 3 kg of fresh algae was required to inoculate 1 m^{2} of substrate, but the liberated spore yield was extremely variable and depended on the fertility of fronds. Plants were first cleaned and then put in the dark at 4^{o}C overnight before placing into aerated 10^{o}C 1-µm filtered seawater for one hour during which time the spores were released. The spores were counted in suspension and introduced to larger tanks where they were settled on to the substrate medium. Fluorescent lights suspended 0.5 m above the water surface, maintained light levels of 50 µmol photons m^{-2} s^{-1} with a photoperiod of 12 hr. Bubbling was initiated 2 days after spore settlement. Spores were settled on roughened white polystyrene plates. Best growth was obtained with added nutrients and water exchange once per week.

A similar methodology to Le Gall *et al.* (2004) was used by Pang and Lüning (2004; 2006) for culturing *P. palmata* but without radical shock treatments to initiate spore release. A biomass of 0.5-1 kg fresh weight of tank-grown short day tetrasporangial thalli was cleaned three times with seawater and then put into seawater in a 10-litre plastic container with mild aeration over a period of three days. Thalli were picked out every morning, released tetraspores were allowed to sink down to the bottom for 1 hour, supernatant water was removed and new seawater was added to wash the spores. This procedure was repeated 3 times. Clean spores were collected and cultured for one month in 10% Provosali's Enrichment Solution (PES) at 10°C in fluorescent white light (10

µmol photons m^{-2} $s^{-1,}$ 12 hr light per day). The culture medium was renewed every week. At 1-2 mm size the sporelings were transferred into a 5-litre glass bottle and cultured for a further month in full PES at elevated irradiance (40-60 µmol photons m^{-2} s^{-1}), again with weekly renewal of the medium. The young sporelings were subsequently transferred into an indoor 100-litre tank and later into a 2000-litre greenhouse tank, both supplied with flowing seawater and no further addition of nutrients. No substrate was used for settlement, rather the thalli were allowed to develop in the free floating state.

Advantages of the Le Gall *et al.* (2004) and Pang and Lüning (2004; 2006) methods include the potential for controlling spore numbers and the cleaning of the spores as the mucilage attached to the spores on release has been identified as a potential problem for spore survival (Kadel, pers com). Both methods also result in an even distribution of spore on the substrate surface. All the above methods for culturing *P. palmata* have been trialled during the term of this project.

Laminaria saccharina

L. saccharina is most common on rocky reefs but may also be found growing on rock and shell fragments on sand. The alga is found throughout the north Atlantic from the coast of New York in the west to France in the east, and north to Greenland and Norway. The alga has a life cycle typical of other *Laminariales*, consisting of an alternation between the large macrophytic sporophyte and a microscopic gametophyte. The diploid sporophytic stage is the plant we are most familiar with. It grows to 3-5 m in length and is found primarily in sheltered waters on hard substrates. The sporophyte releases motile haploid spores from sori on the plant's surface. The germinating spores give rise to filamentous gametophytes (www.algaebase.org). In the laboratory, this stage can produce motile male antherozoa within 2-3 weeks. These then fertilize the

female oogonia to produce the sporophyte.

In the growth season of 2004 (winter-spring), culture of *Laminaria saccharina* and *Palmaria palmata* were trialled adjacent to fish farm cages at Calbha. In 2004, fish biomass was at a maximum for the Calbha site (see Chapter 1). The algae were outplanted at varying times during the season and at varying distances from the fish cages adjacent to cage group 'D'. Epiphytes were monitored because they have been identified as a potential problem for cultured seaweeds, particularly those grown adjacent to fish farm cages.

Methods

Palmaria palmata

Seasonality of spore release

To determine the seasonality of tetraspore production by *Palmaria palmata* in the vicinity of SAMS marine laboratories at Dunstaffnage, thirty *P. palmata* plants were collected regularly each from three sites on a rocky coast near Easdale on the Isle of Seil, 30 km south of Dunstaffnage. The sites were in close proximity (within 50 m) and differed in perceived environmental conditions in order to determine possible small scale geographic variations in seasonality of tetraspore production. The sites were around a small bay at the northern end of the township. Plants were collected from the east (EAST) and west (WEST) sides of a promontory and in the bay subtidally (BAY).

On the more wave-exposed eastern side within the bay, plants were sampled from the lower intertidal on reef (EAST) and from the upper subtidal on *Laminaria hyperborea* holdfasts (BAY). Plants were sampled from the western side in the lower intertidal and upper subtidal from the rocky reef and attached to *Fucus serratus*. Tetrasporic plants were identifiable by the marbled appearance of the surface of the fronds and the presence of tetraspores were confirmed using a binocular microscope. The plants were scored for the presence or absence of tetraspores. The wave-exposed side was not always sampled due to wave action.

The majority of plants for culture were sourced from Isle of Seil but additional sites included the Falls of Lora 5 km north of Dunstaffnage, in front of the SAMS marine laboratories, and at Calbha, one of the Loch Duart sites in north west Scotland.

Substrate

Spores for each method were settled on to Kuralon string. A significant advance in this project was the development of a frame that maximised the length of string that could be evenly seeded with *P. palmata* spores. The efficient output of amounts of seeded string necessary for large scale culture is a potential constraint for commercialisation of *P. palmata* culture. At harvest, one to two kilograms fresh weight of *P. palmata* is obtained for each metre of seeded string (Browne 2001) or, for a crop of ten tonnes, 10 kilometres of seeded string is required. *Palmaria palmata* spores are not motile so settlement is restricted to upward facing surfaces. Current culture techniques seed lengths of string of tens of metres rather than thousands of metres.

In this project, frames were developed that exposed maximal string surfaces to spore settlement. This was done using two innovative frame designs. The first used string wound around closely spaced pegs at two ends of culture tanks and the second was wound around an open frame with the lower strings bought to the same level as the upper strings (see Figure 4.1). Future large scale seeding should consider Japanese and Korean technology for seeding *Porphyra* spp which includes using nets for seeding.

In the settlement tanks, microscope slides were placed at the bottom to monitor spore numbers and survival.

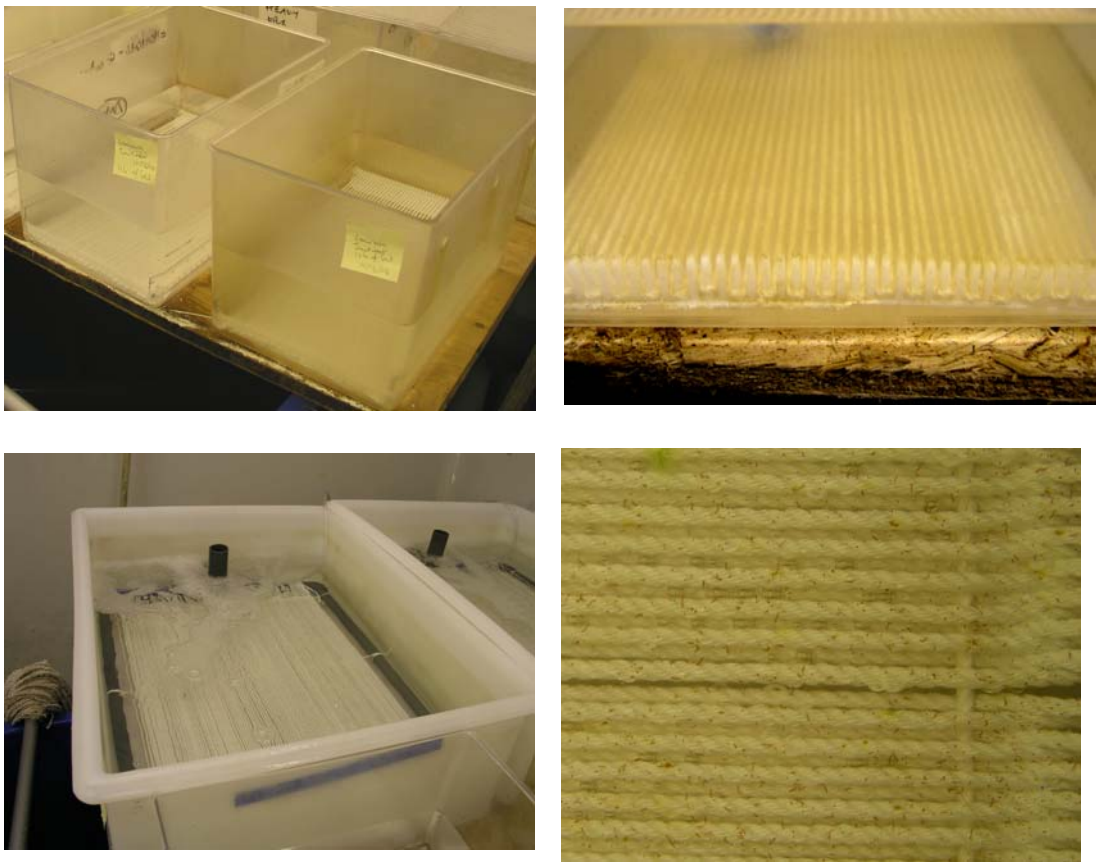


Figure 4.1 Two frames types were used for seeding string.

Above: string is looped on to 'teeth' at each end of the tank and **Below:** string is looped around a frame with an insert keeping lower strings on a level with top strings.

Spore release

In culture trials in 2004 and 2005, three different methods of spore release were successfully trialled. The methods were those of Browne (2001) which I have termed the 'free' method, Le Gall *et al.* (2004) termed the 'shock' method and Pang and Lüning (2004; 2006), called the 'tumble' method.

Free Method

P. palmata fronds were collected, washed with UV- treated seawater and approximately 100 g of the washed plant material was added to tanks of 0.05 m^2 basal area. The plant fragments in the culture medium were exposed to 2-6 hours of high light and then left for up to 48 hours with light aeration on low light of 10-20 µmol photons m^{-2} s^{-1} at 16:8 hours light:dark.

Shock Method

Outer surfaces of tetrasporic plants were wiped to detach loosely attached epiphytes and excess moisture. They were then wrapped in paper towelling, placed in the dark at 4°C and left overnight before being introduced to the culture medium. They were left here with aeration for one hour before the seawater containing the spores was filtered through a 43-µm filter into separate tanks containing substrate.

Tumble

A biomass of up to 500 g fresh weight consisting of tetrasporangia-bearing thalli was rinsed in 1 µm-filtered seawater and then air tumbled in 10-litre plastic containers filled with 8 l of filtered seawater. All thalli were picked out every morning and used repeatedly for tetraspore release during the following three days. After the thalli had been taken out, the released tetraspores were allowed to sink down to the bottom for 1

hour, excess water was removed and new seawater was added and spores washed through a 43 μm filter into separate tanks containing substrate.

Growth conditions

For the first 3-7 days, the *P. palmata* spores were grown at 10-12^{o}C, long day (16:8) with low light of 10-20 μmol photons m^{-2} s^{-1} after which the seawater was exchanged for seawater containing f/10 (Guillard and Ryther 1962) nutrients and GeO_2 to limit diatom contaminats (Markham and Hagmeier 1982). At this stage microscope slides at the base of the tanks are sacrificed to do spore counts. The number of spores were counted on transects of the slides and values extrapolated for the tank area. The percentage of living (red healthy colour) were noted against dead (clear to green patchy colour). The seawater medium was changed every 7 days and after two to four weeks the light levels were raised to 50-100 μmol photons $m^{-2}sec^{-1}$.

***Laminaria saccharina* culture**

Laminaria saccharina plants used for culture were collected at Isle of Seil and at Loch Creran approximately 20 km north of Oban. Fertile *L. saccharina* fronds were usually older, longer, thicker blades and the sori were identified by raised areas that sometimes appeared darker than the rest of the frond. Sori can occupy areas from a few square centimetres to much of the frond surface with a tendency to be located near the central part of the frond. At least five fronds with sori were selected to maintain a minimum level of genetic diversity. Sections of frond with sori were cut from the blade, the outer surfaces were wiped with a cloth or paper towel to remove loosely attached epiphytes, and the fronds were placed in the darkness at 4^{o}C overnight before placing into sterilized seawater for two hours. The spores that had been released into suspension were introduced to tanks containing the substrate medium.

Laminaria saccharina was grown under similar conditions to *P. palmata* except that it could tolerate - and was grown under - higher light conditions (100+ µmol photons m^{-2} s^{-1}). Spores were released on to the same substrate as that used for the *P. palmata*: Kuralon string. Seawater medium was changed every 7-10 days. Sporophytic plants were obvious on the string within four weeks and counts of the plants indicated more than 30 per centimetre of string.

Nutrients (f/10) were introduced to spore cultures of both species when the seawater was changed with apparent positive effects. If, however, the number of epiphytes also increased, compromising spore survival, both *P. palmata* and *L. saccharina* appeared to survive well without nutrient additions. Not using nutrients appeared to be a good means of managing epiphyte contaminants.

Cultured algae were outplanted on site at Calbha on three occasions. The first outplant to the Calbha site occurred on 19/2/04 and consisted of a mixture of two *L. saccharina* batches (initiated 23/12/03 and 9/1/04) and the first of the *P. palmata* batches ('**Feb**', initiated 9/1/04).

The second outplants were deployed on 16/3/04 and consisted of a *P. palmata* batch produced by colleagues at Queens University Belfast marine station at Portaferry ('**Mar No 1**', initiated 20/1/04 & 12/2/04) and a second *P. palmata* batch from SAMS ('**Mar No 2**', initiated 3/2/04). The third outplant ('**May**', 11/5/04) consisted of an *L. saccharina* batch (initiated 1/4/04) and the final batch of *P. palmata* (initiated 3/3/04, see Table 4.1 for synopsis).

Table 4.1 Dates for culture initiation and time before deployment to sea for algae outplanted to Calbha.

Stock Source	**Culture initiated**		**Date of Outplant**	**Days after initiation**
	Palmaria palmata	***Laminaria saccharina***		
Seil		23/12/2003	19/02/04	58
Seil	09/01/04		19/02/04	41 (**Feb**)
Creran		09/01/04	19/02/04	41
Seil	03/02/04		16/03/04	42 (**Mar No 2**)
N. Ireland	20/01/04		16/03/04	56 (**Mar No 1**)
	12/02/04		16/03/04	33 (**Mar No 1**)
Seil	03/03/04		11/05/04	69 (**May**)
Creran		01/04/04	11/05/04	40

Frames

For deployment on site, *P. palmata* seeded string was cut into metre lengths. Loops were tied in each end of the strings to give a length of 80 cm and attached to clips on buoyed frames (see Figure 4.2). The buoyed frames consisted of two 1.5 m length PVC pipes, each with four clips, attached to a 12 mm mooring rope. The 12 mm mooring rope was attached to a buoy at the surface and at approximately half depth or at least 6 m depth above the bottom at low tide; a 2-3 kg weight was attached that kept the upper section of the line vertical. Each line was moored at the base with a 20–30 kg weight. The top of the frames were 2.0 m from the surface. The mooring lines were set up so that the frames could be bought into a small boat at low tide for maintenance and monitoring of the seeded strings. Using frames provided multiple strings for each site, thus increasing replication. Four *P. palmata* seeded strings were supported by each of the frames.

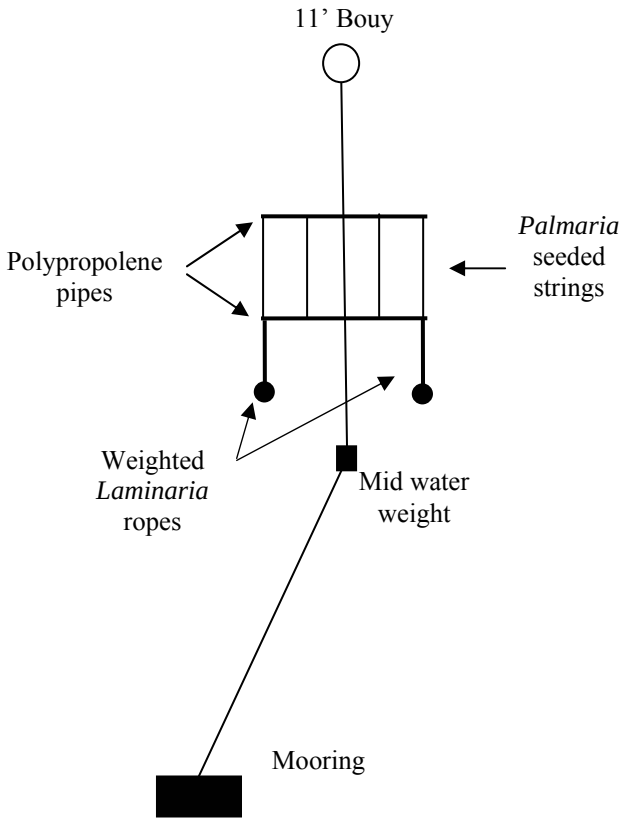


Figure 4.2 Buoyed frames used for outplant of cultured *Palmaria palmata* and *Laminaria saccharina* at Loch Duart.

Frame Placement

The aim was to determine how placement relative to the salmon cages would affect growth and quality of the cultured seaweeds. The original plan was to have frames in triplicate at various locations with respect to the salmon cage group. However to accommodate farm operations and loss of some of the frames, frames were grouped depending on their distance from the farm cages (see Figure 4.3). Frames located close to the salmon farm cages (to 50 m) were attached to two horizontal surface lines which ran east from the eastern corners of the cage group. Frames were attached to these lines with a 1-2 kg weight at their base.

The final arrangement was 10 frames between 0 and 50 m on the east side of the salmon cage group ('**Close**'), 6 buoyed frames between 70 & 80 m on the west side ('**Mid1**'), 4 buoyed frames at 120 to 220 m ('**Mid2**') and 5 at 500+m ('**Far**', see Figure 4.2) for giving a total of 25 frames. Note that while the eastern group of '**Far**' frames are over 900 m from the western cage group, they are only 500 m from another set of salmon farm cages on the eastern side of the lease site that had a similar biomass of fish at the time. These frames are thus grouped with the second southern '**Far**' group of frames.

L. saccharina was also cultured on the frames. To do this, seeded *L. saccharina* string was cut into 10 cm sections. Two of these sections were interwoven into the lay of each 80 cm length of 10-mm three strand polypropylene rope of which there were two on each frame. One string was inserted ten cm from the top and the second ten centimetres from the bottom of the ropes. These ropes were attached to the bottom corner of the frames with a small lead weight (sinker) at the base of the ropes to keep them vertical. Where only one rope was attached to a frame a weight was attached to the opposite corner to balance the frame.

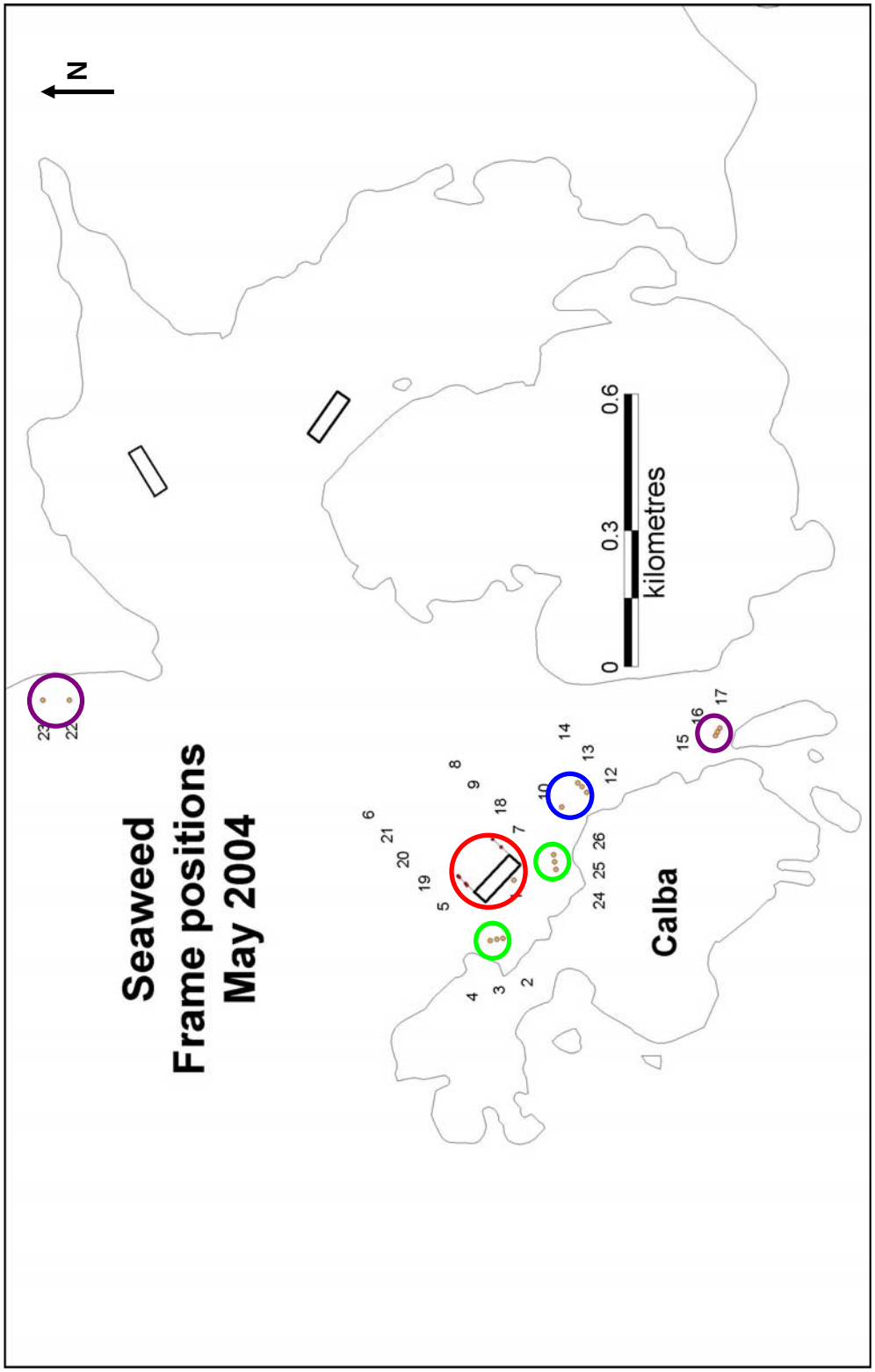


Figure 4.3 Position of frames at Calbha in 2004 grouped according to distance from salmon farm cages. Red: '**Close**'; Green: '**Mid1**'; Blue: '**Mid2**' and Purple: '**Far**'. Numbers refer to frame numbers.

Figure 4.3 Frame positions at Calbha, 2004.

***Palmaria palmata* growth measurement**

Palmaria palmata sporelings were checked on the seeded string before deployment into the field. Maximum size of the plants at deployment was consistent 2-3 mm in length across batches. On each visit to the site, the seeded *P. palmata* strings were unclipped

from the frames and photographed on a plastic tray with rulers for later image analysis. Growth of plants was monitored using the length of the five longest plants on each string. Measurements were obtained from photographs of the strings using the image analysis program ImageJ. Subsequent verification in 2005 proved the validity of using either this attribute or photographic area (see Figure 4.4 a-c) as a proxy for biomass increase. The non-linear relationship between plant area and weight is due to the overlapping of fronds with the larger plants. There may also be some thickening of the thallus with increasing plant size.

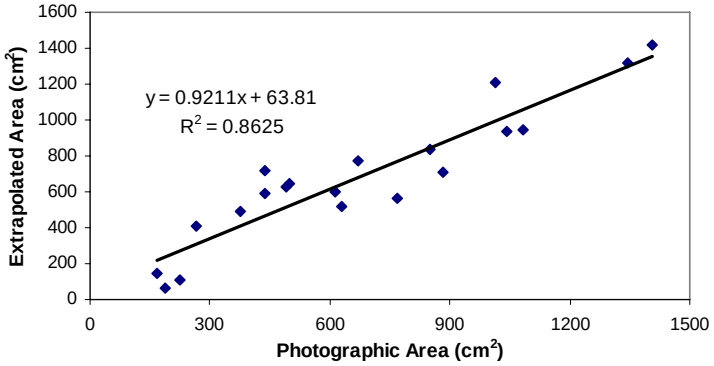


Figure 4.4 a) Extrapolated area (product of length of string with *Palmaria palmata* and mean plant height of the five longest plants) versus photographic area. Measurements were taken at harvest in June 2005 (for details see chapter 5).

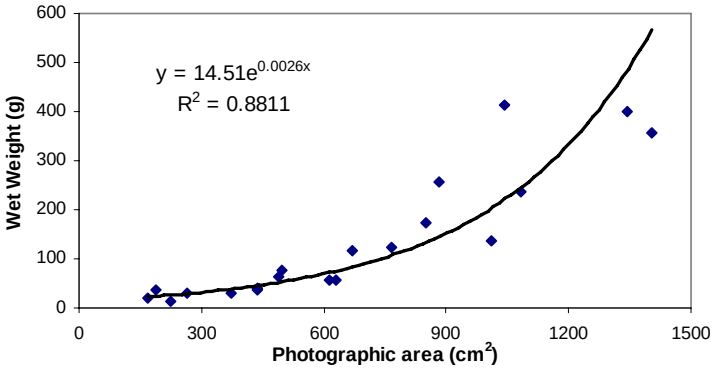


Figure 4.4 b) *Palmaria palmata* photographic area versus wet weight. Measurements were taken at harvest in June 2005 (for details see chapter 5).

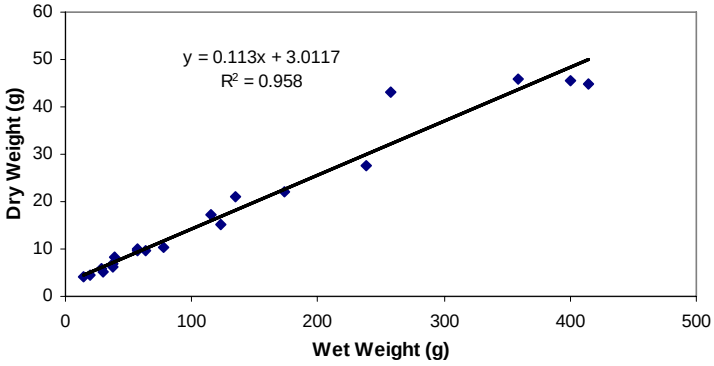


Figure 4.4 c) Wet to dry weight for *Palmaria palmata* harvested from strings on frames. Measurements were taken at harvest in June 2005 (for details see chapter 5).

Epiphytes

Epiphytes are a common problem for cultured macroalgae (e.g. Fletcher 1995; Buschmann *et al.* 2001a). A measure of epiphyte cover was developed based on the quantity of epiphytic growth occurring on the bare sections of string. This was done using image analysis. The length of the string with epiphytes was measured and the apparent surface area taken up by the epiphytes calculated using ImageJ. This gave for each string, a value for the epiphytic cover per length of string (area per length of string: $cm^2\ cm^{-1}$). Epiphytes were identified to species level where possible; otherwise, they were grouped to an identifiable classification level e.g. 'fine red algae' which would have included species such as *Ceramium* spp. and *Polysiphonia* spp.

***Laminaria saccharina* growth measurement**

The length of the five longest *L. saccharina* plants were determined for each buoyed frame at harvest (15/6/2004). Until late summer, length of *L. saccharina* blades directly reflects growth rate and productivity. After late summer, blades of first year plants begin to erode from the tips and the hole punching technique (Parke 1948) is a more appropriate method for estimating growth rates. *Laminaria saccharina* grows from the base of the blade and erodes from the tips after mid summer. After mid summer growth can be monitored by holing the thallus in the middle of the blade, usually with a 0.5 cm hole punch, 15 cm up from the stipe-blade junction. The change in distance between the stipe-blade intersection and the hole between time intervals is the rate of growth of the plant (see Chapter 5)

Subsequent verification demonstrated the utility of using length measurements as a

proxy for growth and production of *L. saccharina* bundles (a bundle of *L. saccharina* arises from each 10 cm section of *L. saccharina* seeded string, see chapter 5 and Figures 4.5 a & b). On 15 June 2004, the first batch of *L. saccharina* plants was harvested for analysis of novel polysaccharides (Cumashi *et al.* unpubl.) and measured. Measurements were continued on the second batch of *L. saccharina* outplanted in May but loss of the plants due to poor growth and fouling limited the integrity of these data and so they are not presented.

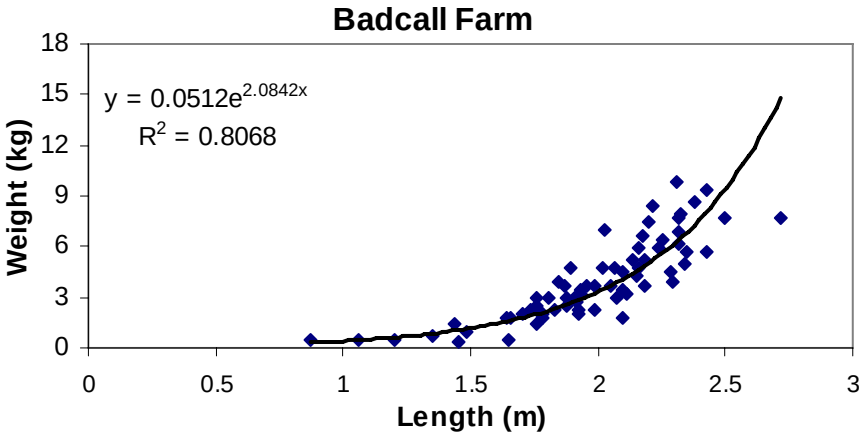


Figure 4.5 a) Mean length of the five longest plants as a predictor of bundle weight, Badcall Farm, 2005.

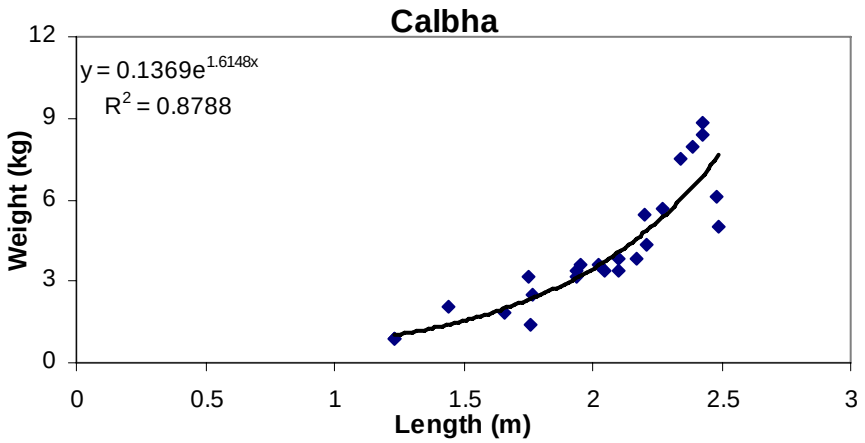


Figure 4.5 b) Mean length of the five longest *Laminaria saccharina* plants as a predictor of bundle weight, Calbha longline (growth period: 25/01/2005 – 22/06/2005).

Palmaria palmata harvesting

In the first week of December 2004, all seeded lines were harvested from the frames. Each line was photographed, and then divided into sections based on dominant organisms. These were: fine red algal epiphytes, mussels or *P. palmata*. The lengths of each of these sections was measured and weighed.

Statistical analysis

Data were grouped for frames on the basis of their distance from the fish cage groups and comparisons were made using analysis of variance (ANOVA) using General Linear Modelling (GLM). Data was tested for normality (Anderson-Darling test) and homogeneity of variance (Bartletts test). Data was transformed where required. Post hoc comparisons were done using a Tukey test. Statistics packages used were Minitab and JMP IN (SAS). On all graphs error bars are 95% confidence intervals unless otherwise specified.

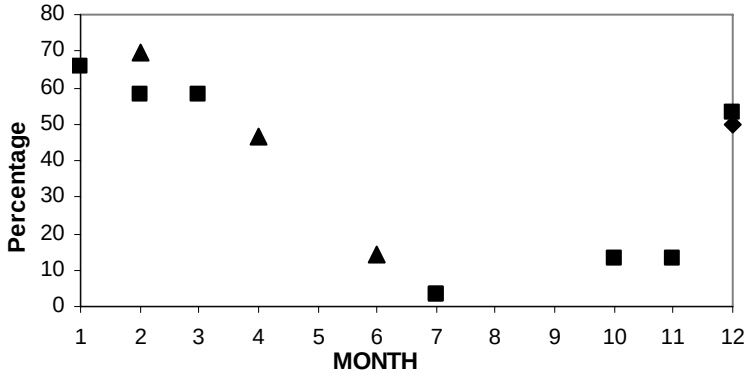
Results

Seasonality of spore release – *Palmaria palmata*.

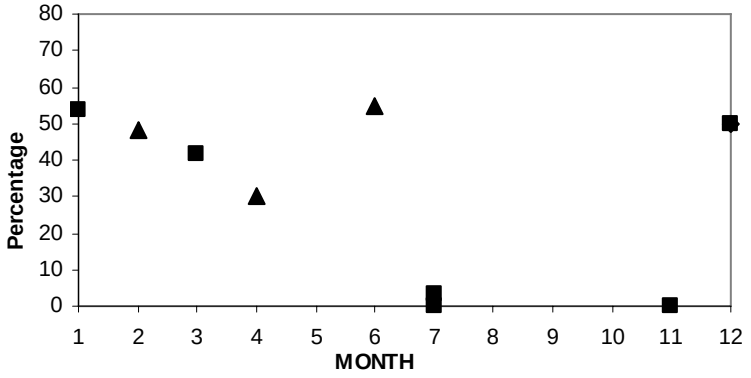
More than 50% of the 2003-2005 WEST site plants were tetrasporic from December through to April with a few being found to be tetrasporic as early as October (Figure 4.6). Seasonality of the intertidal BAY and EAST plants was less obvious. The EAST intertidal plants followed a similar pattern to the WEST except for an anomalously high point in June of 2005. The season for tetrasporic plants at the BAY site began later than the other two sites and extended later into the summer with tetraspores being found on these plants until at least July (2004 & 2005). Both of the eastern sites (EAST and

BAY) were sampled less frequently due to the more wave-exposed nature of the site resulting in fewer collections for the winter months in particular.

a) WEST intertidal site



b) EAST intertidal



c) BAY subtidal

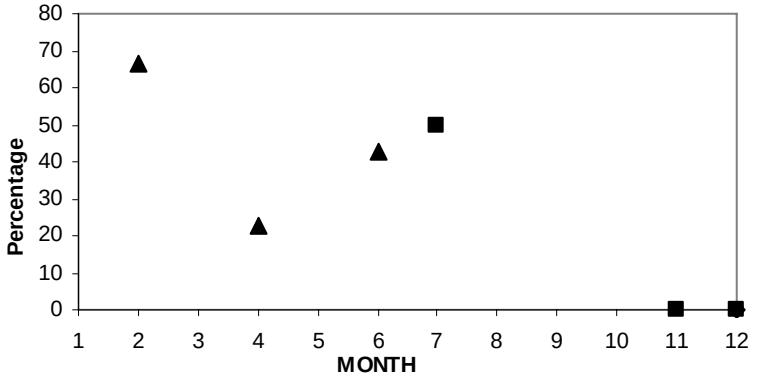


Figure 4.6 Percentage tetrasporic *Palmaria palmata* plants determined for three sites at Easdale, Isle of Seil**:** 2003: diamonds, 2004: squares and 2005: triangles. Numbers 1 to 12 are the months January to February.

Method comparison – *Palmaria palmata*

A synopsis of the cultured batches for *Palmaria palmata* is contained in Table 4.2. A successful culture was one that resulted in an even spread of plants over the substrate of at least 10 spore for each centimetre of string. Culture success varied between methods with the 'tumble' having the higher proportion of successes. The 'free' method in particular resulted in higher epiphytism rates, more variable settlement and occasional batches where there were no germinating spores for without any obvious reason. There appears to be little difference in spore output per amount of plant material between the different methodologies for a given month. However, there appears to be a greater output for months February to March when compared with November to January.

East versus west side of peninsula

For the batch initiated 4/3/2004, source plants were collected from two sites, one each side of the small peninsula at Easdale and eastern (EAST) and western (WEST) spore survival contrasted. Both lots were treated similarly however all the WEST cultures failed within two weeks. This indicates possible high variability in spore survival even from plants sourced from within 50 m sections of the coast.

Contaminants

Algal contaminants in the cultures were common, more so with the 'free' method. First colonisers were diatoms. Later colonisers were green algae including *Enteromorpha* spp (*Ulva*), a small flagellate green alga and *Cladophora* spp. A tubular small brown alga was occasionally seen as well as the results of cross contamination from *Laminaria saccharina* cultures. Two filamentous red species were observed and tentatively identified as *Ceramium* spp and *Polysiphonia* spp. Diatom contamination was controlled through the addition of GeO_2 and other contaminants were controlled by

limiting the addition of nutrients.

Table 4.2 Synopsis of culture details for algae cultured 2003- 2005**.**

Run No.	Culture initiated	Type	Thallus Area (cm^2)	Wet wt (g)	Area / bit (cm^2)	Wet / Dry wt ratio	Spores / area thallus (cm^2)	Spores / wet wt (g)	Successful
1	23-Dec-03	Shock	599.3	22.5	7.5		73.7	1965	No
2	09-Jan-04	Free	630.0	23.6	8.2				Yes
		Shock	1321.5	49.6	11.3				Yes
3.1	15-Jan-04	Free	523.5	19.6	7.8	10.8	93.9	1875	No
3.2		Free	462.8	17.4	8.4	10.5	41.9	938	No
3.3		Free	628.4	23.6	7.5	9.3	64.2	1625	No
		Shock	1196.0	44.9	7.8	8.4	135.2	3831	No
6-E1	04-Mar-04	Free	238.0	8.9	6.4	10.6	673.6	13136	Yes
6-E2		Free	273.1	10.2	4.6	9.9	351.5	10442	Yes
6-E3		Free	350.8	13.2	4.9	9.8	405.9	12140	Yes
6-W1		Free	424.5	15.9	10.1	8.3	290.4	8638	No
6-W2		Free	430.4	16.1	9.2	8.5	187.4	5321	No
6-W3		Free	515.0	19.3	18.4	7.5	177.1	5727	No
2	03-Nov-04	Free	2854	107			26.9	714	Yes,2 out of 3 tanks
2	09-Dec-05	Tumble	12829	481			39.6	1058	Yes
3	11-Jan-05	Tumble	16884	633			168.1	4485	Yes
4	11-Feb-05	Tumble	12589	472			266.1	7098	Yes
			Max				673	13136	
			Min				41	938	
			Mean		8	9	226	5266	

Laminaria saccharina culture results

Cultures of *Laminaria saccharina* were always successful; in fact, there were problems with the alga being too successful. On occasion, *P. palmata* cultures were contaminated by *L. saccharina* cultures. *Laminaria saccharina* cultures proved to be very robust, withstanding adverse environmental conditions such as being transferred by car to and from Loch Duart and extended times in culture without maintenance. The cultures were also resistant to overgrowth by incidental epiphyte contamination.

Palmaria palmata growth on site

Batches of *P. palmata* grew best from April to July with maximum mean RGRs of .025-

.03 cm cm^{-1} day^{-1} (Figure 4.7, Table 4.3) Across all batches, position relative to the cages made little difference to growth rates (Figure 4.8, Table 4.4). However, results for individual batches shows that while this appears to be the case for the February outplants, for the March and May outplants, the plants farthest away from the farm cages appear to grow better than plants grown close to the cages (Figure 4.9).

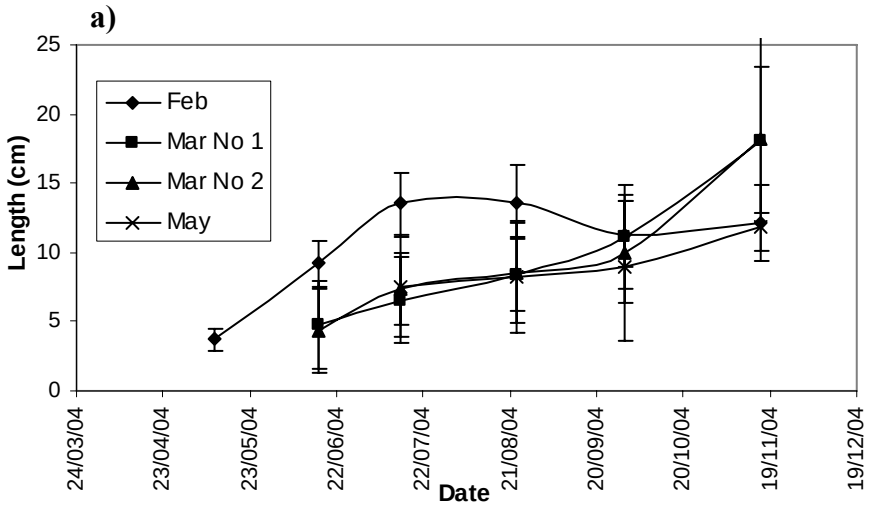


Figure 4.7 a) Change in length for *Palmaria palmata* batches deployed at Calbha in 2004 (vertical bars indicate 95% CI).

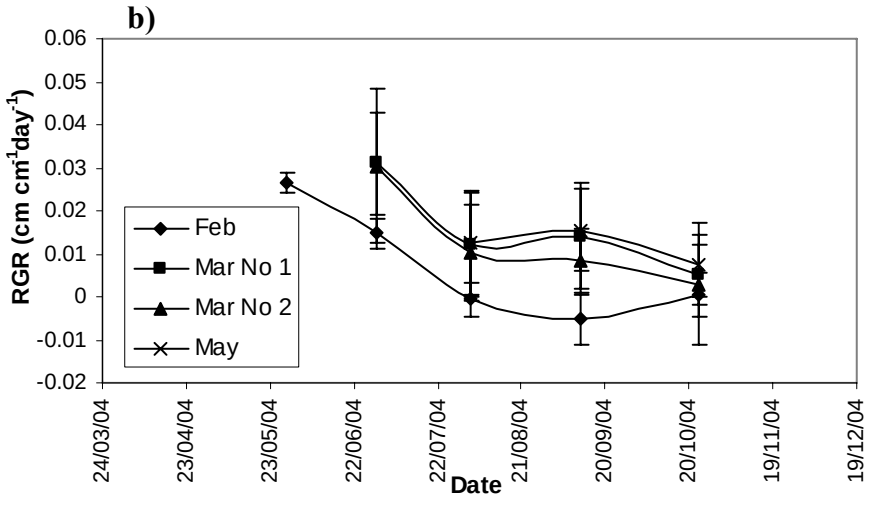


Figure 4.7 b) Change in RGRs for *Palmaria palmata* batches deployed at Calbha in 2004 (vertical bars indicate 95% CI).

Table 4.3 Number of measured lines used for data presentation in the graphs in Figure 4.7.

Date	Feb	Mar No 1	Mar No 2	May
11/5/04	**19**			
16/6/04	**20**	**6**	**5**	
14/7/04	**20**	**11**	**7**	**4**
23/8/04	**19**	**13**	**13**	**11**
30/9/04	**18**	**13**	**12**	**8**
16/11/04	**11**	**8**	**5**	**6**

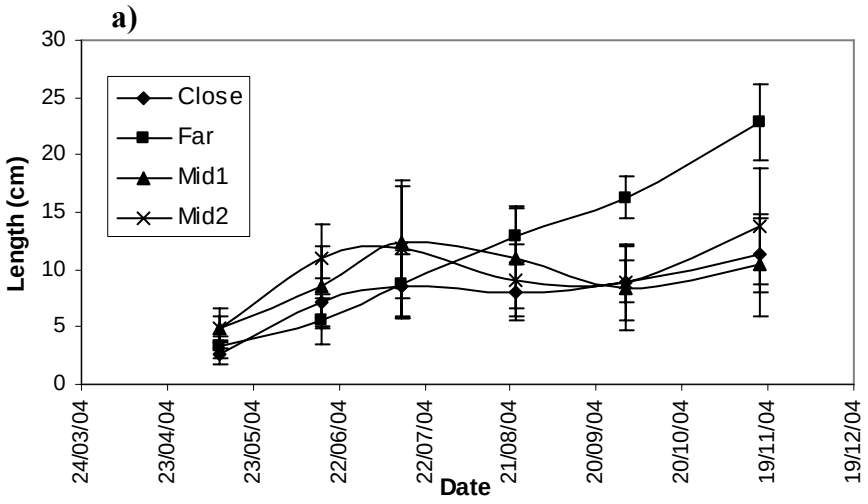


Figure 4.8 a) Change in length for *Palmaria palmata* deployed at Calbha in 2004 at varying distances from fish cages (vertical bars indicate 95% CI).

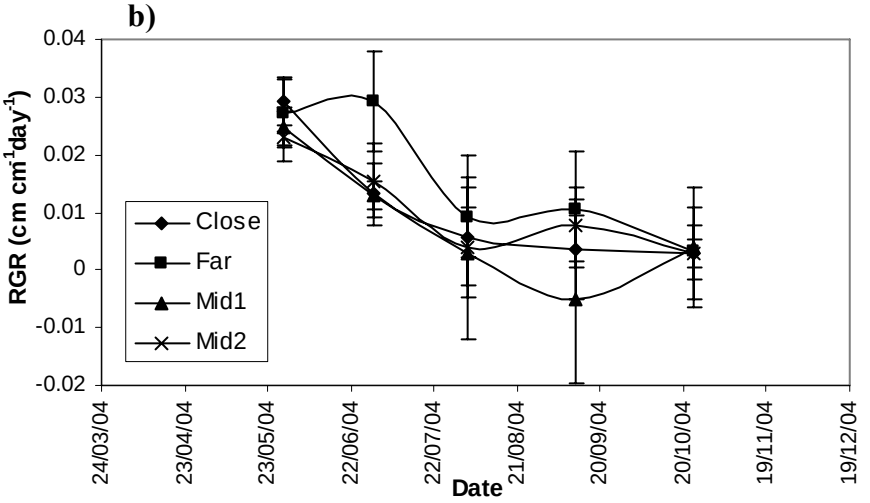


Figure 4.8 b) Change in RGRs for *Palmaria palmata* deployed at Calbha in 2004 at varying distances from fish cages (vertical bars indicate 95% CI).

Table 4.4 Number of measured lines used for data presentation in the graphs in Figure 4.8.

Date	Close	Far	Mid1	Mid2
11/5/04	7	4	5	4
16/6/04	9	11	8	4
14/7/04	12	15	8	6
23/8/04	18	17	10	8
30/9/04	18	12	8	10
16/11/04	12	8	6	3

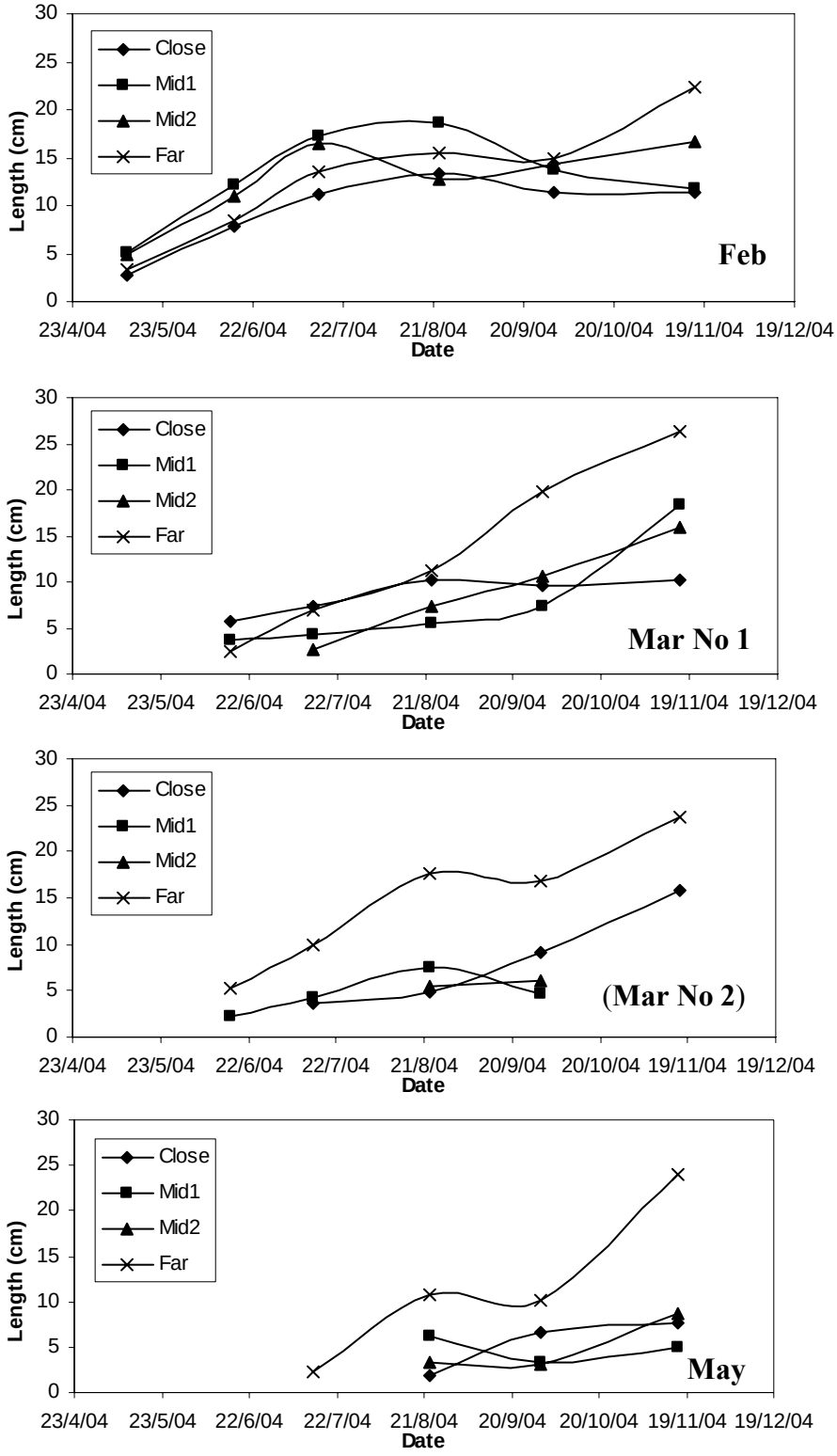


Figure 4.9 Mean of the mean five plant lengths for *Palmaria palmata* frames grouped by distance for each batch. Each point represents measurements for 1 to 8 frames with an average of 3.5 for May, June, July and August, 3 for September and 2 for November.

Palmaria palmata string harvest

Two way ANOVA, unbalanced, for factors of batch and distance on the total harvest weight of strings from the frames in November 2004 shows highly significant results for batch and distance with a non significant interaction. Tukey post hoc tests show the final batch (**May**) to be significantly lighter than the first (**Feb**) and the **Mid1** strings to be significantly lighter than strings from the other three sites (see table 4.5). When analysed for component organisms, ANOVA for fine red algae and mussel weights reflected these results. Differences for *P. palmata* on its own with distance were not significant.

Table 4.5 Results of two way analysis of variance for harvested seeded strings. Superscript letters indicate results that are significantly different. Results that share the same letter are not significantly different Two way ANOVA: Batch $F_{(3, 64)} = 5.78$, $p = .001$, Distance $F_{(3, 64)} = 12.74$, $p = <.0001$ Interaction non significant. .

Batch	**Mean Wt (g)**
Feb	166.91 [a]
Mar No 1	108.74 [b]
Mar No 2	111.36 [b]
May	68.06 [b]
Where	**Mean Wt (g)**
Close	163.45 [a]
Mid1	38.15 [b]
Mid2	114.35 [ab]
Far	139.12 [ab]

Epiphyte cover

Total cover of epiphytes on the strings peaks in August and the strings that were in the vicinity of the cages had the most cover (see Figure 4.10). Cover declines after August to September with a slight rise for November. This pattern is consistent across batches however it is particularly accentuated for the February outplants.

Epiphyte species

Epiphytes species were determined principally from photographs which limited the level of discrimination. The principal groupings were 1) 'fine red algae' which included *Ceramium* spp and *Polysiphonia* spp, 2) '*Palmaria palmata*' and 3) 'mussels' believed to be *Mytilus edulis* but this grouping was a suite of animal species including encrusting bryozoans and an herbivorous snail species: *Lacuna vincta* (Montagu 1803). Other minor species included *Cladophora* spp, *Ulva* spp, *Enteromorpha* spp, fine filamentous browns and the occasional thallus brown.

Patterns across the sampling sessions and batches (Figures 4.11 & 4.12) showed early settlement, development and dominance by fine red algae, followed by *P. palmata* and then mussels from September onwards. Coincident with the establishment of the mussels was a decline in the fine red algae and *P. palmata*. The earlier outplanted strings (**Feb**) had a higher later incidence of mussels. Of the sites, the **Mid2** sites showed greatest incidence of mussels followed by the Farm site with the least at the **Mid1** sites. *Palmaria palmata* was most successful at the Far sites and for the earliest batch (February outplant).

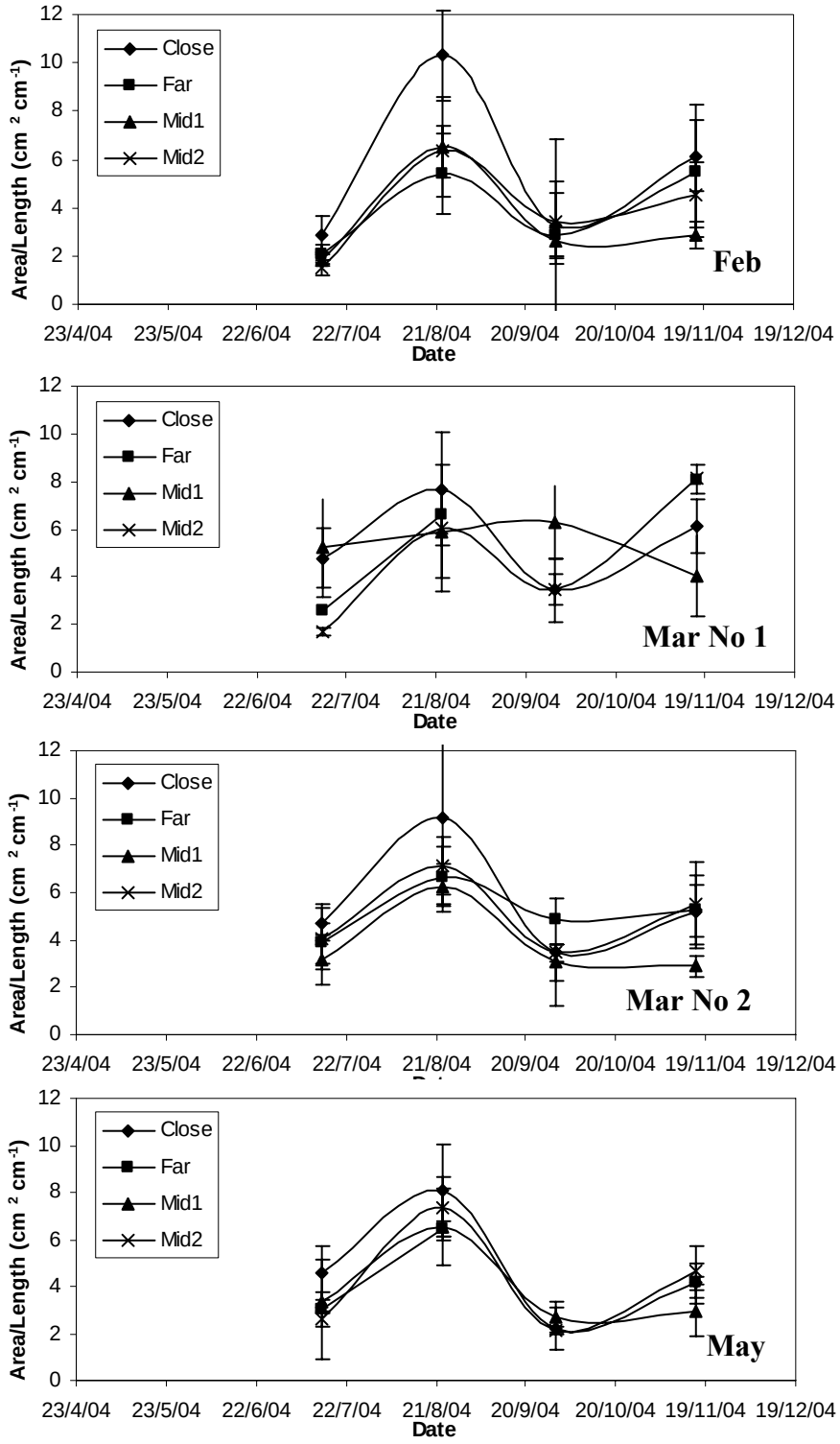


Figure 4.10 Epiphyte growth on culture strings by batch and distance from the cages as measured by area of epiphytes per length of string (vertical bars indicate 95% CI).

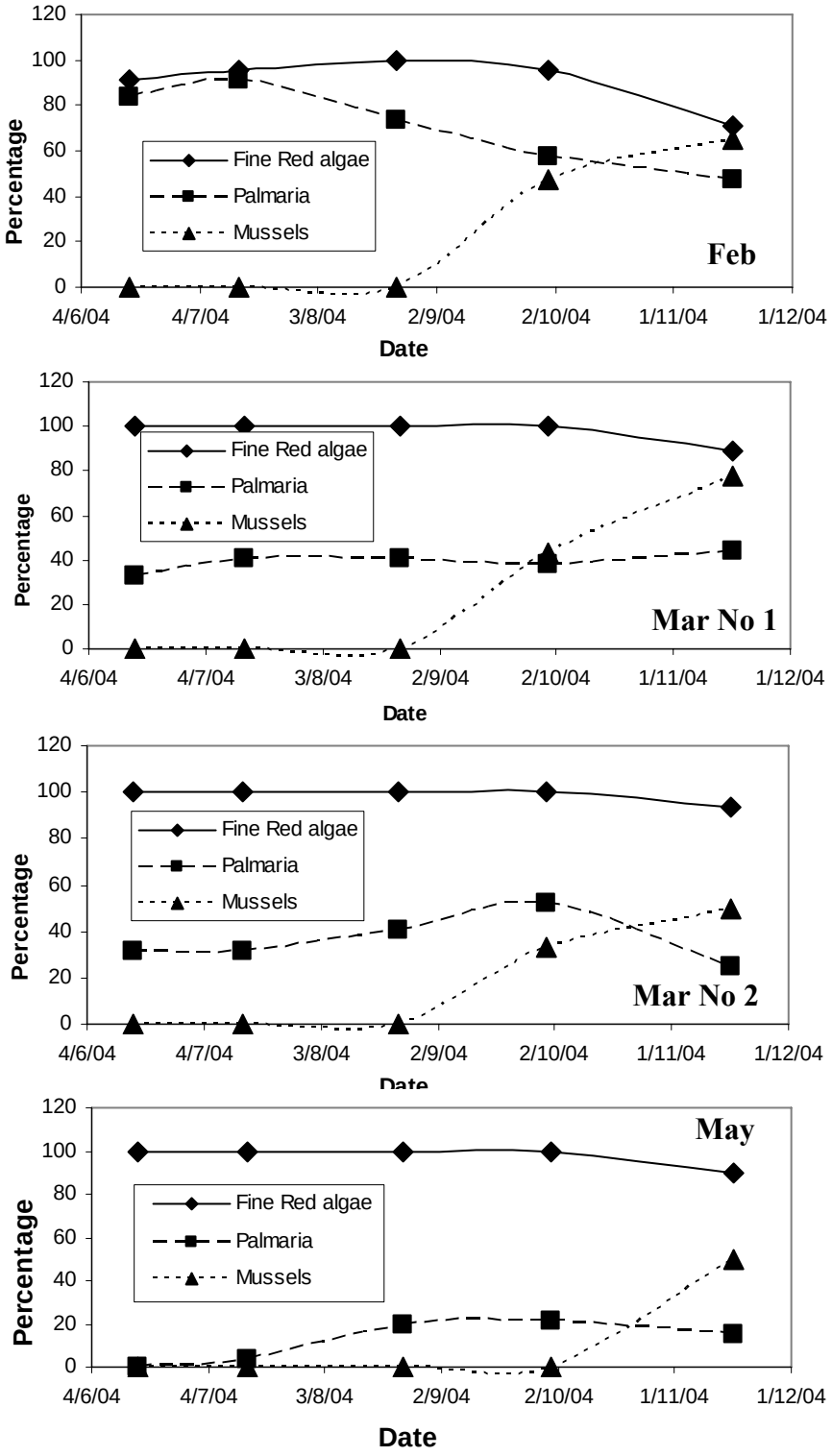


Figure 4.11 Percent incidence of seeded strings with the epiphytes: fine red algae, *Palmaria palmata* and mussels for each batch.

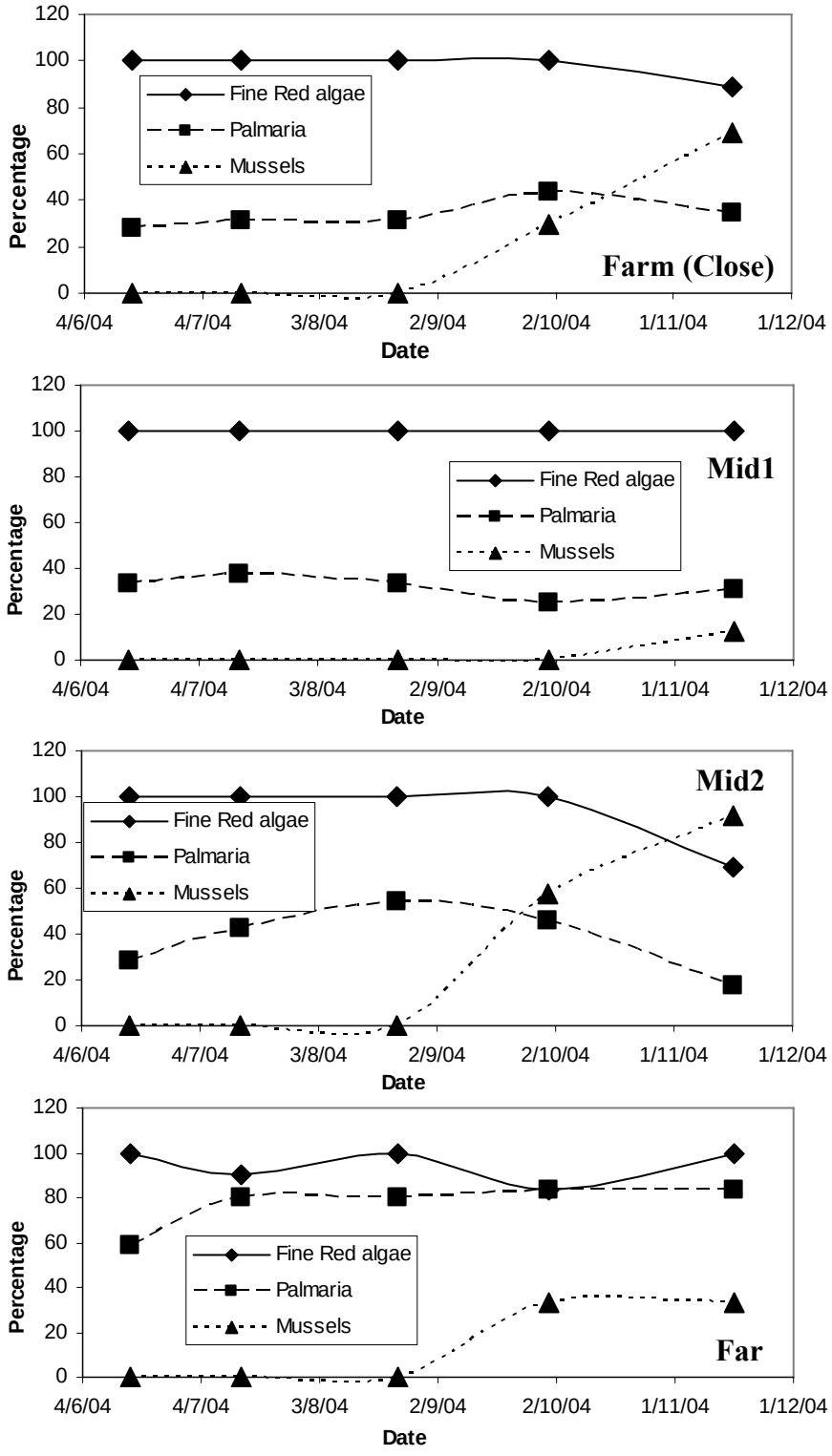


Figure 4.12 Percent incidence of seeded strings with the epiphytes**:** fine red algae, *Palmaria palmata* and mussels with distance from the farm cages.

***Laminaria saccharina* growth**

Mean lengths *Laminaria saccharina* for frames as measured at harvest at Calbha on 15/6/04 were converted to frond weights as per the length-weight relationship (Figure 4.13) determined for cultured *L. saccharina* fronds in the following years harvest (Badcall harvest, June 2005, see chapter 5). This makes it possible to estimate weight differences for *L. saccharina* fronds with distance from farm cages (Figure 4.14) which would be directly related to potential crop harvest differences. Mean maximal weight for fronds grown 500 m from the farm cages is 42.3 ±10.1 g (s.e., n = 3) which compares to a value of 90.8 ± 0.8 g (s.e., n = 17) for *L. saccharina* grown within 250 m of the cages i.e. an increase of 215 %.

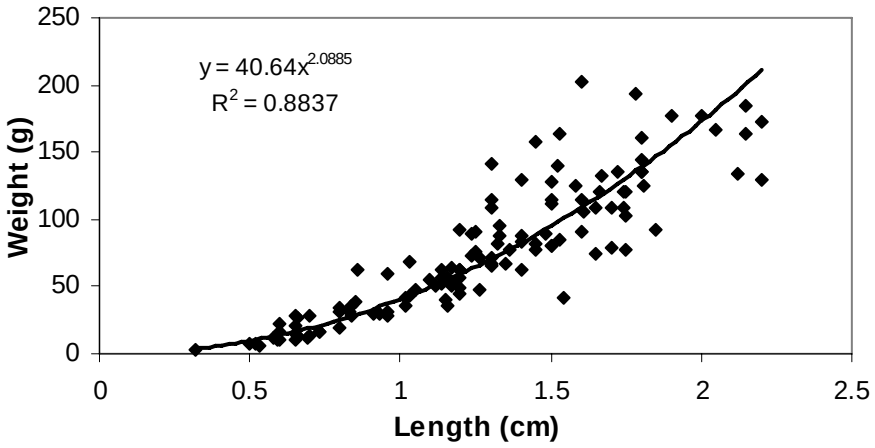


Figure 4.13 Graph showing the relationship between blade length and weight for *Laminaria saccharina* for June 2005 harvested plants at Badcall Bay.

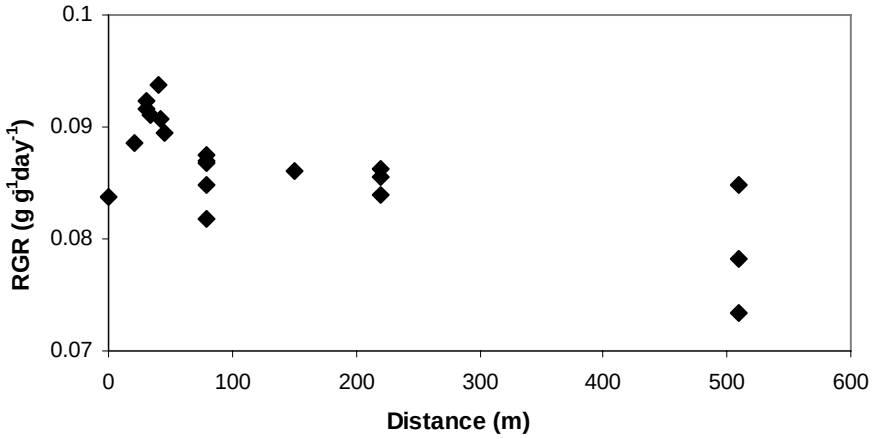


Figure 4.14 Relative Growth Rates for *Laminaria saccharina*(19/2/2004 to 15/6/2004) based on weights of the five longest plants of frames against distance of the frame from the fish farm cages. Lengths converted to weights based on length / weight conversion obtained at June 2005 harvest, see chapter 5 for details:

$$Lam_{wt} = 40.64 * Lam_{length}^{2.0885}.$$

Lam_{wt} = *Laminaria saccharina* blade weight.
Lam_{length}.= *Laminaria saccharina* blade length.

Discussion

Palmaria palmata culture

Mass cultivation of *P. palmata* will be dependent on having efficient means of cultivating the alga at the necessary scales. Some estimates of farm production using the string and longline method require upwards of 20 kilometers of string to be seeded per hectare (Browne pers com; 66 x 7m 'droppers' per longline, 40 longlines per hectare). Current methods seed string sections of 20 to 100m. For commercial realization, techniques will have to be scaled up two orders of magnitude. The trials of the three differing techniques as part of this project show that the tumble method has the greatest potential for seeding large quantities of substrate. It has the advantages of:

- More even distribution of spores on culture surfaces,
- low labour requirements,
- the ability to determine the density of spores on the substrate giving a more even distribution,

- more control over the quality of the spores through being able to 'wash' them before introduction to substrate media,
- less stress on the spores themselves as there is no 'shock' incentive to spore release potentially giving greater survival,
- greater control over epiphytes by minimizing the chances of their introduction in the first instance and
- the ability to obtain larger quantities of spores (through continual spore release) and thus seed more area.

For the 'tumble' method of spore release, while plant fragments were tumbled for three days and spores collected over this period, there would appear to be no reason why the cultures could not be maintained indefinitely, keeping conditions optimal for growth of 'brood' stock plants so that the spores could be continually produced and released for as long as possible.

The more even distribution of spores over culture surfaces using the 'tumble' and 'shock' method arises because the spores are non-motile. With these methods, the spores are evenly distributed in the culture solution when added to the tanks resulting in an even distribution throughout the tank and on the substrate as they settle. With the 'free' method, spores fall directly from fronds on to the substrate resulting in a very patchy distribution.

A study on the reproductive periodicity of *P. palmata* in the UK on the Isle of Man by Kain (1982) found most plants were reproductive during the first five months of the year, with about half the plants bearing tetraspores and most of the remainder bearing spermatia after January. All were sterile from August to October. All plants sampled by Kain were epiphytic, inhabiting the distal end of *Laminaria* stipes. Kain (1982; 1989), in a comparison of fertility of nine species of red algae, found differences in reproductive status within a species depending on position on the shore and depth but she believed a lot of the differences were attributable to the size and the age of the plants.

In this study, a small proportion of plants of *P. palmata* were tetrasporic in October and this is in agreement with the study by Le Gall *et al.* (2003) from field observations on the presence of fertile tetrasporophytes made from October to May along Brittany and Normandy coasts. However, mature tetrasporophytic fronds of *P. palmata* were found earlier at Roscoff (October) than further East in the English Channel (January), corresponding to earlier occurrence of lower temperatures in late autumn at Roscoff. Kain's studies were based approximately 180 km south of Oban and the later season there may be due to warmer waters in this area compared to Oban.

Pang and Lüning (2004; 2006) attribute tetraspore initiation to short days and low temperatures indicating that these together may function as an "early warning system" as in many other seaweed species (Lüning 1990; Dring 1991), ensuring the new *P. palmata* male gametophytes and juvenile tetrasporophytes are present for vigorous vegetative growth in spring at optimum irradiance and daylength conditions. From a biogeographical view, Kain (1986) states that low temperature and short days as

possible triggers for reproduction in *P. palmata* may be a reflection of environmental conditions in the centre of the geographical range of this species, which is in the Arctic region.

The plant's growth cycle may also influence seasonality of reproduction. For a population of *P. palmata* in northern Spain, two distinct phases in the growth cycle were reported, a phase of active growth from March to August and a quiescent phase with predominance of frond breakage (negative elongation rates) from August to March (Martinez and Rico 2002; Faes and Viejo 2003).

This current study suggests variation in seasonality of spore production in *P. palmata* over short geographic distances of less than 50 m. The seasonal cycle for the subtidal plants was delayed by one to two months. This may reflect the lower light levels experienced by the predominantly subtidal algae and the lower variations in temperature due to immersion in seawater for much of the time. The results indicate that it may be possible to find reproductive plants (tetrasproric) for a much greater proportion of the year if all possible habitats are searched for fertile plants at times normally considered out of season, however the overall relative abundances will be a lot less. It may be possible to initiate cultures at times now considered as not possible using plants sourced from the field. If cultures can be initiated in autumn, the yield at harvest would be greater in the following summer before bleaching and fouling can occur. Future culturing of *P. palmata* should include investigations into the relative viability of spores collected through the year from differing habitats.

Pang and Lüning (2004; 2006) induced tetrasporic plants from sterile plant fragments within two months in the laboratory through manipulation of light (short days) and

temperature (10^{o}C). They also successfully maintained *P. palmata* in tank culture with high levels of production. These two factors could also be utilized to produce spores throughout the year by modifying culture conditions including temperature and light. While Pang and Lüning (2004; 2006) claim the principal factors are daylength and temperature in tetrasporic formation they did find some tetrasporic plants in June.

An attempt was made to induce tetraspory in plants as part of this project. October collected plants were maintained in 10 x 10 liter containers, each with a starter 10 g wet weight of material, at 10^{o}C, 100 μmol photons m^{-2} sec^{-1} and short days on full nutrients for three months from 8/10/04 to 7/1/05. Relative growth rates were recorded of 0.05 cm^{2} cm^{-2} day^{-1} however no significant levels of tetraspory were achieved during this period. The findings here are fairly inconclusive but indicate that more work needs to be done to ensure tetrasporic production on demand. One possible influencing factor is the importance of thallus size (and age?) for reproduction (see Kain 1982, 1986).

Current models for *P. palmaria* production suggest a requirement for 20 km of seeded string per hectare (Browne 2001, pers com). This assumes droppers on the longlines at spacings of 15cm which may be unrealistically close due to the risk of tangling the lines. One possibility may be to seed nets, using similar methodology as that used for *Porphyra* cultivation in Asia (Sahoo and Yarish 2005). Another shortfall in the current cultivation system is the substrate used: Kuralon string. This is sourced from Korea which is not convenient. While Browne (2001) experimented with a number of possible substrates, the range of types have not yet been exhausted and white multifilament nylon twine as currently sourced commonly from ships chandlers and hardwares may be a possibility. *Porphyra* culture net which had been tried by Browne (2001) and apparently discounted on the basis of its colour (orange) not being suitable for seeing

developing spores should probably be revisited. The methodology used for seeding *Porphyra* nets in Asia should also be more closely examined to see if some of the technology can be adapted for culture of *P. palmata*.

Laminaria saccharina culture

Of the two algae, *L. saccharina* was by far the easier of the two to culture. Also, yield per cm of seeded string was greater and the seedlings were very robust all reflective of this alga's 'weed' like growth strategy. In the field, ten centimeter sections of seeded string were inserted within the strands of 10 mm polypropylene droppers. Each of these 10 cm sections of string gave rise to more than 10 kg of *L. saccharina* (see also chapter 5). This contrasts with *P. palmata* which gave rise to a maximum 100 g from similar lengths of string.

The spores of *L. saccharina* are motile which means that spores of *L. saccharina* can more readily attach to vertical surfaces. *Palmaria palmata* spores in contrast are not motile, so seeding is restricted largely to horizontal surfaces. Seeding with *L. saccharina* can include string that is wound on to cylinders which can sit vertically in the inoculating media thus reducing the area in tanks taken up by substrate.

Palmaria palmata outplants

The best results were obtained for the February outplants of *P. palmata*. This follows logically as the plants were deployed earlier in the growth season and thus had a greater time period to establish. The greater proportion of frames for this outplant with successful *P. palmata* may be a reflection of plants establishing before epiphytes are prevalent. Progressively later batches had a proportion of frames with *P. palmata* but

this may also be reflective of decreasing optimal growth conditions with warmer waters and decreasing available ambient nutrients (see chapter 1).

Growth of the smaller *P. palmata* plants for the February outplant and later appeared best away from the cages which is not expected if the nutrients near the cages are enhancing growth. However, once the plants are greater than 10 cm in length, they appeared to grow well wherever they are. This may be a reflection of increased competition with epiphytes and/or particulate matter on the lines when they are less than 10 cm.

The reduction in growth for most batches for August-September may be partly due to low ambient nutrients however some bleaching of the plants was observed early in this period. Bleached plants often die off with individuals that were large and mature in July senescing from the tips in September and November. August-September is also the time when epiphytic animals such as mussels start to become obvious and smother the plants and this continues up until December. Settlement of herbivorous snails is also likely to be an influencing factor.

The '**Far**' sites were subject to greater wave exposure than the rest of the sites thus potentially confounding influencing factors. Farm cages are sited in wave sheltered areas, and most of the '**Far**' sites were in more wave exposed locations. Changes in nutrient concentration with distance were thus not the only variable that was differing with the more distant sites. Wave action may serve to keep the strings relatively clear of epiphytes. The southern 'Far' sites however, which were more protected from wave action showed similar patterns in success and growth of *Palmaria palmata* to the northern 'Far' more protected sites indicating this may not be that significant a factor.

There appeared little difference in results for the N. Ireland and SAMS cultured *P. palmata* (**Mar No 1** & **Mar No 2**). The N. Ireland plants were slightly more successful in terms of the number of frames that yielded *P. palmata* but growth rates were similar. A visual comparison of plants showed the fronds of the N. Ireland plants to be less dissected with longer internodal lengths.

Laminaria saccharina outplant

Laminaria saccharina plants grown close to the cages (<250 m) were more than twice as heavy as plants grown away from the cages at harvest suggesting a benefit from being grown in the proximity of the cages. Some inconclusive growth rate comparisons (relative growth rate values: RGRs) comparing growth rates close versus those at distance from the cages suggest varying results (not presented here), with growth rates being favoured either close or at distance from the cages for time periods of one-two months. The harvest of plants after the same time interval suggests that integrated effects are for greater growth and productivity closer to the cages. Plants in the second crop did not grow well after August-September and often had epiphytic animals such as the bryozoan *Membranipora* sp. Plants from the original crop on droplines when harvested in December had only stipes remaining, the rest having apparently been eaten.

Summary

The earlier in the season that the seeded lines are put in to the water the greater yield of *P. palmata* and *L. saccharina* before they become susceptible to swamping by epiphytic animal species in the following summer. For both plants, outplants could go into the water as early as September when the seasonal settlement of larval animals from the previous season is likely to have finished. This correlates well with the timing of

Japanese ‘forced’ cultivation of the biennial crop *Laminaria japonica*. This plant normally matures over two years when initiated in winter which is when it is normally reproductively mature. By manipulating the life cycle, the plant is made reproductive in early autumn and the alga then completes its life cycle by the following summer resulting in larger yields of the alga in a shorter time. This way also, the plants are very well established by the time algal epiphytes are prevalent in early spring.

Cultures of *P. palmata* may be initiated out of the currently accepted season by sourcing ‘brood stock’ plants naturally from micro habitats that support conditions suitable for later tetraspore development such as dark cool areas.

Cultures of *P. palmata* may also be initiated out of the currently accepted season by artificially manipulating their reproductive cycle in the laboratory or hatcheries. Potentially, plants seeded in one season could be held over to the following season by slowing their growth or by freezing such as is done for *Porphya* culture in Japan.

The ‘tumble’ method of spore release for *P. palmata* currently appears to have the best potential for seeding lines in the quantities required for commercialization.

Investigations should be initiated into seeding nets. These have better potential for providing commercial quantities of *P. palmata*.

For this trial, while the growth of *L. saccharina* appears to be enhanced by growing close to salmon farm cages, the effect, if present at all, was not as evident for *P. palmata*. On the contrary, early development of *P. palmata* may be inhibited. This may be through competition with epiphytes or particulate matter on the lines.

Declining ambient nutrients in late spring, with lengthening days and conditions of still clear weather can result in bleaching of *P. palmata*. This results in lower quality crops and can lead to loss of plants. The bleaching is caused by UV damage to the plants or prolonged nutrient depletion or a combination of both. Bleaching of *P. palmata* was first noted in these trials in June. For optimal quality *P. palmata*, harvesting is best conducted before bleaching occurs unless bleaching can be prevented e.g. by lowering the crops in the water column.

Settlement of epiphytes and nutrient depletion is exacerbated in areas of low water motion. Growth and condition of cultured algae, particularly *P. palmata* is likely to be improved in areas of moderate water movement. This may be in areas of tidal currents or exposure to swell wave action. In Calbha Bay proper, water motion was relatively low (see chapter 2).

Harvest of *P. palmata* and *L. saccharina* is best done before the establishment of the epiphytes. Epiphytic animals include mussels and bryozoans which cover and swamp the algae resulting in decreasing growth and even loss of plants. The epiphytes also mar the quality of the harvested algae. The epiphytes become obvious in July.

If *P. palmata* settlement densities are high, epiphytes are likely to be limited and/or prevented from establishing. Nutrients from the salmon cages are likely to enhance growth of both fine filamentous algae and cultured seaweeds. By seeding lines with dense numbers of the desired plants, epiphytes have no space in which to become established.

CHAPTER 5

YIELD OF *PALMARIA PALMATA* AND *LAMINARIA SACCHARINA* ADJACENT TO FISH FARM CAGES.

Introduction

The aim of this section was to determine the effect of proximity to fish farm cages on yield of cultured macroalgae, specifically *Palmaria palmata* and *Laminaria saccharina*, over a growth cycle. Water motion has been identified as a factor affecting macroalgal production (Hurd 2000) and possibly a confounding factor for *P. palmata* grown in 2004 at Calbha (Chapter 4), so this factor was also investigated for these two species.

Results of the previous season's culturing at Loch Duart Ltd. indicated that, for *P. palmata* at Calbha, the earlier in the growing season the alga was deployed at the farm site, the more likely was the success for the alga's ongoing growth and yield (see chapter 4). A growing season was considered to be from early winter to early summer. Sporelings put out later than February were found to be inhibited in their early development, and more likely to be over-grown with epiphytes. If the alga can be deployed in the winter or even earlier, the alga appears to be more able to compete with epiphytes such as other algae.

The previous season's cultured *P. palmata* suffered from bleaching in June. The bleaching coincided with extended periods of clear skies and calm seas and is likely to be caused by either high light (Hanelt and Nultsch 1995; Sagert and Schubert 1995; Cordi *et al.* 1997) or nutrient deprivation, or a combination of both (Harrison and Hurd 2001). Often the affected algae did not recover, died back from the tips and were

subsequently more susceptible to enhanced settlement by epibionts. Bleaching of the alga is undesirable as is believed to contribute to a lowering of the customers perception in quality thus affecting its commercial value.

Both *P. palmata* and *L. saccharina* that were outplanted in late winter or early spring were, by November, covered by epibionts such as mussels, bryozoans and herbivorous snails (chapter 4). For optimal quality of harvested *P. palmata* and *L. saccharina*, the algae must be out of the water by July or even June. Multiple harvests of either alga do not appear to be a possibility if the algae are to be left in the water later than June, due to loss in quality and settlement by other organisms.

These results may be particular to the less wave-exposed areas of this part of the coast, such as where the salmon cages are sited, but these are the conditions under which the algae must be grown if they are to benefit from salmon farm-derived nutrients. *Palmaria palmata* in particular is normally found in wave-exposed rocky shore areas and thus is able to withstand, and is likely to thrive, in high energy areas. Under conditions of exposure to wave action or high currents, *P. palmata* may be resistant to epiphytes.

For this part of the project a growth cycle was considered to be from when the sporelings are put into the ocean, before February, to when they are harvested before July.

The principal interest is in growing commercial amounts of these algae. In order to incorporate variation in yield over a growth cycle, a number of sites both close to and far from farm cages was required. Culturing at a commercial scale requires longlines

and setting up enough of these to rigorously test for yield differences was beyond the resources of this project. Also, culturing these quantities of algae without guaranteed markets is difficult to justify, so a proxy was developed. The proxy was the use of buoyed frames that supported smaller numbers of seeded lines. These could be deployed more easily than longlines and enabled a number of sites to be set up away from the cages (Figures 5.1 & 5.2).

Three long lines were also established, two away from the cages and one adjacent to a farm cage group. Buoyed frames set up adjacent to these longlines would allow comparisons to tell us how representative these sites were of the area. The gauge of whether or not there were differences between yields of algae close to, and away from, the farm was based on the quantities of the algae harvested at the end of the growth cycle. Growth of algae both adjacent to and away from the farm cages was also monitored for shorter time periods. Chemical content of the algae, specifically nitrogen content was tested on the harvested algae to estimate how much nitrogen was absorbed by the algae (see chapter 7 for results). Water samples were taken at each of the sites to confirm differences in nutrient availability between each of the sites (see chapter 2 for results).

Exposure to water motion has been identified as a principal factor affecting the growth and production of macroalgae (Hurd 2000). Water motion can serve to break down boundary layers at the algal surface to facilitate nutrient uptake, but too much water motion can lead to breakage and loss of the plants. Water motion can also assist the alga in competing with epiphytes where the motion of the plants under the influence of water movement ‘sweeps’ the surrounding area clear of less structurally sound competitors.

Buoyed frames were put out at a number of sites of varying exposure to water motion to investigate the influence of this factor on culture of *P. palmata* and *L. saccharina*.

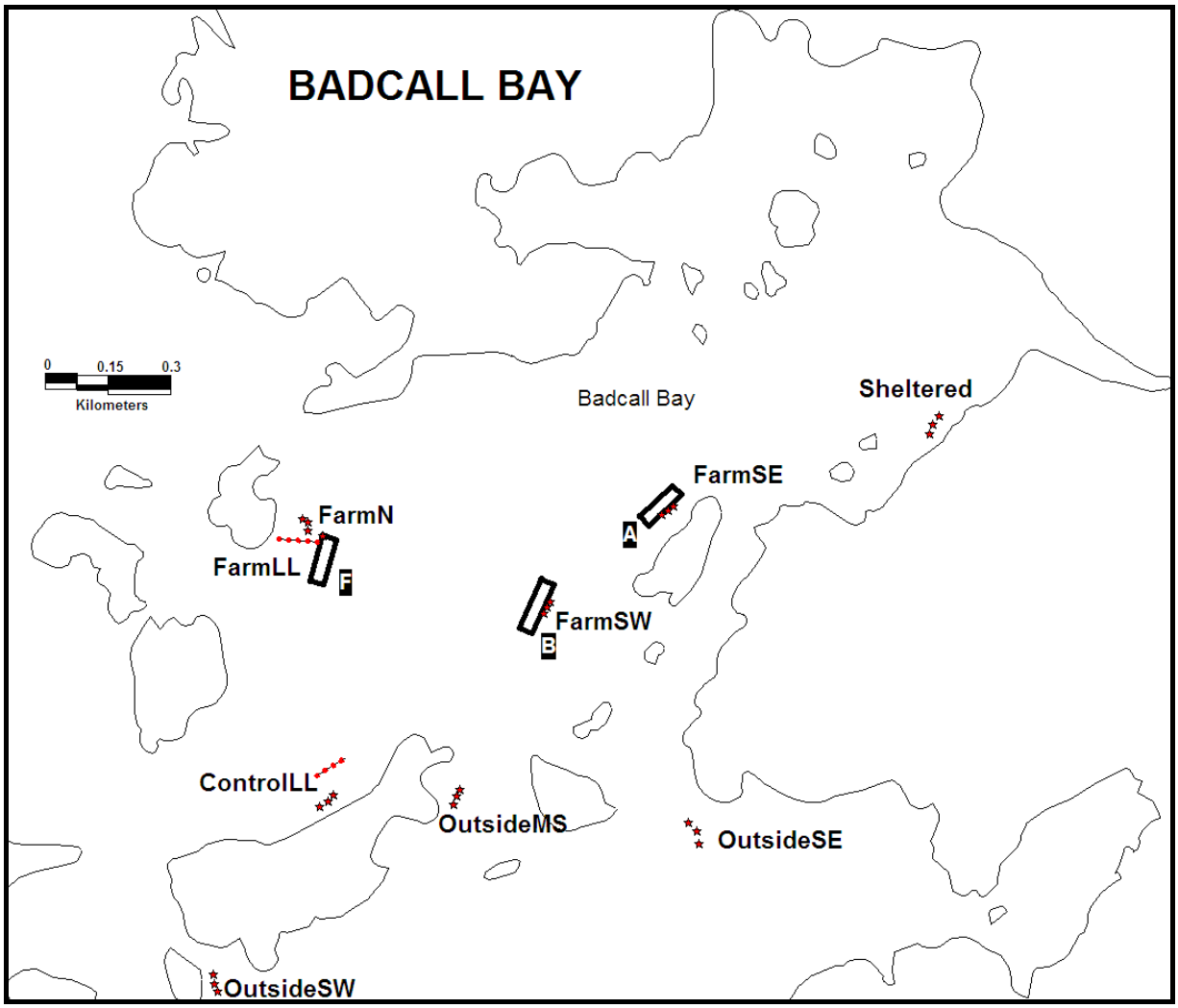


Figure 5.1 Map showing the location of the salmon cages at Badcall Bay (walkways A, B & F, with fish 2004/5), frames (small stars) and longlines (lines with circles).

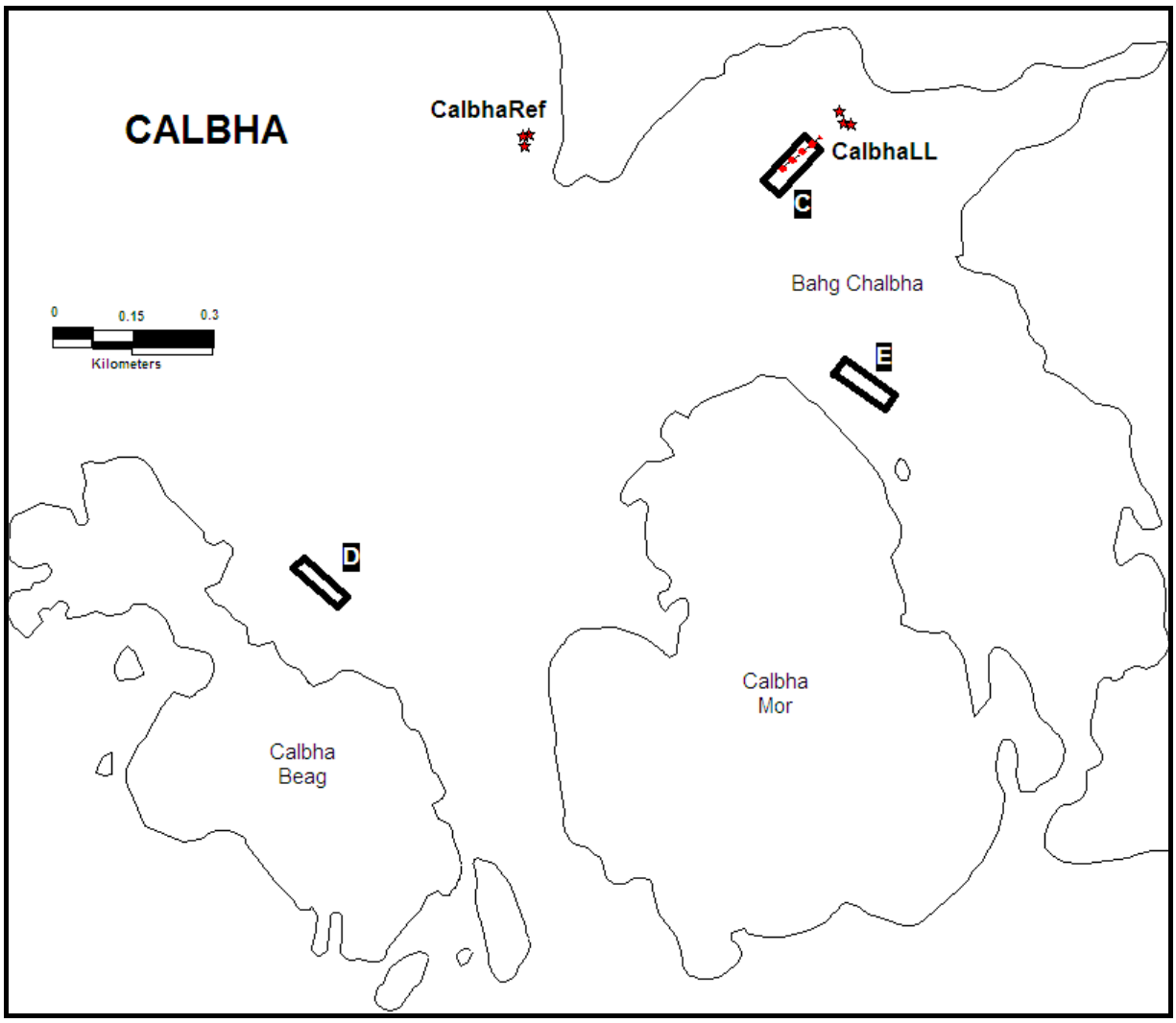


Figure 5.2 Map showing the location of the salmon cages at Calbha (walkways C, D & E, with fish 2003/4), frames (small stars) and longlines (lines with circles). Note longline located where cages had been the year before.

Methods

Frames

To obtain comparisons across multiple sites from a range of environmental conditions, frames to support *L. saccharina* and *P. palmata* (see Figure 5.3) were developed. The inner section of the frames supported up to four seeded strings (*P. palmata*) while two weighted short 80-cm lengths of 10-mm polypropylene ropes hung from the bottom side of the frame corners supporting the *L. saccharina*. A ten-cm section of *L. saccharina* seeded string was intertwined at each end of these 80-cm ropes (i.e. four seeded strings

per frame). Each seeded 10-cm length of string gave rise to a 'bundle' of mature *L. saccharina* plants. The strings seeded with *P. palmata* were at 2 m depth and the *L. saccharina* ropes were at 3 m depth. A weight was attached mid-water on the mooring line so that the line with the buoyed frame hung vertically and minimised the chances of the *L. saccharina* being abraded by the mooring line (see also Figure 4.2, chapter 4).

At Calbha and Badcall Bay, ten sites were established: three buoyed frames were deployed at each site to give a total of thirty frames. Three sites were adjacent to fish farm cages (at Badcall Bay: **FarmN**, **FarmSW** and **FarmSE**) and seven at other sites distant from fish farm cages (**Sheltered**, **OutsideSW**, **OutsideMS**, **OutsideSE** and **CalbhaRef**; see Figures 5.1 and 5.2) including adjacent to the two reference longlines (**CalbhaLL** and **BadcalLL**). The sites away from the farms were situated in areas of varying exposure to water motion in order to determine how this factor might influence plant yield. At the farm sites, the frames were deployed within 5 to 20 m of the fish farm cages. At FarmSE and FarmSW, the frames were 5–10 m from the southern side of the cages and at FarmN, the buoyed frames were 10-20m from the north eastern end of the cage group.

Laminaria saccharina frame harvest

The two rope sections, each with two bundles of *L. saccharina*, were harvested from each frame. Some bundles were estimated to have as many as 100 plants although commonly there were 20-50. Plants did not attach directly to the rope due to its smooth surface texture, but their haptera wound around the rope. This meant that the hold of the bundles on the rope became more challenged as the bundles grew, particularly in the more wave-exposed locations, and a few were lost. Each of the ropes, with attached *L. saccharina*, was weighed using a spring balance on the boat, and the presence and condition of the bundles noted. Some bundles appeared to have been abraded against the mooring rope (a few of the mid-water weights became detached). Three *L. saccharina* plants from each frame were analysed for nitrogen and carbon content (see chapter 7 for results).

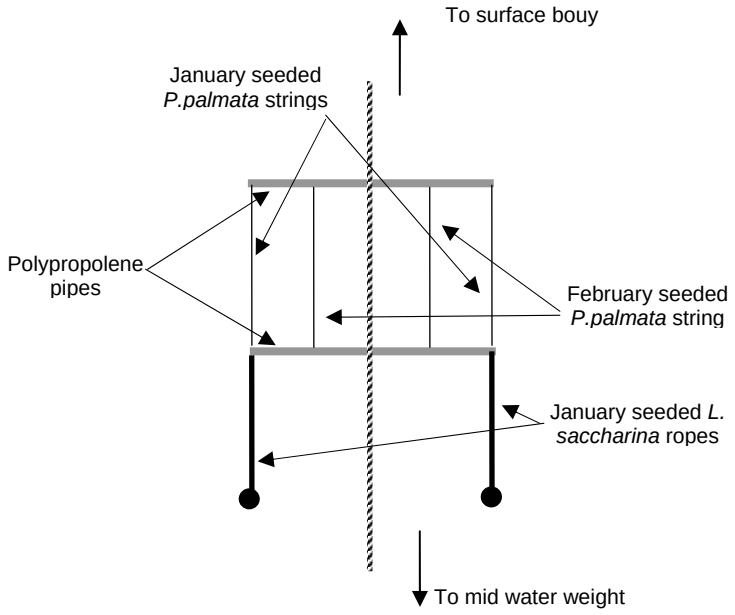


Figure 5.3 Construction of frames to support strings of *Palmaria palmata* and ropes with bundles of *L. saccharina*.

Palmaria palmata frames

Palmaria palmata seeded string was put onto the frames on 25/1/05 and 24/2/05 (two batches). On each occasion, two 80-cm sections of string were attached to each frame giving four strings in total (six per site per batch). The frames were 1.5 m wide, so the distance between the strings was approximately 37 cm. Plastic clips were attached to the frames to hold the lines for easy deployment and to take on and off for monitoring if necessary. Only batches with good even settlement were used on the frames and settlement was assumed to be consistent across all seeded strings.

Palmaria palmata frame harvest

At harvest, each of the four strings with attached algae from each frame was photographed and weighed. A sample of at least five plants from each frame was retained for analyses of nitrogen and carbon isotopes. Photographs of strings with attached algae were analysed using ImageJ (http://rsb.info.nih.gov/ij/) to determine the length of the five longest *P. palmata* plants, the length of line colonised by the plants and the surface area taken up by the plants as presented in the photograph.

A selection of 20 harvested lines was used to obtain relationships between photographic derived measurements and wet and dry weights. After weighing wet, the algae were dried in an oven at 90^{o}C until a constant weight was achieved and recorded (see chapter 4 for graphed results).

Palmaria palmata growth rate

Seeded lines from a subset of frames were photographed on 29 April 2005 (CalbhaRef, CalbhaLL, OutsideE, OutsideW, FarmSE and FarmSW). Adverse weather and time prevented photographing all frames. Photographs were analysed using the image analysis program ImageJ (http://rsb.info.nih.gov/ij/) to obtain the length of the five longest plants, the length of line colonised by plants and the area presented to the photograph taken up by the plants. Growth was compared between this sample period and at harvest (22/6/05) using differences in the length of the longest five plants on each string to calculate relative growth rates (RGR) where

$$RGR = \frac{\ln (L_i/L_0)}{\Delta t}$$

L_i = initial mean length (g)

L_0 = final mean length (g)

Δt = time (days)

Longlines

The surface section of the longlines consisted of 50 to 80 m lengths of 3 cm diameter polypropylene rope with 11-inch trawl floats every 10 m to ensure that the line stayed afloat after attachment of droppers and to make the line obvious to shipping to limit the chances of it being run over. The droppers consisted of either:

1/ *Palmaria palmata*: 1-6-m lengths of seeded *P. palmata* string (depending on the total length in the batch) with a weight at the bottom to ensure the droppers hung vertically and did not tangle; or

2/ *Laminaria saccharina*: Ten centimetre lengths of seeded string were intertwined into the lay of 7 m lengths of 10-mm three strand polypropylene rope also with a weight at the base to keep the line vertical.

The fish farm cage longline was attached to 'F' walkway at Badcall Bay (see Figure 5.1). Here, one end of the longline was attached to the fish farm cages and the other anchored to the adjacent coast approximately 60 m distant.

One reference longline was deployed at the head of Badcall Bay (BadcallLL, Figure 5.1) at least 500 m from the closest fish farm with a second reference longline at Calbha (CalbhaLL, see Figure 5.2). Calbha is approximately 3.5 km south of Badcall Bay. The Calbha longline was placed over a site where there had been salmon cages the year before. The two reference longlines consisted of 100+ m of 3-cm diameter polypropylene line with a 100+ kg anchor at each end. Two large cylindrical buoys approximately 1.5 m long by 0.9 m in diameter were attached at each end of the 50+ m long surface section of the longline.

***Laminaria saccharina* longline**

Culture of *L. saccharina* sporophytes was conducted as described in Chapter 4. Sporophytes of *L. saccharina* approximately 6 weeks of age and 0.5 mm in length seeded on to Kuralon string were outplanted into the field. The density of spores on the string was even and in the order of tens of plants per centimetre. Culture of *L. saccharina* was conducted in 10-litre rectangular containers. Each container produced 10 m of seeded string. The string was cut into 10-cm sections on site and then entwined into three-strand 10-mm polypropylene rope. This rope formed the 'droppers'. Each dropper was 7 m long and attached to the head-rope of the longline. Droppers were weighted at their base usually with a half a house brick to keep them vertical. One seeded 10-cm string was intertwined into the rope at depths of 1, 2, 3, 4 and 5 m depths on each dropper.

Droppers with *L. saccharina* were deployed twice in the 2004/5 growing season. A small number were put out on 23 December 2004 on the farm and Badcall reference longlines. The weather turned for the worse when deploying these plants with rain and gale force winds. This, or the time of the year they were put out, caused these lines to give poor results and are not considered further. The principal batch was deployed on all three longlines on 25/1/05. Three groups of five droppers were attached to each longline in order to provide sufficient replication to allow comparisons within longlines and between the farm and the reference sites. Droppers were 1 m apart and the groups were distributed evenly at least 10 m apart over the length of the line. Groups from the farm longline were termed Farm1, Farm2 and Farm3 numbering out from the cages.

***Laminaria saccharina* longline harvest**

For droppers on the longlines (as for the frames), numerous *L. saccharina* plants arose from each 10-cm section of seeded string forming bundles of plants. Each bundle was considered as an individual unit for site and depth comparisons. On the farm site, each bundle of *L. saccharina* was weighed separately using a spring balance. On the two reference longlines, each bundle was weighed separately for a random subset of five droppers. For each of the weighed bundles for the farm and Badcall longlines, the length of five of the longest plants was also measured. For the remaining droppers, total weight was obtained which included all bundles from each dropper.

Three plants were kept from 1, 2, 3, 4 & 5 m depth from each group of longlines to analyse for total nitrogen and carbon, and for nitrogen and carbon isotopes. Sampled plants were kept cool, and the following day in the laboratory, sub-samples were taken mid-lamina from a point approximately 10 cm above the stipe-blade intersection. These were freeze dried for later analyses. Plants from the farm and Badcall longline sites were used for length and weight measurements back at the laboratory. The data were

necessary for production estimates from length increment results (see chapter 4 for graphed results).

Palmaria palmata longlines

Palmaria palmata was seeded on string as described in chapter 4. To determine the optimal time for outplant of *P. palmata*, droppers consisting of weighted sections of seeded line were put out on three occasions from three separate plant batches (see Table 5.1). These were on 21/12/04, 25/1/05 and 24/2/05. On the first occasion, only the Badcall longlines were available. The seeded line (26 m) was divided up to give 7 droppers at BadcallLL and 6 at the FarmLL each approximately 2 m long. On the second and third occasions, the amount of available seeded line (approximately 150 m and 300 m) was divided to give three groups of five droppers on each of the three longlines. Each dropper was approximately 3 m long for the 25/1/05 outplant and approx 6 m long for the 24/2/05 outplant.

As the seeded lines were only 2 mm in diameter, they were not attached directly to the longline. Instead they were attached with clips to 1.5 m sections of 10-mm polypropylene ropes that were tied off on to the longlines. The top of the seeded string was set at 1 m depth because growth below this depth had been shown to be optimal by Browne (2001) and the results the tethered plants (see chapter 3). Droppers were weighted with circular net weights and were approximately 70 cm apart on the longlines. Groups of droppers were evenly distributed over the longline. The size of the *P. palmata* plants at time of outplant varied up to 3 mm in length. Plant densities on the lines varied depending on the success of settlement. One of the sub-batches (one of two put out on 25/1/05) had patchy settlement. Sub-batches were evenly distributed between sites and groups.

Palmaria palmata longline harvest

Because the *P. palmata* string droppers were of differing lengths, only the top metre was considered in detail for comparative purposes. This was cut off from the remainder, weighed and the number of plants estimated. The lengths of five of the longest plants from each string were measured. Where there was good growth on lower sections of the dropper, this was divided into metre lengths and each length was weighed, providing an estimation of weight variation with depth.

Representative plants were sampled from each metre, from each group, for analyses of total nitrogen, carbon and nitrogen and carbon isotopes (see chapter 7).

Table 5.1 Culture history for algae outplanted to longlines and frames at Loch Duart Ltd. 2004/5.

Source	Culture initiated		Date of Outplant	Days after initiation	Harvest date	Days at sea
	P. palmata	*L. saccharina*				
Seil	03/11/2004		21/12/2004	48		
Seil		03/11/2004	21/12/2004	48	15, 22/06/05	176, 183
Seil	09/12/2004		25/01/2005	47	15, 22/06/05	141, 148
Seil		09/12/2004	25/01/2005	47	15, 22/06/05	141, 148
Seil	11/01/2005		24/02/2005	44	15, 22/06/05	111, 118
Seil	11/02/2005		29/03/2005	44	15, 22/06/05	78, 85

Harvest Timing

Based on the previous year's experience (2004; see chapter 4), for optimal quality *P. palmata*, the crop must be harvested before the plants are bleached and before the establishment of epiphytic animal communities. After bleaching, most of the larger plants die off and are more subject to epiphyte colonisation. Bleaching occurs in early summer when there are prolonged periods of calm weather with clear skies. This was predicted to occur in late June. However in 2005, bleached plants were observed in late May and the decision was made to harvest all the seaweeds from the longlines and

frames as soon as possible after that date. All longlines were harvested between 15th & 17th June 2005 and frames on 22nd and 23rd June 2005.

Laminaria saccharina growth rates

A selection of *L. saccharina* plants was retained from the BadcallLL harvest for redeployment at sites adjacent to and at distances from the farm cages to look at short term growth rate differences during summer using the hole punch technique (Parke 1948). One hole was punched 10 cm above the stipe-blade intersection using a 5 mm hole punch. Four plants were attached to 2 frames to give 8 plants at each of the three Badcall farm cage groups and three 'away' sites (BadcallLL, Sheltered and OutsideMS). The frames on the cages were hung from the walkway on the south facing sides of the cages. Frames were put out on 30/6/05 and were reassessed on 23/8/05 after an interval of 54 days.

Environmental variables

The environmental variables of principal interest for seaweed culture were ammonium concentration and water motion. Other environmental variables measured were nitrate, nitrite and phosphate concentrations, water clarity and water movement. Nutrient concentrations were measured as detailed. Results were presented in chapter 2. Water motion differences were estimated using the dissolution rate of plaster of Paris cylinders (see below) and irradiance variation between the sites was determined using a Secchi disk. On two occasions, profiles of salinity and temperature were measured using a CTD (Conductivity-Temperature-Depth Instrument: Sea-Bird Electronics Inc. Bellevue WA USA: 19 CTD profiler, profiling mode, sampling rate 2Hz) and temperature data across the sites were obtained from the salmon farmer (see Appendix 1). Fish farm personnel measure temperatures at 5 m depth and Secchi depths at least twice a week at sites where fish farming is in operation.

Water movement

Relative water movement differences were estimated using a variation of the plaster clod method first used in marine plant ecology by Doty (1971) and most recently reviewed and modified by Jokiel and Morrissey (1993) and Porter *et al.* (2000). Dental plaster (JW Super Yellow, www.johnwinter.co.uk) was used and mixed at the rate of 2.9 kg of plaster to 900 ml of water. Polypropylene pipe was used for moulds to give cylindrical casts 44 mm in diameter, 50 mm high and weighing approximately 150 g. After casting, the cylinders were dried at 50^{o}C for 48 hours and then weighed. They were then attached to plastic bases with epoxy and reweighed before attachment to the frames in the field. After retrieval, the cylinders were dried at 50^{o}C for 48 hours and reweighed and loss of plaster calculated.

Plaster cylinders were used for water movement estimates twice, the first from 2/6/05 to 16/6/05 (14 days) and the second from 16/6/05 to 23/6/05 (7 days). Over the first period, the plaster was coated with marine varnish (polyurethane) on the sides to limit the dissolution rate. This was done as it was believed that a measure of the dissolution rate might be obtained from height measurements of the cylinders as they dissolved from the upper exposed surface. Also, painting the sides was believed to slow the dissolution rate, so that the cylinders could be left in the water (and thus integrate water motion) over a longer period of time. For this first session, two cylinders were attached on each frame (i.e. six replicates per site). All cylinders were facing upwards at the top of the frames (see Figure 5.4) to minimise the chances of the cylinders rubbing against the frame structure and to standardise their exposure to currents and water motion types.

Once on site and in the water, seawater penetrated the polyurethane coating resulting in patchy dissolution of the plaster and uneven dissolution rates from the cylinder surfaces.

The plaster thus did not dissolve only from the upper surface, making measuring of height of the cylinders as an indicator of water movement unfeasible. The results are useful, however, as the high level of replication for sites gave good estimates of relative water movement rates.

Figure 5.4 Plaster cylinder facing upwards on top of buoyed frame at deployment.

For the second session, the cylinders were not coated and only one cylinder was placed on each frame. All cylinders were facing upwards at the top of the frames (see Figure 5.4) at 2 m depth to give a good indication of relative water movement exposure between sites. The frames were subject to some vertical movement, and the cylinders incorporated this effect. While currents and wave action are acknowledged to cause water flow over plants, some movement also comes from the vertical motion of the frames as a result of their attachment to surface buoys. The plaster cylinders integrate all water motion contributions.

Calibration of the cylinders was achieved in the laboratory by exposing them to different extents of laminar seawater flow and monitoring weight loss through time at a constant temperature (12.2^{o} C). This was done by mounting cylinders in a 2.0 m diameter tank on a rotating arm at various distances from the central axis. The speed of rotation was set (one revolution per minute) and dissolution monitored through weighing once a day for 7+ days. The rotating motion of arms caused the water in the tank to rotate due to entrainment effects, meaning that the speed of the water over the arms was reduced. To estimate the speed of rotation of the water, a small glass vial half filled with water was introduced to the tank at a point half way along the arms. The time for the vial to complete a full rotation of the tank was measured on a number of occasions throughout the trial. The time of rotation was assumed to be proportional to the speed of entrained water and was subtracted from the known speed of the arms to give the actual speed of water across the arms.

Porter *et al.* (2000) claimed that plaster clods are good for estimating either turbulent or laminar flow, but results for a combination of water movement types are equivocal. While water movement exposure in the tanks does not necessarily directly relate to water motion exposure in the field, the calibration standardisation process enables comparison with other studies.

Light

An estimate of water clarity was obtained using a Secchi disk. A Secchi disc is 20 cm in diameter with alternating quarters of its upper surface painted black and white. The disc is lowered through the water until it is lost from view and then pulled up until it reappears. The depth at which it reappears is noted, this is done three times for a site

and the mean Z_{SD} used.. Kirk (1994) describes the Secchi disc as a crude visual method for estimating the attenuation coefficient, K_d, where

$$K_d = 1.44 / Z_{SD}$$

The Secchi disc is thus an easy cost effective method for estimating water clarity. From K_d, we can estimate the 1% irradiance depths which have relevance for photosynthetic organisms. The 10% and 1% irradiance depths are the mid and lower points of the euphotic zone within which significant photosynthesis occurs. Downward irradiance diminishes in an approximately exponential manner with depth. This may be expressed by the equation:

$$E_d(z) = E_d(0)e^{-K_d * z}$$

Where $E_d(z)$ and $E_d(0)$ are the values of downward irradiance at a depth of z m, and just below the surface, respectively, and K_d is the average value of the vertical attenuation coefficient over the depth interval 0 to z m. At a depth at which irradiance is 1% of surface light, $z_{1\%} = 4.6 / K_d$. From secchi disc measurements we can thus obtain a gauge of the lower limit of light for growth of kelps.

A standard Secchi disk was used to estimate water clarity on visits to each of the sites. Readings were conducted by the same operator to reduce errors due to differences between operators. All sites were not measured on every visit. Readings were not conducted if it was judged that the disk could not be seen reliably when the water surface was too disturbed as a result of strong winds.

Statistics

Comparisons were made using analysis of variance (ANOVA) with General Linear Modelling (GLM). Data were tested for normality using an Anderson-Darling test and homogeneity of variance was tested using a Bartletts test. Data were transformed where required. Post hoc comparisons were conducted using a Tukey test. Statistics packages used were Minitab and JMP IN (SAS). Error bars on graphs are 95% confidence intervals unless otherwise specified.

Results

Laminaria saccharina growth rate.

Survival of the hole-punched *L. saccharina* plants used for monitoring growth over the summer period was low and, for some of the surviving plants, it was difficult to distinguish the hole that had been punched in the lamina at the end of the sampling period due to the poor condition of the lamina. Where it was not possible to distinguish the hole, the plants were excluded from the analysis. Many plants also had epiphytes, such as the bryozoan *Membranipora* sp., covering them. Mean increments for *L. saccharina* blades using the hole punch technique (Parke 1948) over this sample period (30/6/05 to 23/8/05, 53 days) showed greater growth for farm based plants (FarmN, FarmSE & FarmSW, mean increment = 50.5 cm) than for non-farm sites (BadcallLL, Sheltered and OutsideMS, mean increment = 31.3 cm; ANOVA site: $F_{(4,18)} = 0.19$ nested within farm; $F_{(1,18)} = 9.31$, also see Fig 5.5).

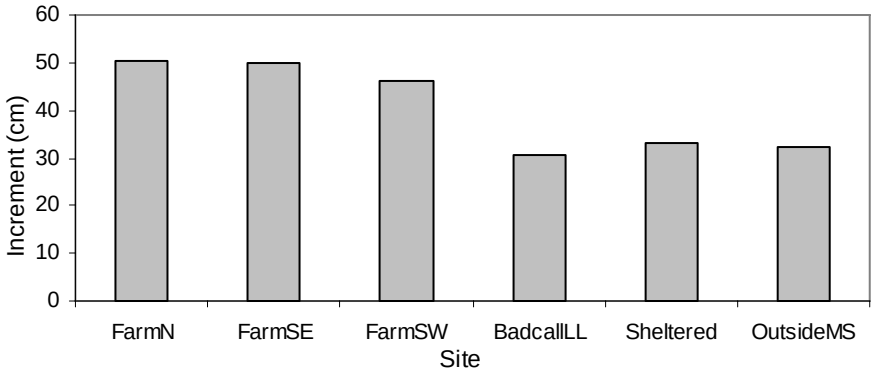


Figure 5.5 Mean length increment *in Laminaria saccharina* plants at four sites between 30/6/05 and 23/8/05. Numbers of plants measured for the six sites were: 7, 3, 4, 4, 4, 2.

Palmaria palmata growth rate

Growth rate differences as RGRs show a significantly greater growth rate for the farm grown *P. palmata* than non-farm for the period 28 April 2005 to 22 June 2005 for both batches (Table 5.2). However, the initial lengths of the farm plants were smaller than those of plants grown away from the farm indicating slower growth and/or development up until that time for farm based plants (Figure 5.6).

Table 5.2 ANOVA results for *Palmaria palmata*. RGRs between 28 April and 22 June 2005, comparisons for farm *versus* other sites for the two batches (see also Figure 5.5); degrees of freedom, F ratio and significance level.

Batch	Mean RGR (cm $cm^{-1}day^{-1}$)		DF	F	*p*
	Farm	Other			
25/01/2005					
Farm	0.018567	0.013791	1, 15	9.62	0.011
Site (nested)			4, 15	7.21	0.005
24/02/2005					
Farm	0.036582	0.024891	1, 15	10.62	0.009
Site (nested)			4, 15	7.21	0.425

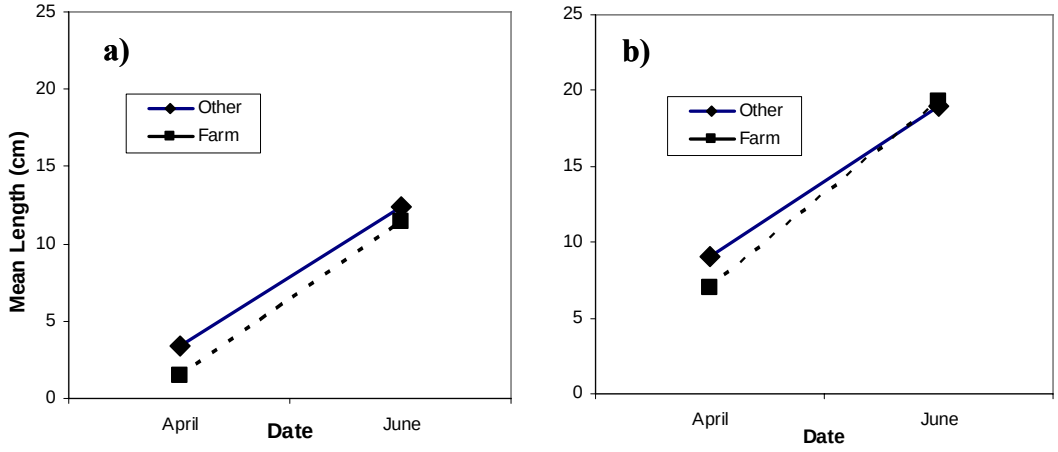


Figure 5.6 Mean lengths of *Palmaria palmata* plants on 28 April and 22 June 2005 from farm sites **(**FarmSE and FarmSW and 'other' or non-farm sites (CalbhaRef, CalbhaLL, OutsideE and OutsideW):: **a)** 24/2/05 batch, **b)** 25/1/05 batch. Lengths presented are the mean of mean *Palmaria palmata* lengths for each site.

Frames with *Laminaria saccharina*

Harvesting of frames revealed some losses of *L. saccharina* bundles which were likely to have arisen due to either exposure to wave action or tangling in the mooring lines as a result of the loss of some of the mid-water weights (all bundles lost or compromised from CalbhaRef). Nevertheless, 49 out of a possible 60 ropes yielded results with a total harvest of 764 kg of *L. saccharina*. Without excluding damaged bundles from the analysis and using total weight of *L. saccharina* for frames, there was a significant difference between farm associated (mean wet weight of *L. saccharina* on frames: 17.4 kg) and other site frames (mean weight: 11.5 kg, ANOVA Site: $F_{(8,16)} = 1.13$ nested within Farm: $F_{(1,16)} = 5.34$).

When excluding damaged and missing bundles (ropes with only one bundle or with damaged bundles excluded) there was a highly significant difference ($p < 0.001$) between farm (mean rope weight: 9.9 kg) and non-farm sites for individual rope weights (mean rope weight: 7.8 kg, Figure 5.7). Damaged and missing bundles were excluded as

there are factors other than nutrient availability from the farm cages affecting their weights. Water motion for instance, would cause loss and damage of bundles.

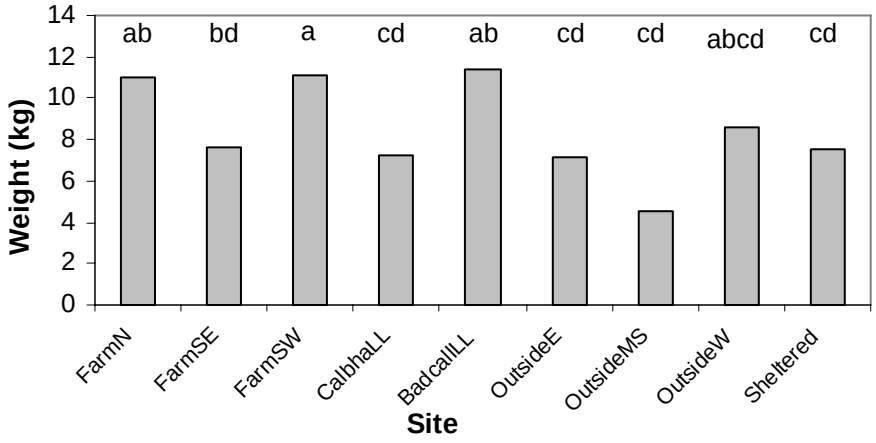


Figure 5.7 Mean rope weights with *Laminaria saccharina* for each site. ANOVA Frame nested: $F_{(10,13)} = 2.50$ within Site: $F_{(7,13)} = 6.15$ nested within Farm $F_{(1,13)} = 19.12$. Values which share a letter are not significantly different at $p = 0.05$.

Frames with *Palmaria palmata*

A total of 18.6 kg of *P. palmata* were harvested from all the frames. The earlier batch outplanted on 25/1/05 gave the greater yield of the two batches. For analysis of data from the earlier batch, seeded strings with less than 10 cm coverage of *P. palmata* were taken out of the analysis. Lines with greater than 10 cm coverage of *P. palmata* were more likely to have fewer epiphytes, or to have been subject to mishaps and had a more even settlement of *P. palmata* along the length of the string (5 strings excluded from 62). Results of ANOVA show that Farm grown lines had greater biomass (Mean wet weight: 347.2 g/80 cm) than non-farm sites (Mean weight: 213.5 g/80 cm, Site nested within Farm, Farm: $F_{(1,46)} = 6.75$, Site: $F_{(8,46)} = 1.84$, Figure 5.8). While frames were placed to optimise their exposure to light, soon after the initial deployment of seeded strings at FarmSE, salmon cages were moved as part of farm operations and placed very near the frames at this site resulting in shading of the frames. FarmSE gave the lowest yield of the farm frames for *P. palmata* (and *L. saccharina*).

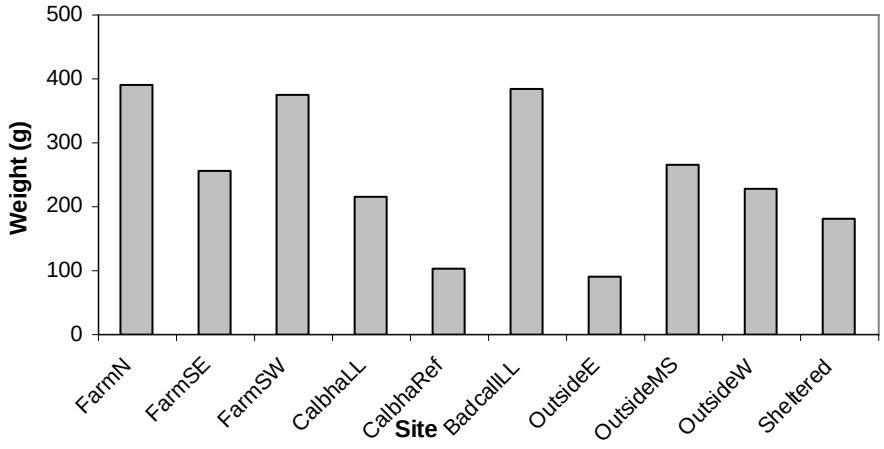


Figure 5.8 Mean *Palmaria palmata* string weights for 25/1/05 outplants for each site. No significant difference was observed between individual sites, but the combined results from the three farm sites were significantly higher than all other sites.

Palmaria palmata strings outplanted on 24/2/05 showed no significant difference between sites (ANOVA, Figure 5.9) or between farm and non-farm string weights. The mean yield per string across all sites was nearly a fifth of the outplant from the previous month (58.8 g and 249.9 g, respectively). The relative weight yields between the sites for the two cultured algae: *P. palmata* and *L. saccharina* show similarities for the 25/1/05 outplant and there was a highly significant correlation between the two species (Pearson's correlation coefficient: $r = 0.65$, $p = <0.01$, Figure 5.10).

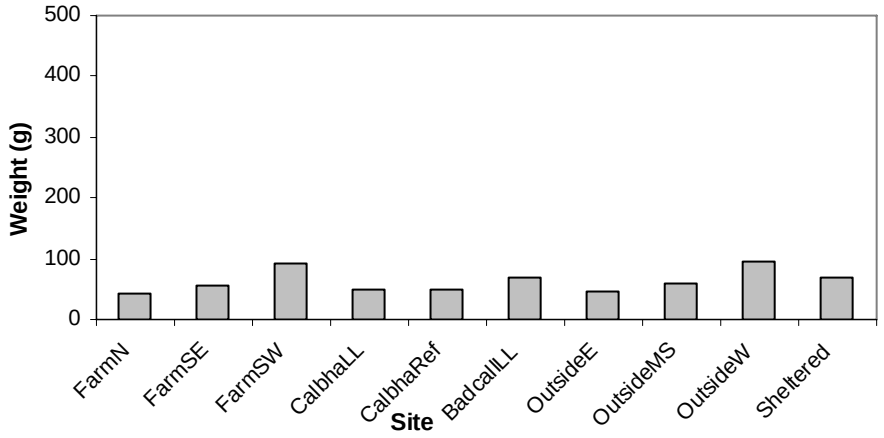


Figure 5.9 Mean *Palmaria palmata* string weights for 24/2/05 outplants for each site. No significant difference was observed between sites.

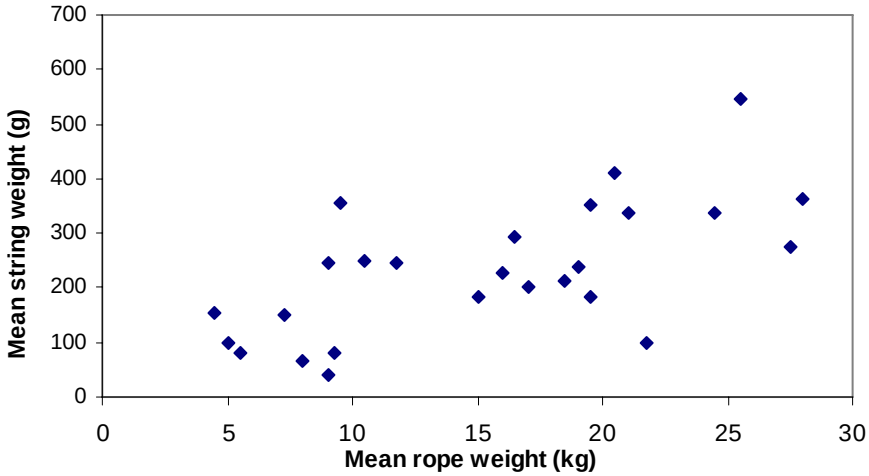


Figure 5.10 Correlation between *Laminaria saccharina* (mean rope weight) and *Palmaria palmata* (mean rope weight) for each frame (25/1/05 outplant only) for each site (Pearson's correlation coefficient: r = 0.65, df = 24, $p < 0.01$).

Longlines with *Laminaria saccharina*

All 10-cm *Laminaria saccharina* seeded strings gave rise to bundles of *L. saccharina* except the 1-m depth bundles from group 2 of the farm longline. There was no evidence of *L. saccharina* on group 2 ropes at 1 m depth except the original bare 10-cm pieces of string. A possible explanation for this may be that a boat had crossed the line and cut the upper bundles free. To simplify comparisons, all Farm group 2 bundles were eliminated from all analyses. All longline bundles were harvested between the 15th & 17th June except for a line from two of the Badcall longline groups. These were kept for later harvest for chemical analysis and to provide plants for the hole punch growth comparison. The harvested plants gave a grand total from the longlines of 940 kg of *L. saccharina*.

There are no obvious trends for biomass along any of the longlines, either for the farm longline with distance from the cages, or for the Calbha and Badcall longlines with distance from either end (which might result from orientation with prevailing currents etc.). Analysis of variance for the total weight of droppers between longlines shows that

the BadcallLL droppers have a greater biomass than either the Farm or Calbha longline groups (Figure 5.11).

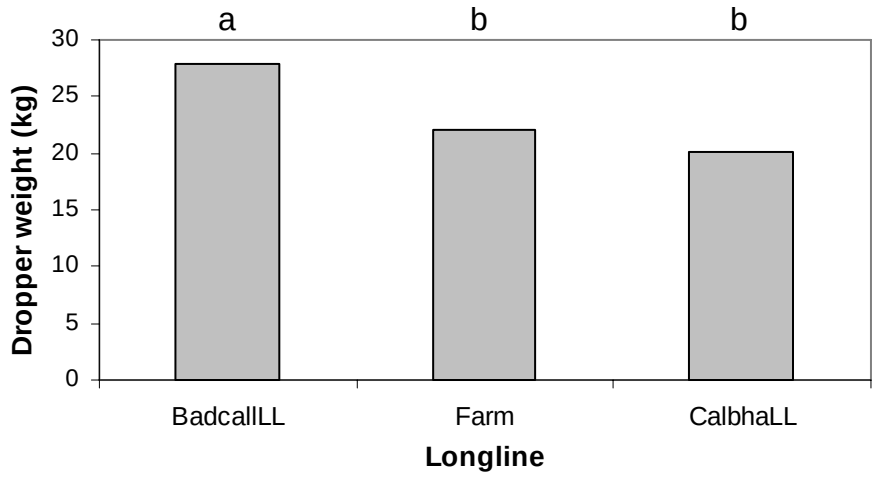


Figure 5.11 Mean weight of *Laminaria saccharina* harvested from each dropper for each of the three longlines: the farm longline and the two reference longlines at Badcall and Calbha. Values which share a letter are not significantly different at $p = 0.05$. ANOVA group: $F_{(5, 30)} = 1.27$ nested within longline $F_{(2, 30)} = 25.95$. To compare group mean dropper weights, group 2 droppers at the farm were eliminated because of the loss of the 1 m bundles.

Individual bundles were weighed for all the groups on the farm longline and for five droppers from the reference longlines at Badcall and Calbha. The Farm 2 dropper bundles were not included in any analyses due to the loss of the 1 m depth bundles. A two-way analysis of variance of all the bundle weight data showed highly significant differences among groups and depths, but no significant interaction between groups and depths. The mean weight of the BadcallLL bundles was greater than the Farm 3 bundles and the CalbhaLL bundles (Figure 5.12). The 1 m bundles were significantly heavier than those at all greater depths, and the bundles at 2 and 3 m were heavier than those at 4 and 5 m (Figure 5.13). An exponential curve shows a good fit to the weight distribution of the bundles with depth, and probably reflects the reduction in available irradiance with depth.

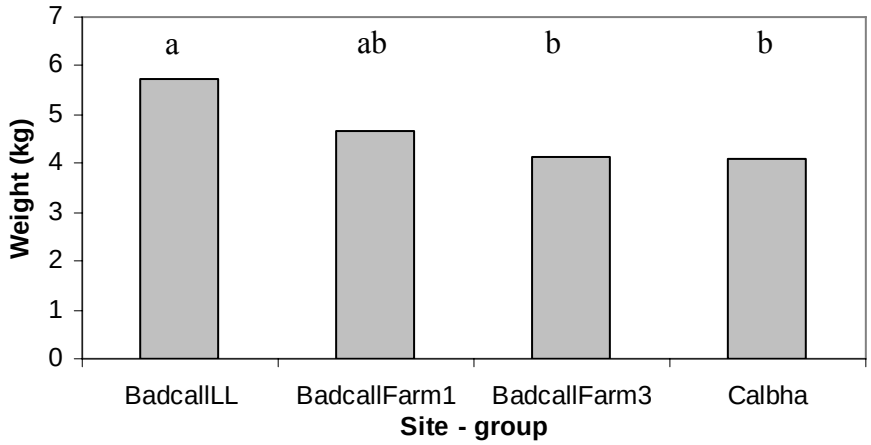


Figure 5.12 *Laminaria saccharina* harvested per bundle from each dropper from each of the three longlines. Values which share at least one letter are not significantly different at $p = 0.05$. Two way ANOVA Site/group: $F_{(3,79)} = 7.13$, Depth: $F_{(4,79)} = 47.14$, no interaction.

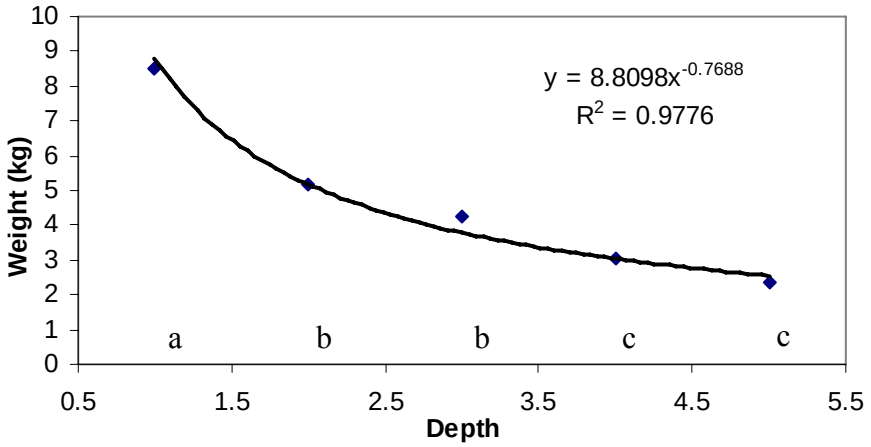


Figure 5.13 Yield of *Laminaria saccharina* harvested per bundle for depths across all groups. Values which share at least one letter are not significantly different at $p = 0.05$.

Longlines with *Palmaria palmata*

The *Palmaria palmata* longlines were of variable success. Two factors working against their success were 1) the loss of longlines due to inadequate structural support in rough seas and 2) variable seeding success. Structural support failed where the seeded lines slid along the longlines, and merged and tangled. Also, not all lines were evenly seeded. A 'batch' refers to all plants that were deployed at a given time; a 'sub-batch' refers to individual bins from which the lines may have been seeded in the laboratory. While every care was taken to seed all bins evenly, checks on the lines in the bins before deployment showed variation between sub-batches (but not for the frame-seeded lines). However these sub-batches were divided evenly amongst the groups on the longlines. Hence the assumption is that variation between groups was similar when they went into the water. Table 5.3 summarises the seeding history. The total mass of *P. palmata* harvested from the longlines was 18.1 kg.

Table 5.3 Culture history of *Palmaria palmata* droppers. The 'Droppers remaining' column relates to the number of droppers relocated from each batch at harvest in June 2005.

Date	Where	Group	Original number strings	Droppers remaining	Droppers with >20 plants in first metre.
21/12/04	BadcallLL	1	7	6	3
	Farm	1	6	6	4
25/01/05	BadcallLL	1	5	5	
		2	5	4	4
		3	5	1	
	CalbhaLL	1	5	3	2
		2	5	5	1
		3	5	5	1
	Farm	1	5	4	1
		2	5	3	
		3	5	3	1
24/02/05	BadcallLL	1	5	1	
		2	5	5	4
		3	5	4	2
	CalbhaLL	1	5	4	
		2	5	5	1
		3	5	4	
	Farm	1	5	5	1
		2	5	5	2
		3	5	5	3

Longlines: *Palmaria palmata*: plant numbers in the first metre

Plant numbers were taken as an indicator of plant survivorship. Plant numbers were estimated for the first metre of the line. Statistical comparisons were conducted using the Mann-Whitney non-parametric post hoc test. For the December batch that showed the best overall yield, and for the January batch, there were no significant differences between the site droppers in the number of plants initiated in the first metre of the string (see Figure 5.14). For the February batch, BadcallLL had significantly higher numbers of plants than CalbhaLL, but not significantly different from FarmLL. The survivorship of the FarmLL plants was not significantly different from that of the Calbha longline.

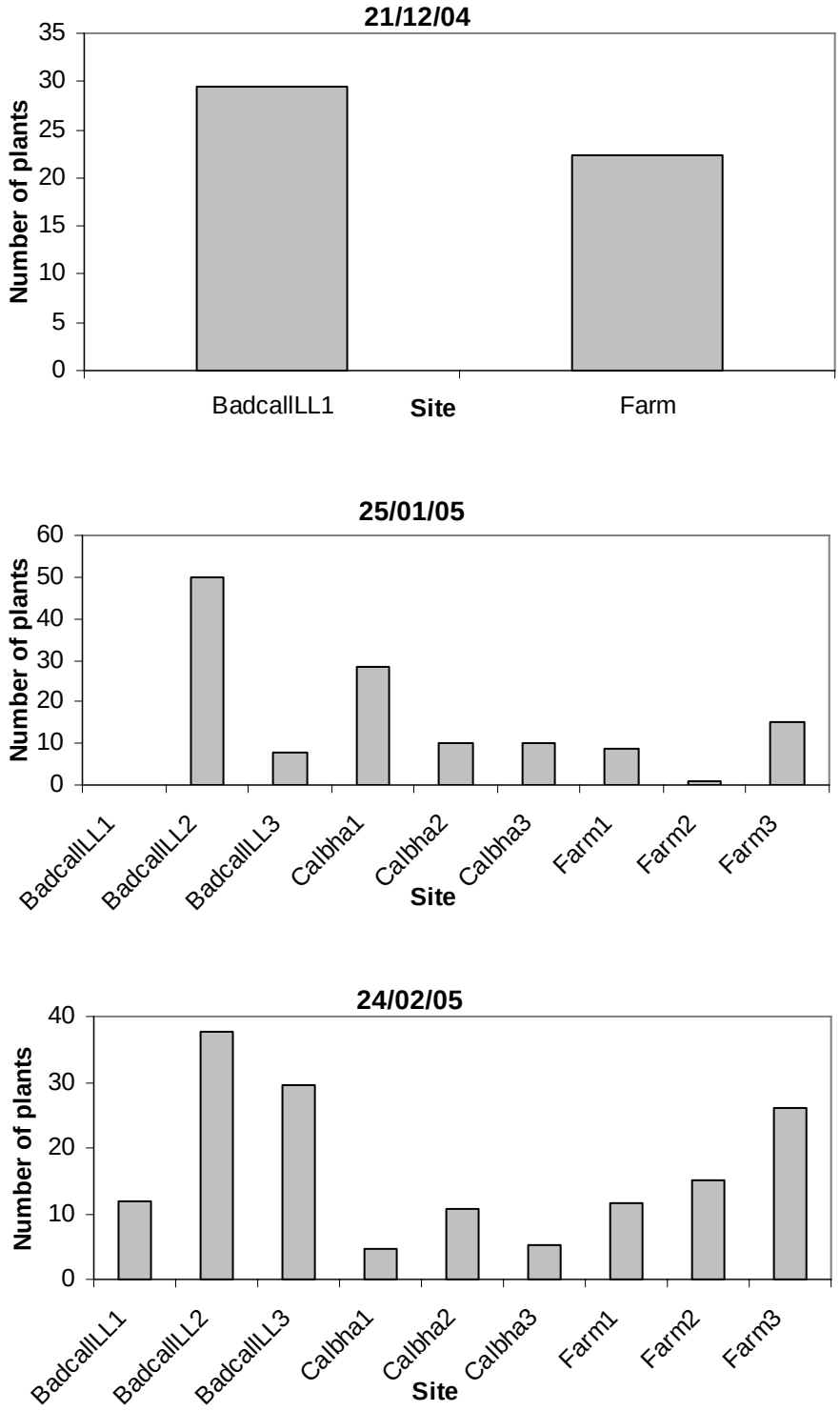


Figure 5.14 Mean estimated number of plants in the first metre of the droppers for *Palmaria palmata* outplanted in **a)** 21/12/04, **b)** 25/1/05 and **c)** 24/2/05 at harvest.

Longlines: *Palmaria palmata*: yield

For productivity comparisons, the length of string with *P. palmata* attached (>10 cm for frame strings) or number of plants in the first metre of droppers (>20) is taken as the mark for a successful seeding. These cut-off marks were used because lines remaining in the analysis had good plant cover with plant sizes that were reflective of site results generally. Seeded lines at harvest with greater than 10 cm coverage of *P. palmata* were more likely to have fewer epiphytes and were less likely to have been subject to adverse conditions that may have rubbed plants off. This has necessitated pooling across groups within longlines for statistical analyses.

For the droppers outplanted on to the longlines on 21/12/04, the yield of the Badcall reference longline droppers was greater (Mean fresh weight: 937.3 g in the first metre, Figure 5.15) than the farm droppers (Mean fresh weight: 267.7 g, ANOVA $F_{(1,4)}$ = 17.4). There was no significant difference between sites for the 25/1/05 and the 24/2/05 outplants between farm and non-farm outplanted droppers. Figure 5.16 shows the relationship between wet weight of *P. palmata* and depth from the longlines when compared to the first metre weight.

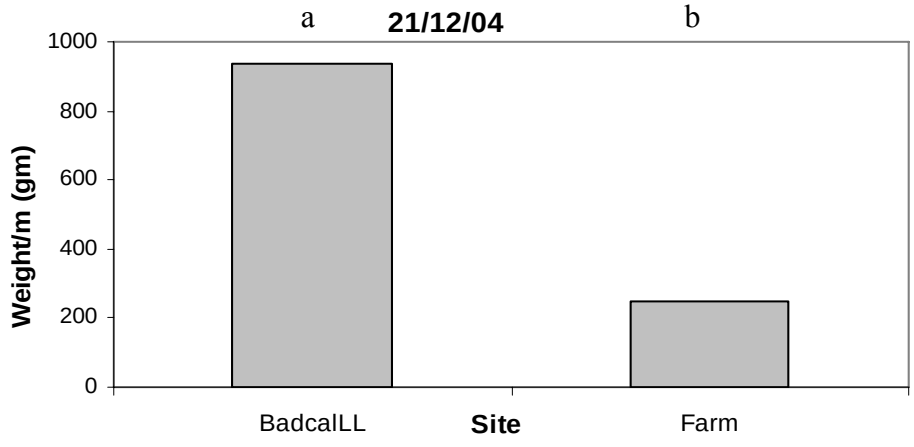


Figure 5.15 Mean mass of plants at harvest in the first metre of the droppers for *Palmaria palmata* outplanted in 21/12/04 (g, fresh weight).

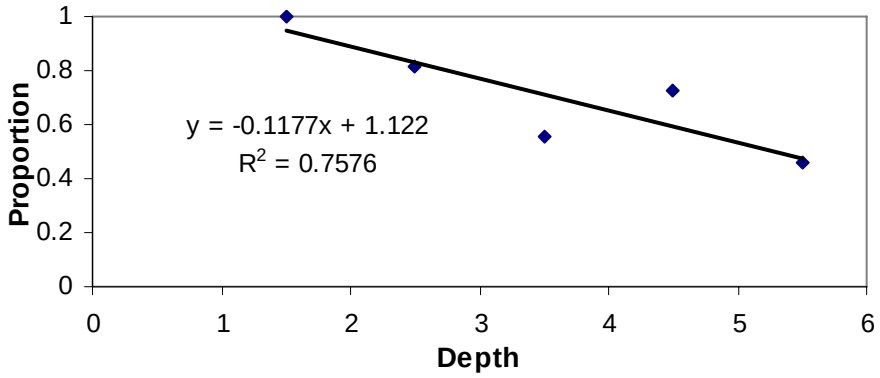


Figure 5.16 Mean *Palmaria palmata* yield per metre depth as a proportion of the mass of plants in the first metre of the dropper. This decline gives an indication of how yield of *Palmaria palmata* varies with depth.

Light

Secchi disc readings show no significant differences between sites, although there was a tendency for farm sites and the sites that were further towards the head of Badcall Bay to have reduced Secchi readings (Table 5.4). Secchi mean values for the sites ranged from 8 to 10 m. This corresponds to light values of 1% of surface irradiance varying between 25.6 and 32 m depth and 10% at 12.8 and 16 m depth or Jerlov coastal water types 1 – 3 (Jerlov 1976). The amount of light reaching seaweeds can determine growth differences and is dependent to a large extent on the transmittance of the water (Lüning 1990, Dring 1991). Here, transmittance is unlikely to have influenced growth differences between sites as there is little difference in water clarity and the 1% and 10% surface irradiance depths were consistently greater than the depth at which the algae were grown.

Table 5.4 Secchi disc readings taken at each of the sites during the term of the project.

Date	25/02/05	29/03/05	27/04/05	01/06/05	22/06/05	29/06/05	Average
BadcallLL	10.7	8.5		8.8	9	12.5	**9.9**
CalbhaLL	9.2	12.8	8.7	7	9	12	**9.8**
CalbhaRef	9.2	10.9	9	6.2	9	12.6	**9.5**
FarmN	7.6	10.5	6.4	7.9	8	11.2	**8.6**
FarmSE	7	9	7	7.9	7	10.8	**8.1**
FarmSW	8.5	9.9	7.7	7	8.5	10.5	**8.7**
OutsideE	10	11	8.5	8	9.7		**9.4**
OutsideMS	9.8	11		7.4	9		**9.3**
OutsideW	9.5	12		6.7	8.5		**9.2**
Sheltered	8.3	9		7.3	7	8.5	**8.0**
Average	**9.0**	**10.5**	**7.9**	**7.42**	**8.47**	**11.2**	**9.0**

Temperature

Water temperature readings for the three sites are presented in Appendix 1. Over the three years of the project they showed an annual water temperature variation going from a minimum of 7^{o} C in March to a maximum of 15^{o} C in July and August across the three sites. The pattern of temperature variation within years does not appear to vary greatly between the sites although, in 2003, water temperatures reached 16^{o}C.

Water movement

Percentage loss of plaster from cylinders for the two sessions is presented in Figure 5.17. The first session cylinders were coated with the polyurethane. Despite there being six replicates there is higher variability than for the second session. One advantage of the coated cylinders was the slower dissolution rates meaning that water movement could be integrated over longer periods of time. There is a good correlation for the ten sites between the two sessions ($r = 0.7$, $df = 8$, $p < 0.05$) but because of the lower variability for the second session, these results were used for correlation against algal yield.

For the calibration exercise, measurement of entrained currents by the stirrer arm indicated a lap speed of 2.5 minutes for middle section of the arms with a slight

decrease towards the outer part of the tank (due to resistance of the water current with the tank's outer wall). Figure 5.18 shows the calculated adjusted current speeds experienced at the level of the cylinders. Calibration of the uncoated plaster cylinders showed an even dissolution with time and good reproducibility between cylinders (Figure 5.19).

Correlation of harvest production of *P. palmata* with water movement (Figure 5.20) indicates that water movement may be a factor in optimising yield. There is an apparent reduction in yield with relatively high and low water movements as indicated by plaster loss of the cylinders.

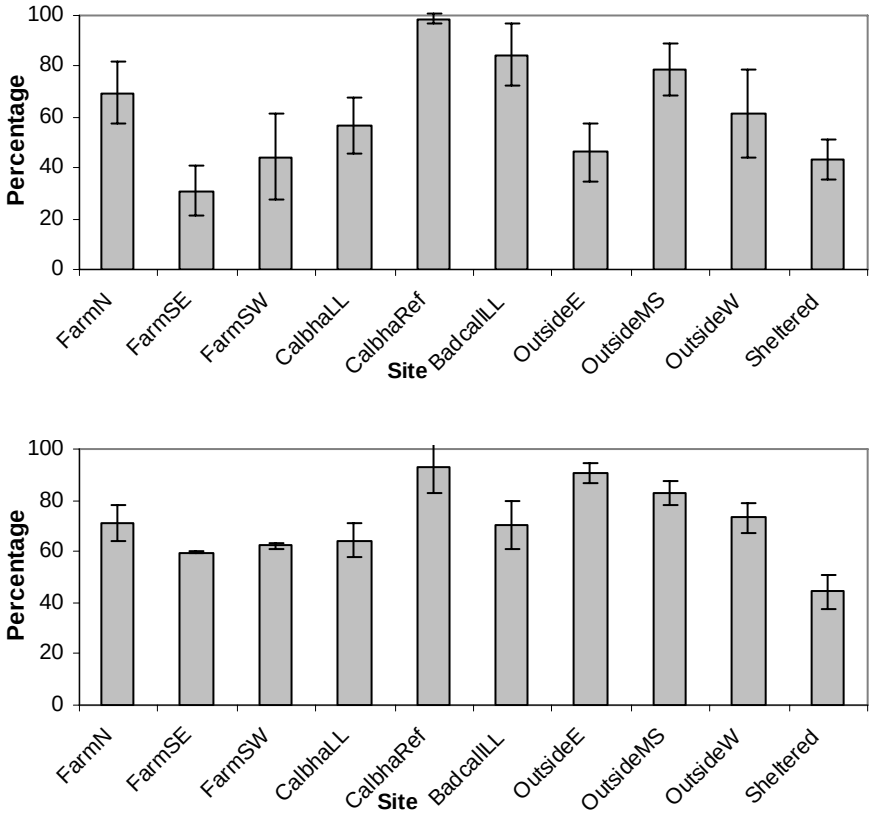


Figure 5.17 Mean percentage loss of plaster from cylinders for each site for: **above)** first session: 2/6/05 to 16/6/05 and: **below)** second session: 16/6/05 to 23/6/05, (±95 % CI).

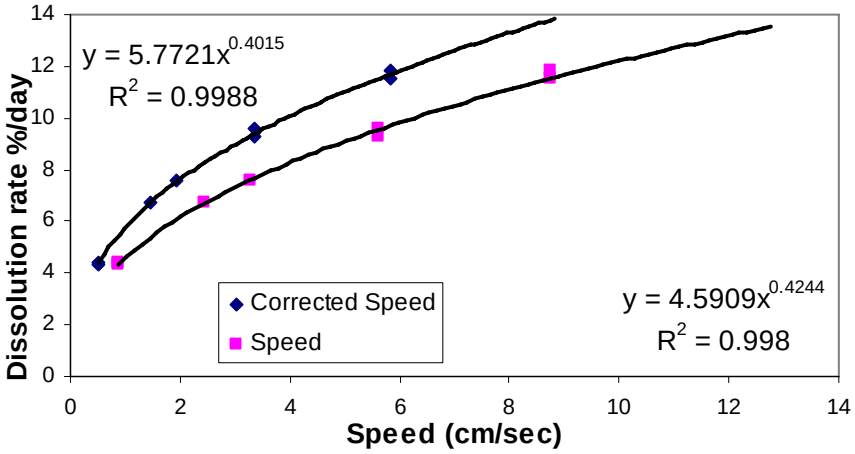


Figure 5.18 Dissolution rate as measured for the plaster cylinders in the tank as percentage per day *versus* actual and corrected current speeds over seven days.

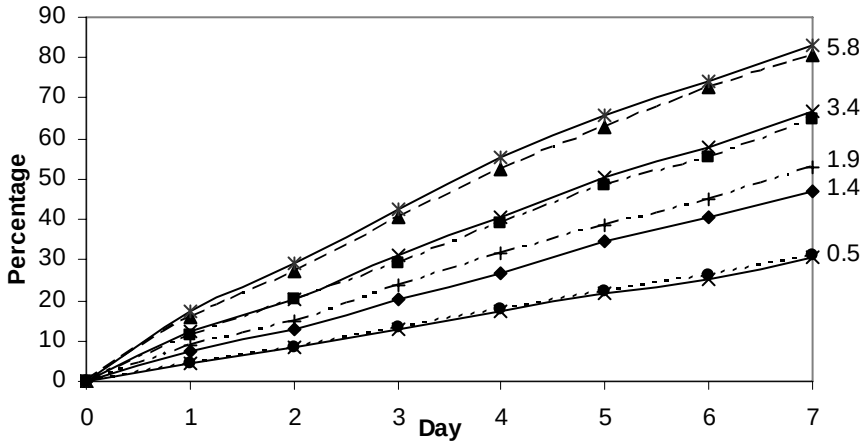


Figure 5.19 Calibration of plaster cylinders: percentage loss of plaster cylinders with time under various current speeds**.** Values on the right are corrected current speeds in cm s^{-1}. There were duplicate runs for 0.5, 3.4 and 5.8 cm s^{-1}.

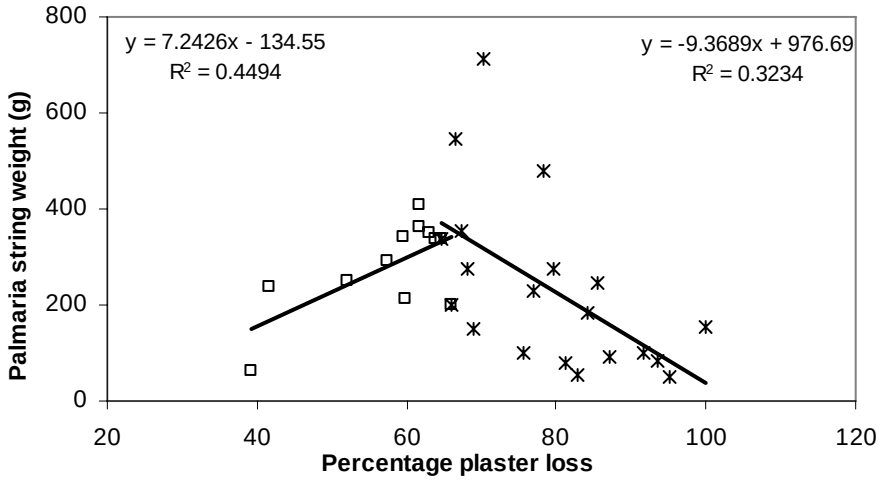


Figure 5.20 Mean *Palmaria palmata* harvest string weight *versus* plaster loss for each frame. Linear regressions are fitted for values greater and less than 65% plaster loss showing possible trends. There are apparent low yields of Palmaria palmata under high and low relative water movement as reflected in plaster loss from the cylinders.

Discussion

Short term measurements of growth of *L. saccharina* (summer) and *P. palmata* (late spring) both show evidence of enhanced growth close to the cages: *L. saccharina* increased by 61% and *P. palmata* by 38% and 44% (January and February deployed batches). However, the initial lengths of the *P. palmata* plants appeared to show better growth prior to these growth measurements for the plants away from the farms.

Yields at harvest over a growing season for both *L. saccharina* and *P. palmata* show enhanced yields for the farm based algae, with *L. saccharina* frames and individual ropes showing a 51% and a 27% increase and *P. palmata* strings from the January outplant showing a 63 % improvement when compared to yields away from the farm.

These findings are comparable with a 40% increase in growth rate for *Gracilaria chilensis* (specific growth rate; 7% day^{-1}) cultivated at 10 m distance from salmon farm cages compared to growth at 150 m and 1 km distance in southern Chile during two months in summer (Troell *et al.* 1997). A later study at the same site in Chile (Halling *et*

al. 2005), showed little difference between *G. chilensis* grown 30 m from the cages when compared to algae grown 300 m away. This second study however was conducted from Autumn to Spring when ambient nutrients may have been higher. Nutrient levels were not measured as part of the latter project.

RGRs of *P. palmata* of 0.013 to 0.036 cm $cm^{-1}day^{-1}$ for the late spring period compare well with values calculated for *P. palmata* for Calbha in 2004 (e.g. 0.03 cm $cm^{-1}day^{-1}$ in June) and Loch Laxford in 2003 (e.g. 0.03 $cm^2cm^{-2}day^{-1}$ in May), but are lower than values of 0.1 g $g^{-1}day^{-1}$ for outplanted *P. palmata* plants measured by Browne (2001) and land based tank measurements exceeding 0.1 g $g^{-1}day^{-1}$ for Browne (2001) and as part of this project. Morgan and Simpson (1981c) and Demetropolous and Langdon (2004) recorded SGRs in excess of 10% per day for tank based cultures of *Palmaria mollis*.

The enhanced growth of plants near the farms indicates that these algae benefited from being close to the cages over a growth cycle, although most of the benefit may have been during the late spring to late summer period when ambient nutrient availability is usually low. Analysis of nitrogen content of the algae harvested in June showed significantly higher amounts in the plants grown close to the cages (chapter 7).

The data suggests that growth is not always enhanced by growing the algae, in particular *P. palmata*, close to the cages. There may even be factors inhibiting growth of the algae relative to sites away from the cages prior to summer. This may include competition from naturally occurring filamentous algal contaminants whose growth is also enhanced near to the cages. There may also be some influence from other farm wastes such as particulate matter arising from feed and faeces. This is sometimes found

coating algae close to the cages and influences the quality of the plants. This is in agreement with the findings in the previous chapter where early development of *P. palmata* appeared to be inhibited.

Contrary to general trends, the Badcall longline away from the fish farm cages had a better yield than the farm based longline. The results of the buoyed frames show that the Badcall longline site had exceptionally high yields when compared to the other non-farm site results. Nitrogen isotopes indicate that nitrogen in the Badcall longline plants may have been of farm origin (chapter 7).

The trend of decreasing yield of *Palmaria palmata* with depth on droppers from the longlines is consistent with the significant difference between growth of tethered plants at 2 m and 7 m depth as described in chapter 3. The results from the tethered plants suggest growth rates (change in area with time) was halved at 7 m. Similarly yields at 5 m are half of those at 1 m on the longlines. The logarithmic decline in yield of *Laminaria saccharina* with depth is consistent with the decline in available light. Extrapolating yields of *P. palmata* over the whole depth range on the basis of yields from the first metre should take into account the decline in likely yield with depth.

While proximity to the farm cages appears to favour the growth of *L. saccharina* in particular, water movement relates better to the yield of *P. palmata*. This is in agreement with the natural habitat of these algae. Healthy populations of *L. saccharina* are found in waters sheltered from wave action while *P. palmata* is found in more wave-exposed sites. Large plants of *P. palmata* up to a metre long have been found in high tidal current areas. This suggests that *P. palmata* is not as suited as *L. saccharina* to being grown adjacent to salmon farm cages in low current areas. Salmon farm cages

are usually located in relatively sheltered waters, although tidal currents aid in dispersion of waste products. If *P. palmata* is to be grown adjacent to salmon farm cages, then exposure to water motion such as tidal currents will improve the growth of the alga.

Overall, the December outplants of *P. palmata* gave the greatest yield at close to 1 kg per metre. Harvest yield may be increased by an earlier deployment to sea than December, perhaps August–September after the abatement of threats to the crop from epiphytes or larval settlement. How early, may be limited by the availability of fertile *P. palmata* plants for culture. This study has shown that *P. palmata* has great variability in reproductive status within microhabitats from small sections of coast (see chapter 4). For example, upper subtidal plants growing on kelp holdfasts may have a delayed reproductive season and thus provide reproductive plants for out-planting in August-September. Another possibility for early maturation of plants may be through manipulation of the reproductive cycle through exposing growing plants to short days and cooler water (Pang and Lüning 2004; 2006) in on-land culture facilities.

Browne (pers. comm.) has obtained yields of up to 1.8 kg fresh weight of *P. palmata* per metre of longline. Yields at Loch Duart Ltd. may be increased to these levels and higher by outplanting earlier in the season. Multiple cropping may be possible if the algae are of a sufficiently large size early enough in the season before cutting. This may allow growth before being re-cropped prior to the presence of epiphytes in July. Larger plants and multiple cropping may also be more feasible if the alga is grown in higher water motion areas, such as in tidal currents, that might limit the settlement of epiphytic plants and encrusting animals. Higher current areas may also keep the plants clear of any particulate wastes emanating from the farm cages.

SUMMARY

Short term measurements of growth rate of *Laminara saccharina* in summer show a 61% increase for algae grown close to cages (< 20m) when compared with sites at distance.

Short term measurements of growth rate of *Palmaria palmata* in late spring show a 38-44% increase for algae grown close to cages (< 20m) when compared with sites at distance.

The yield of cultured *Laminaria saccharina* grown over a growth cycle from January to June was enhanced by 12.7-51% for algae grown close to fish farm cages (< 20 m distance) when compared to sites away from the cages.

The yield of cultured *Palmaria palmata* grown over a growth cycle from January to June was enhanced by 63% in algae grown close to fish farm cages (< 20 m distance) when compared to sites away from the cages.

On *Palmaria palmata* seeded droppers, maximal yields of 940 g m^{-1} were achieved. This may be improved by deploying seeded lines earlier in the growing season (i.e. before December).

Yields of 28 kg per dropper were achieved for *Laminaria saccharina* on longlines. These also may be improved by deploying seeded lines earlier in the growing season i.e. before December).

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Yields of cultured *Palmaria palmata* are improved under conditions of moderate rather than either low or high levels of water movement.

CHAPTER 6

AMMONIUM AND NITRATE NUTRITION OF *PALMARIA PALMATA*

Introduction

The aims of this section relate to the nitrogen physiology of *Palmaria palmata* and growth of the alga in the vicinity of fish farm cages. The four sections of this chapter address the following questions:

1. Is ammonium inhibitory for *Palmaria palmata* growth? If so what levels are inhibitory?

2. Does *P. palmata* take up ammonium before nitrate? Does ammonium uptake inhibit nitrate uptake?

3. *P. palmata* can store nitrogen – for what periods can it do so? What internal levels of nitrogen are indicative of nitrogen depletion?

4. Where in the thallus is ammonium taken up? Is there translocation of nitrogen to new tissue?

6.1 Is ammonium inhibitory for *Palmaria palmata* growth? If so what levels are inhibitory?

The overall aims of this project included determining the potential for growing commercial seaweeds, principally *Palmaria palmata*, in the vicinity of salmon cages. Early in the project, discoloration had been noted in *P. palmata* when grown adjacent to fish cages at Loch Laxford (Chapter 3). One possibility for the decline in the condition of the alga grown adjacent to salmon cages may be a low tolerance to ammonium. This experiment was conducted to investigate the response of *P. palmata* to different concentrations of ammonium and nitrate.

Ammonium (NH4 +) is a paradoxical nutrient ion in that, although it is a major nitrogen (N) source whose oxidation state eliminates the need for its reduction in the plant cell, and although it is an intermediate in many metabolic reactions, it can result in toxicity symptoms in many, if not all, plants when cultured on NH4 + as the exclusive N source (Britto and Kronzucker 2002). Sensitivity to NH4 + may be a universal biological phenomenon, as it has also been observed in many animal systems, including humans, where it has been implicated in particular in neurological disorders.

Studies on *Chondrus crispus* showed that it grows equally well on NO_3^- or NH_4^+ (Neish and Shacklock 1971). In *Gracilaria tikvahiae* and *Gracilaria cornea*, growth rates were similar when nitrogen was supplied as NO_3^-, NH_4^+ or both forms simultaneously (Lapointe and Ryther 1978; Navarro-Angulo and Robledo 1999). *Gracilaria foliifera* and *Neogardhiella baileyi* grew faster with NH_4^+ as the N source (De Boer *et al.* 1978), while *Gracilaria tenuistipitata* and *Gracilaria cornea* presented similar growth rates

under both N sources (Haglund and Pedersen 1993; Navarro-Angulo and Robledo 1999). Hafting (1999) reported that NO_3^- is a better N-source for growth of *Porphyra yezoensis* than NH_4^+ in high light (160 μmol m^{-2} s^{-1}), but no differences in growth under NO_3^- and NH_4^+ were seen in low light conditions (50 μmol photon $m^{-2}s^{-1}$).

Ammonium toxicity has been noted for two species of *Palmaria*; *P. palmata* and *P. mollis*, when exposed to pulse fertilisation of ammonium for extended periods of time (Morgan and Simpson 1981c; Demetropoulos and Langdon 2004). Demotropolous and Langdon (2004) found evidence of toxicity in *Palmaria mollis* cultures supplied with 2353 μM N day^{-1} as NH_4NO_3 after 49 days, and after 14 days when exposed to 7059 μM NH_4NO_3 supplied every three day with slow seawater exchange (2.6 g l^{-1}, 1 vol exchange day^{-1}). Morgan and Simpson (1981) found decreased growth rates in *P. palmata* with additions of 500 μM NH_4^+ four times a week at 6.25 g l^{-1} and 3-4 tank volumes day^{-1}. In other red algae, daily additions of greater than 10-20 mM ammonium was toxic for *Gracilaria parvispora* in tanks when preparing for oceanic cage culture in Hawaii at 6.8 kg m^{-3} and 5% water turnover day^{-1}. (Nagler *et al.* 2003). Decline in growth at 110 μM NH_4^+ for *Gracilaria* (and *Cladophora*) was noted by Peckol and Rivers (1995) at 1 g l^{-1}. Iwasaki (1967) observed that NH_4Cl inhibited the growth of the conchocelis phase of *Porphyra tenera* at 7.0 mM. This compares with no toxicity found for several native Northeast American species of *Porphyra* cultured over 4 weeks at concentrations of 25-300 μM at 0.3g l^{-1} and 1 volume exchange every 3-4 days (Carmona *et al.* 2006).

For other groups of photosynthetic organisms, Waite and Mitchell (1972) found that NH_4^+ concentrations as low as 50 μM inhibited photosynthesis in *Ulva lactuca* and (Harlin and Thorne 1977) observed that *U. lactuca* grew poorly in a closed fish culture

system with NH_4^+ concentrations of 0.6-1.2 mM. For aquatic angiosperms, levels as low as 25 μM NH_4^+ have been noted to be toxic for *Zostera marina* (van Katwijk *et al.* 1997). Ammonium is toxic to most commercial fish species at concentrations above 100 μM (Wajsbrot *et al.* 1991; Neori *et al.* 2004).

Land plant species are particularly sensitive to, or tolerant of, NH_4^+ as the sole nitrogen source and have been the subject of research and speculation. The postulated mechanisms underlying ammonium toxicity are diverse. Explanations of the mechanisms underlying NH_4^+ toxicity have been hampered by numerous misconceptions regarding this subject, and many often-cited possibilities have more recently been shown to be at best insufficient, partial explanations, or even incorrect. These latter include the uncoupling of photophosphorylation by NH_4^+ in plants; the effects of external pH declines resulting from NH_4^+ acquisition; the role of biochemical pH-stat mechanisms in cells accounting for differences in the internal H^+ balance associated with differences in NH_4^+ and NO_3^- metabolism; the accumulation of free NH_4^+ in plant tissues (including, specifically, the cytosol); and the higher root carbon allocation to amino acid synthesis under NH_4^+ nutrition. More plausible explanations include the involvement of ethylene synthesis and action as a key plant response to NH_4^+ stress; the role of NH_4^+ membrane flux processes, particularly the energy-demanding active efflux of cytosolic NH_4^+; photosynthetic effects, particularly with respect to photoprotection; and displacement of essential cation concentrations from homeostatic set points in subcellular compartments (Britto and Kronzucker 2002).

Methods

Sub-tidal *P. palmata* was collected on 29 July 2004 from Seil Island, Scotland (56.45422^o N, 005.44393 ^{o} W). The apical tips of the field collected *P. palmata* were cut into 3 cm lengths and were cleaned by rinsing with filtered seawater and followed

by gentle wiping to remove epiphytes. The clean tips were then placed into large 10–litre transparent plastic containers filled with 8 l of filtered seawater, that was aerated vigorously. Algae used in the experiment were acclimated by culturing for up to three days in a temperature controlled room at 11°C on a 16:8 light:dark cycle under approximately 100 µmol photon $m^{-2}s^{-1}$ of irradiance.

Twelve apical tips equivalent to 0.8 g fresh weight per container were used. The algae were cultured in seawater with varying levels of nutrients representing eight treatments, each conducted in triplicate. These were: 1) 3520 µM $(NH_4)SO_4$; 2) 880 µM $(NH_4)SO_4$; 3) 500 µM $(NH_4)SO_4$; 4) 100 µM $(NH_4)SO_4$; 5) 50 µM $(NH_4)SO_4$; 6) 3520 µM $NaNO_3$; 7) 880 µM $NaNO_3$ and 8) 50 µM $NaNO_3$. All treatments also received phosphate ($NaH_2PO_4.H_2O$) and trace metals at f/2 medium concentrations (Guillard and Ryther 1962). Once a week, the algae were measured and seawater media with nutrients exchanged. The experiment was run for three weeks.

Data analysis

Relative growth rates (RGR) were calculated using the equation:

$$RGR = \frac{\ln (W_i/W_0)}{\Delta t}$$

Where :

W_i = initial wet weight (g)

W_0 = final wet weight (g)

Δt = time (days)

Where data met with the pre-conditions of normality, homogeneity of variance and independence, the difference between treatments was analysed by using ANOVA using Minitab statistical analysis programme.

Results

Data were tested and found to be normally distributed (Anderson-Darling Test for Normality, $p > 0.05$), with equal variances (Test for Equal Variances, Bartlett's Test, $p > 0.05$) and were then subjected to ANOVA and Tukey's pairwise comparison.

All nitrate treatment concentrations and the two treatments with the lowest concentration of ammonium (100 µM NH_4^+ and 50 µM NH_4^+) sustained the highest RGR (0.061-0.071g $g^{-1}d^{-1}$, Figure 6.1) and reached the largest end weight (2.4-2.8 g, Figure 6.2.). Intermediate RGRs and end weights were experienced under treatments with medium concentrations of ammonium (880 µM NH_4^+ and 500 µM NH_4^+,). In the treatment with the highest concentration of ammonium (3520 µM NH_4^+) no growth was observed after week 1.

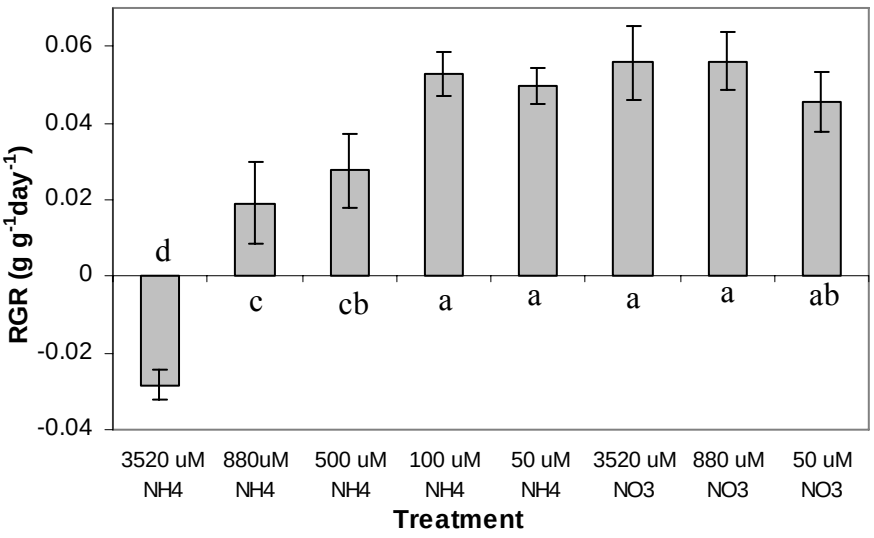


Figure 6.1 Relative Growth Rates of *Palmaria palmata* in eight treatments with differing NH4+ and NO3- concentrations over the experimental period of 21 days (+/- 95% CI). Seawater with nutrients was replenished weekly. Values which share at least one letter are not significantly different at $p = 0.05$.

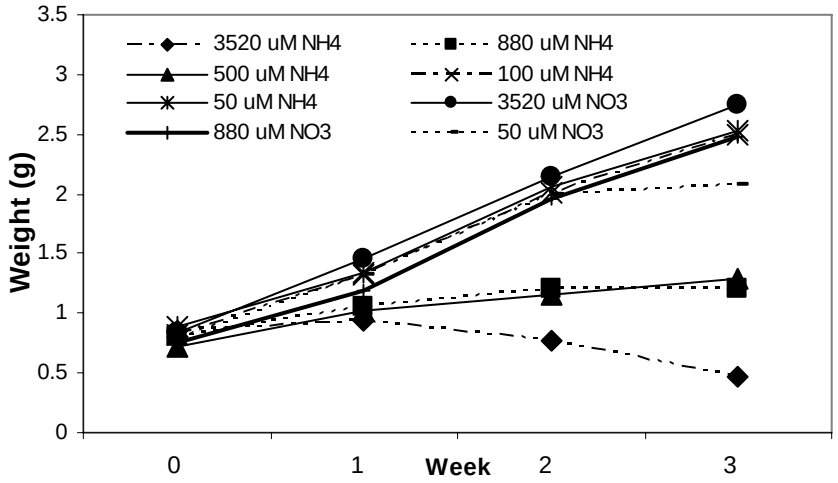


Figure 6.2 Cumulative weight of *Palmaria palmata* cultured in different NH_4^+ and NO_3^- concentrations for three weeks. Seawater with nutrients was replenished weekly.

Discussion

Palmaria palmata was found to grow in media enriched with nitrogen as either ammonium or nitrate. Growth rates achieved during this project are comparable to those that have been found previously for *P. palmata* (Morgan and Simpson 1981) and are similar to those found for other red seaweeds (Deboer *et al.* 1978; Troell *et al.* 1997; Demetropoulos and Langdon 2004)) growing under nutrient enriched conditions.

Low concentrations of ammonium (< 100 μM) sustained the same level of growth as did high concentrations of nitrate. High concentrations of ammonium caused ammonium toxicity in *P. palmata* which has also been reported in other macroalgae (e.g. Waite and Mitchell 1972; Demetropoulos and Langdon 2004). The results of Morgan and Simpson (1981) show consistently improved growth rates for nitrate over ammonium however the concentrations of ammonium used in their study were higher than those used here. More meaningful comparisons could be derived from experiments aimed at determining the effects of temperature, and light on growth taking into account

differences induced by pulse additions and continuous exposure to variable concentrations of ammonium. These relationships would also be of use when extrapolating to field situations. Matos *et al.* (2006) reported that in tank cultivation of *P. palmaria*, the alga could not survive water temperatures above 21^{o}C when TAN (total ammoniacal nitrogen) was near 75.4 μM. It is possible that ammonia at these levels in conjunction with temperature might be toxic.

During this experiment, the algae were exposed to high concentrations of ammonium over three weeks. However, it is possible that inhibition may have been evident at lower concentrations than 100 μM if the ammonium concentrations had been present on a continual basis. In this experiment, the ammonium was renewed once per week so the algae were exposed to the higher concentrations for short periods of time only once per week. The ammonium concentrations would have been gradually reduced during the week due to uptake by the plants. More exposure to more constant lower concentrations may produce inhibitory effects as has been found for *Ulva* (50 μM; Waite and Mitchell 1972) and *Zostera marina* (25 μM; van Katwijk *et al.* 1997).

Under the culture conditions presented here, mass balance calculations indicate 2 g fresh weight (at the end of three weeks) of *P. palmata* growing at an RGR of 0.1 g g^{-1}day^{-1} will use nearly all of the nitrogen in 8 litres of 100 μM N in a week (assuming wet weight to dry weight ratio of 7:1 and 5 % N (dry weight) and no excretion of N compounds. As 100 μM is the upper safe level found here, it is possible that ammonium becomes inhibitory when it remains excess to requirements.

Ammonium concentrations measured in the field adjacent to fish cages in an oceanic environment have been recorded at up to 30 μM (Ahn *et al.* 1998). This was measured

only for a short time interval and mean levels are mostly in the range of 3 to 20 μM (e.g. Karakassis *et al.* 2001). Maximum concentrations measured as part of this project adjacent to Loch Duart salmon cages were 8μM. These levels are much less than those causing toxicity in laboratory grown *P. palmata* and are only likely to enhance growth of *P. palmata* in the field.

6.2 Does *Palmaria palmata* take up ammonium before nitrate? Does ammonium uptake inhibit nitrate uptake?

Nitrogen uptake has been studied for a variety of macroalgae, but few general patterns have emerged concerning N species preference (Hanisak 1990; Lotze and Schramm 2000; Naldi and Wheeler 2002); some algae take up NH_4^+, some take up NO_3^- while others take up either inorganic N source equally well. The process of nitrogen uptake and assimilation in macroalgae involves transport from the water column across the cell membrane, followed by incorporation into proteins and macromolecules for growth (McGlathery *et al.* 1996). For NO_3^-, there is the additional step of reduction to NH_4^+ by nitrate reductase after uptake, but before assimilation (Hurd *et al.* 1995). One explanation for NH_4^+ preference may be that energy otherwise required for nitrate reduction could be saved (Rosenberg and Ramus 1984). However, NH_4^+ storage capacity may be limited due to toxicity (Waite and Mitchell 1972; Haines and Wheeler 1978; Lotze and Schramm 2000; Cohen and Fong 2004) although some algae have the capacity to store ammonium in vacuoles such as *Noctiluca* spp, some species of *Desmarestia* and *Dictyota* and some flowering plants (Raven pers com.) so that the free ammonium concentration is low.

The aim of this section was to determine whether NO_3^- uptake was inhibited by ammonium in *Palmaria palmata*.

Methods

Sub-tidal *P. palmata* plants were collected on the 29th July 2004. Thirty individual plants were collected randomly from Seil Island, Scotland (N56.45422, W005.44393). The plants were kept in flow-through indoor tanks with aeration and illumination prior to the experimental stage.

Palmaria palmata fronds were cleaned by rinsing them with filtered seawater and wiping them gently to remove epiphytes. Full fronds were then placed into 8-litre plastic containers with 5 l of filtered seawater that was aerated vigorously and enriched with f/2 standard medium (Guillard and Ryther 1962), phosphate and trace metals without nitrogen. The fronds were preconditioned for three days in a temperature controlled room at 12° C with 16:8 light:dark cycle under 100 μmol photon $m^{-2}s^{-1}$ of irradiance provided by cool white, Osram Lumilux 58 W/840 fluorescent lights.

The uptake experiments were conducted in 3-litre clear plastic vessels filled with 2 l of 1μm filtered seawater. Each vessel was enriched with phosphate and trace metals as for the preconditioning, moderately aerated and inoculated with approximately 10 g wet weight of *P. palmata* fronds. Prior to the addition of nitrogen enrichments to the medium the *P. palmata* fronds were immersed briefly into the experimental vessels and then taken out. This was to ensure that any introduced ammonium or nitrate from the plants would be accounted for in the initial seawater sample.

Four combinations of NH_4^+ and NO_3^- were added:

NH_4^+ (µM)	**NO_3^- (µM)**
20	0
0	10
20	10
10	20

After the addition of ammonium and nitrate to the vessels, the first water sample was taken to confirm the initial concentration of the nutrients in the medium. Subsequently, each experimental vessel was inoculated with the *P. palmata* fronds. Three extra vessels were used for controls (treatment: 20 µM NH_4^+ and 10 µM NO_3^-) to monitor the potential loss of NH_4^+ and/or NO_3^- due to factors such as microbial activity and vaporisation and no algae were added to these.

The experimental period was 4 h, during which sub-samples were taken at 15, 30, 60, 120, 180 and 240 min. The water samples were taken manually using a syringe. To sample the water, firstly 10 ml of medium was used to rinse the sample bottle and the filter paper and then a 50 ml water sample was taken.

The water samples from the uptake experiment were frozen and analysed for ammonium and nitrate concentrations within two days of the experiment by using a QuickChem 8000 autoanalyser following the method based on Grasshoff (1976). Samples were analysed in triplicate.

Dry weights of the samples were determined by drying eight random 10 g sub-samples of the preconditioned *P. palmata* to a constant weight on aluminium trays in an oven at 55°C.

Data analysis

Uptake rates were normalised to plant dry weight by using the following equation:

$$V = \Delta N * vol/ (\Delta t * X)$$

Where:

V = uptake rate (μmol N g dry weight^{-1} h^{-1})
N = NH_4^+ or NO_3^- concentration of the culture medium
Vol = volume of medium at t = 0
t = time (h)
X = average dry weight of 10 x 10 g wet weight tissue segments

Volumes used in the calculation of uptake rates were adjusted at each time interval for samples taken out (60 ml).

Results

The depletion of ammonium and nitrate from the medium containing approx. 10 g of *P. palmata* fronds during the four hours of the nutrient uptake experiment can be seen in Figure 6.3. Within the first two hours of the experiment most of the ammonium had been removed from the medium. In all of the treatments the ammonium concentrations reached a minimum concentration of 1.2-1.7 μM after which no further decrease occurred. Nitrate concentrations decreased to near zero in all treatments. No significant loss of ammonium or nitrate was detected in the control vessels (see Table 6.1).

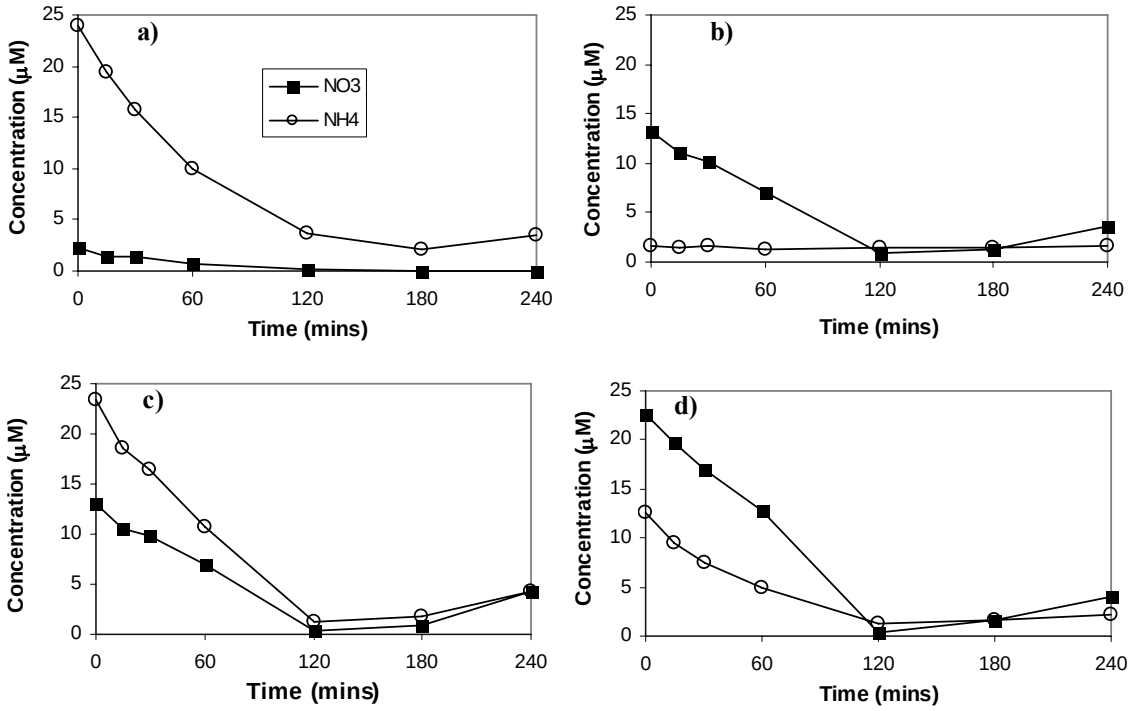


Figure 6.3 Ammonium and nitrate concentrations measured at intervals over 4 hours in containers after addition of 10 g fresh weight of *Palmaria palmata* fronds. The containers had seawater with four different initial NH_4^+ & NO_3^- concentrations. Treatments, **a)** high (20 µM) NH_4^+ & no NO_3^-, **b)** no NH_4^+ & intermediate (10 µM) NO_3^- , **c)** high (20 µM) NH_4^+ & intermediate (10 µM) NO_3^- , **d)** intermediate (10 µM) NH_4^+ & high (20 µM) NO_3^-. The experiment was conducted under saturating light at 12° C with moderate aeration.

Table. 6.1 Concentrations of ammonium and nitrate detected at the beginning and at the end of the experiment.

Concentration of nutrient added	At the start of the experiment	At the end of the experiment
NH_4^+, 20 µM	19.9 µM	20.1 µM
NO_3^-, 10 µM	13.4 µM	14.0 µM

Ammonium and nitrate were taken up simultaneously by *P. palmata.* When nitrate was present in the medium the rate of uptake of ammonium by *P. palmata* was not significantly different from when there was no nitrate was in the medium for any of the following: 0-15min, 15-30 min, 30-60 min or 60-120min (Figure 6.4 a and c). In the presence of ammonium the rate of uptake of nitrate was not significantly different from when there was no ammonium in the medium at: 0-15min, 15-30 min, 30-60 min or 60-120min, (Figure 6.4 b and c). Therefore, there was no evidence for inhibition of uptake of either nutrient by the presence of the other. The highest uptake rate for ammonium

(V_{max} = 24.1 µmol gDW^{-1} h^{-1}) was observed with 10 µM ammonium and 20 µM nitrate. The positive correlation between uptake rate and concentration of ammonium indicates a possible linear relationship between the two. This is not as obvious for nitrate however indicating possible uptake saturation at the concentrations used. The nitrate uptake rates of *P. palmata* did not differ significantly in 20 µM or 10 µM NO_3^- concentrations or in the presence of ammonium.

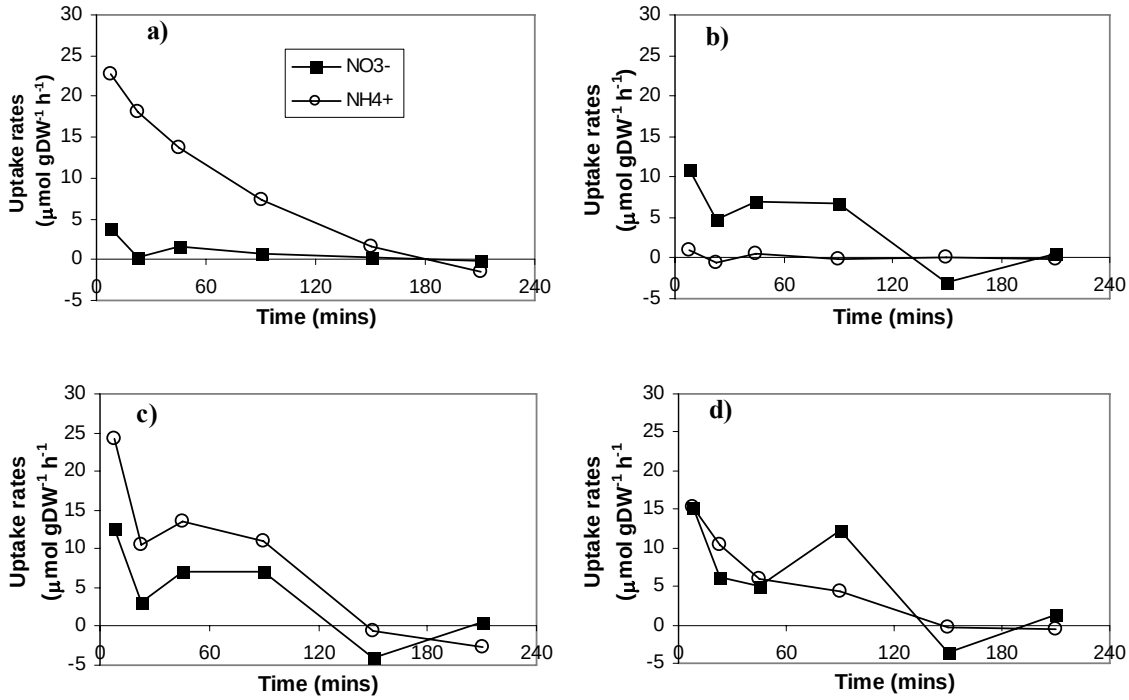


Figure 6.4 Ammonium and nitrate uptake rates of 10 g fresh weight of *Palmaria palmata* fronds in four different treatments at 12°C with aeration, over 0-15, 15-30, 30-60, 60–120, 120-180, 180-240 min from introduction of the algae. Treatments, **a)** high (20 µM) NH_4^+ & no NO_3^-, **b)** no NH_4^+ & intermediate (10 µM) NO_3^- , **c)** high (20 µM) NH_4^+ & intermediate (10 µM) NO_3^- , **d)** intermediate (10 µM) NH_4^+ & high (20 µM) NO_3^-. The experiment was conducted under saturating light at 12°C with moderate aeration.

Discussion

Palmaria palmata was able to take up ammonium and nitrate simultaneously (Figure7.5.) as has been found previously for *P. palmata* (Morgan and Simpson 1981c; Martinez and Rico 2004) and for *Palmaria mollis* (Demetropoulos and Langdon 2004).

The simultaneous uptake of ammonium and nitrate has also been reported for other species of red algae, for example *Gracilaria foliifera* (Delia and Deboer 1978), *G. pacifica* (Thomas and Harrison 1987) and *Neogardhiella baileyi* (D'Elia & DeBoer, 1978) as well as for the kelps *Laminaria saccharina* (Subandar *et al.* 1993; Ahn *et al.* 1998), *L. abyssalis* (Braga and YoneshigueValentin 1996) and *L. groenlandica* (Harrison 1986).

The presence of ammonium has been found to reduce nitrate uptake for a variety of algae such as *Gracilaria foliifera* (D'Elia and DeBoer 1978), *Codium fragile* (Hanisak and Harlin 1978), *Hypnea musciformis* (Haines and Wheeler 1978) and *Ulva fenestrata* and *Gracilaria pacifica* (Naldi and Wheeler, 2002). Ammonium does not seem to inhibit the uptake rate of nitrate in several kelps: *Laminaria longicruris* (Harlin and Craigie 1978), *L. saccharina* and *Nereocystis lutkeana* (Ahn *et al.* 1998).

In this study, for *Palmaria palmata*, the rate of uptake of ammonium was similar to that of nitrate at the concentrations investigated. These findings and the rates are comparable to the rates determined by Martinez and Rico (2004) for *Palmaria palmata*. For the concentrations investigated, there was a trend towards a linear relationship between concentration and uptake rate of ammonium, so that at higher concentrations than those tested here, ammonium may have higher uptake rates than nitrate as nitrate appears to becoming saturating at the concentrations investigated. Morgan and Simpson (1981a) found higher uptake rates for ammonium than for nitrate, but they had concentrations of ammonium of 0.5 – 2 mM in their study.

Martinez and Rico (2004) found a V_{max} of 19.4 μmol gDW^{-1} h^{-1} for ammonium for summer plants which suggests that over the range of concentrations tested here, uptake

saturation for ammonium may not have been reached. Martinez and Rico (2004) stated that saturation with either NO_3^- or NH_4^+ may be dependent on the nutritional history of the plants. Winter plants may have even lower saturation concentrations for ammonium because of higher thallus nutrient content. Morgan and Simpson (1981) found that despite faster ammonium uptake, nitrate supported higher growth rates. Nitrate is expected to be a better N source for the mariculture of *P. palmata*. Despite this, when ammonium is the only N source available, the algae will utilise this source at the concentrations experienced in the field.

6.3 *Palmaria palmata* can store nitrogen – for what periods can it do so? What internal levels of nitrogen are indicative of nitrogen depletion?

Generally, uptake rates for nitrate, ammonium and phosphate are higher in opportunistic, filamentous and tubular or monostromatic algae with a high SA : V ratio, while long-lived, late-successional algae with parenchymatous-like thalli and a low SA : V ratio have lower uptake rates and correspondingly slower growth rates (Littler and Littler 1980; Wallentinus 1984; Lobban and Harrison 1996). *Palmaria palmata* has a thin flat thallus with a moderately high growth rate. Growth strategies are further enhanced for *P. palmata* by the capacity for storage of nitrogen (Martinez and Rico 2002).

Close to the salmon cages, nutrient interception by *P. palmata* is likely to be patchy depending on the proximity to the cages and location with respect to current patterns. Lack of exposure to nutrients for longer periods can be expected to favour algae that can store nutrients. These factors are particularly critical during the summer months when ambient nitrate levels are low. This section aims to determine how long stored nitrogen supports growth of the *P. palmata* and critical percentage nitrogen content. These values are to be compared with two macroalgae of contrasting morphologies *Enteromorpha* (*Ulva*) *intestinalis* and *Polysiphonia lanosa*.

The assumption that *Palmaria palmata* can store nitrogen to the same extent when supplied as either ammonium or nitrate is equivocal. Knowledge of levels of nitrogen in

P. palmata that are reflective of nitrogen depletion is necessary for identification of nitrogen-limited plants in the field.

Methods

Experimental seaweeds were gathered from the rocky shore near Oban on 5 March 2005. Three species were selected primarily on the basis of differences in their morphologies. The species chosen were the red foliose alga *Palmaria palmata,* the green tubular monostromatic green alga *Enteromorpha* (*Ulva*) *intestinalis*, and the red filamentous alga *Polysiphonia lanosa. Enteromorpha intestinalis* and *Polysiphonia* were found growing in the intertidal zone. *Enteromorpha* (*Ulva*) *intestinalis* was found growing in rock pools and *Polysiphonia* was growing as an epiphyte on *Ascophyllum nodosum. Palmaria palmata* was an epiphyte attached to *Laminaria hyperborea* stipes in the sublittoral fringe.

The algae were drip-dried in a colander and the wet weighed. *Palmaria* and *Polysiphonia*, and 20 g of *Enteromorpha* were placed into three 10-litre culture vessels. Each vessel contained 8 litres of filtered (1 μm) seawater with f/2 nutrients and was exposed to 100 μmol photon m^{-2} s^{-1} provided by two cool white Osram 58 watt (Lumilux 58W/840) fluorescent lights on a 16:8 hour light:dark cycle for 11 days. The seawater in all the buckets was aerated vigorously using small airstones in a room maintained at 11^{o}C. After 11 days, the algae were weighed, samples reduced to original weights and excess algae frozen and stored for carbon and nitrogen analysis. The algae were returned to containers with filtered seawater but with nutrients at f/2 levels for phosphate, trace metals only and no nitrogenous nutrients.

The wet-weighing procedure was repeated every three to four days until the experiment was terminated 29 days later on 14th April. *E. intestinalis* was reduced to 10 g on 14th March, to bring it in line with the other species. At each weighing, the buckets were replenished with deionised water to the original level marked on the buckets, and algae were reduced to 10 g wet weight and subsampled for later analysis (< 0.5 g sampled once algae had stopped growing).

The samples of macroalgae were washed carefully in distilled water and freeze-dried until constant weight. They were ground to a powder and stored in an air-tight desiccator in the dark until analysed. Total carbon and nitrogen content was determined with a Europa Scientific CHN analyser, using acetilamide as a standard, following the procedure described by Hedges & Stern (1984).

Data Analysis

Relative growth rates (g $g^{-1}d^{-1}$) of the three algae were calculated as described earlier.

Results

Growth rates for *Palmaria* and *Enteromorpha* peaked at 0.11 and 0.14 g $g^{-1}day^{-1}$ within the first week after nitrogenous nutrients were stopped (Figure 6.6, 0-4 days for *E. intestinalis* and 4-6 days for *P. palmata*). The change in growth rate for the *Polysiphonia* over this period was not as marked although this alga also appeared to peak at around this time (4-6 days). Algal growth decreased to a minimum for all three species at 12-15 days after deprivation of nutrients.

There were small changes in the percentage of carbon for the three algae over the time course of the experiment (Figure 6.7), with a slight decrease for *E. intestinalis* and

Polysiphonia from 35.4 % and 34.2 % to 33.2 % and 29.1 % respectively. *Palmaria palmata* remained steady at 40-43 % after a high initial result (49.5%).

Percentage nitrogen content started at a high for *E. intestinalis*, *Polysiphonia* and *P. palmata* of 4.5, 5.3 and 6.3% and finishes at lows of 0.8, 2.7 and 1.7% respectively (Figure 6.8).

Carbon to nitrogen ratios of the three algae (mass) reflected the pattern for nitrogen content (Figure 6.9). All three had ratios of 6-8 initially, finishing on values of 43.8, 25.5 and 10.8 for *E. intestinalis*, *P. palmata* and *Polysiphonia* respectively.

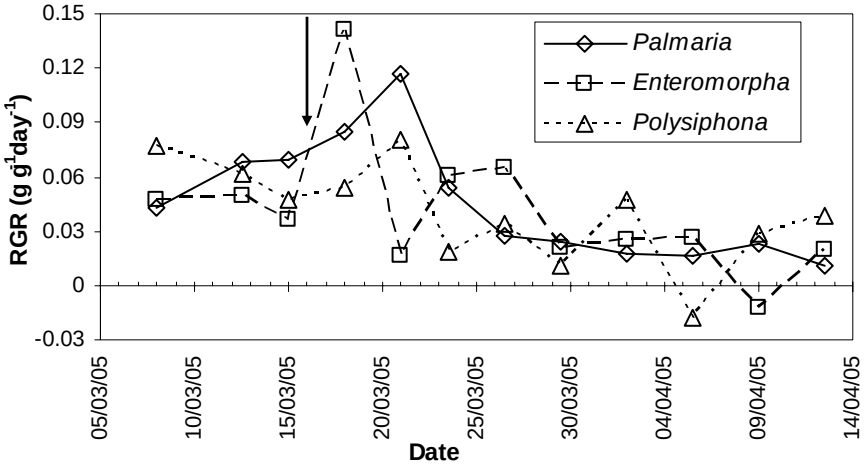


Figure 6.6 Mean relative growth rates of the three species of macroalga in nitrogen-depleted seawater medium, initiated 16/3/05. Growth rates refer to period between sampling events. The arrow indicates when nitrogenous nutrients were stopped.

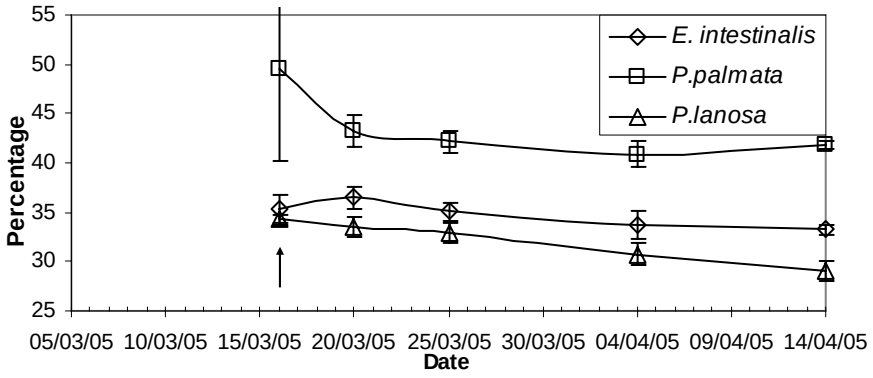


Figure 6.7 Carbon as a percentage of dry weight for the three species of alga over the term of the growth period (vertical bars indicate 95% CI). The arrow indicates when nitrogenous nutrients were stopped.

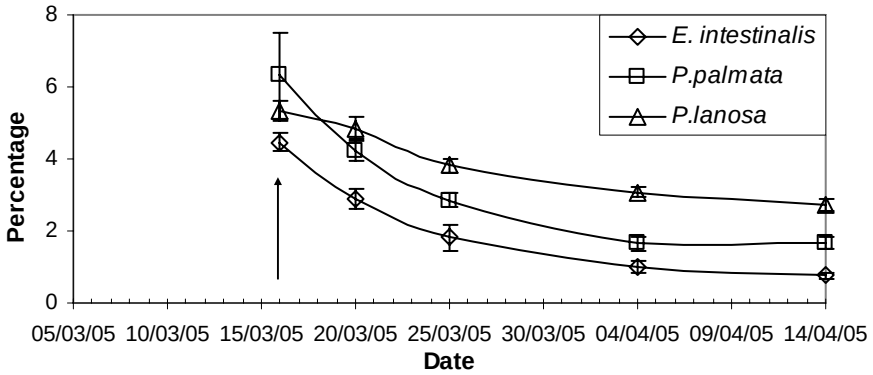


Figure 6.8 Nitrogen as a percentage of dry weight for the three species of alga with time over the term of the growth period (vertical bars indicate 95% CI). The arrow indicates when nitrogenous nutrients were stopped.

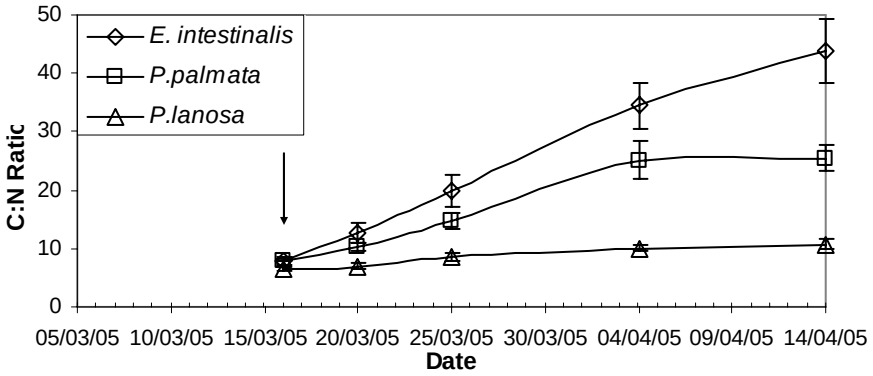


Figure 6.9 Carbon to nitrogen ratio (mass) for the three species of algae with time over the term of the growth period (vertical bars indicate 95% CI). Arrow indicates when nitrogenous nutrients were stopped.

Discussion

Measured growth rates for individual species were quite variable. Growth rates were monitored using the wet weights of the algae and despite due care, some of the variation in calculated growth rates may be due to differing amounts of retained water between sampling. *Palmaria palmata* does not appear to be as prone to such variation as in the other two algae which may be due to this alga's morphology. *E. intestinalis* has a tubular morphology which is likely to hold water and *Polysiphonia* is a semi rigid filamentous alga which retains water in its branches *P. palmata* is a flat alga which is easier to dry.

Culture media for *E. intestinalis* were observed to take on a green tinge between the 14th and 20th of March suggesting that the alga may have initiated spore release. This may have impacted on overall growth rates of the alga although it coincides with the higher growth rates measured.

Maximum growth rates for *P. palmata* and *E. intestinalis* are on a par with previous studies for these algae (e.g. Browne 2001, Nielsen and Sandjensen 1990). At the time of high growth rates, percentage nitrogen contents of the algae compare well with higher levels measured in other studies (e.g. Martinez and Rico 2002). The lowest percentage nitrogen was measured fifteen days after nutrient deprivation which also corresponded to lowest growth rates suggesting that these algae may have been nitrogen depleted at this stage.

These findings concur with those of Demetropoulos and Langdon (2004) for *P. mollis* and Morgan and Simpson (1981) for *Palmaria palmata.* These studies found no significant difference between growth rates for the algae supplied with nutrients every day and those that are supplied every seven days. Hence, the alga can store sufficient

nitrogen for maximal growth over a seven day period. Nagler *et al.* (2003) had similar results for *Gracilaria parvispora. G. parvispora* grown in nitrogen enriched medium prior to deployment to a low nutrient ocean lagoon grew well for 14 days, but after 21 days growth ceased due to depletion of stored nutrients. Nitrogen content of the thalli was 2.5-5% of dry weight before outplanting to the sea and decreased to 1 % as the nitrogen was mobilised to support growth.

The initial nitrogen enriched thalli have C:N ratios comparable to general ratios for quoted for macroalgae (Atkinson and Smith 1983) of 10:1 and the Redfield C:N ratio of 6.6 for phytoplankton. At maximal growth, the macroalgae in this study had similar C: N ratios but change relative to each other as starvation progresses. Percentage nitrogen and C:N ratio for *E. intestinalis* appear to be still declining at the close of the experiment suggesting they may still not be nitrogen limited at 29 days. *Palmaria palmata* and *Polysiphonia* however appear to have been nitrogen limited by 4/4/05 i.e. after 19 days.

The variation in growth rates experienced for these algae limits the conclusions regarding comparison of growth rates and percent nitrogen between species. Growth rates of *Polysiphonia* appear low (although on a par with the RGR measured for *Polysiphonia nigrescens* of 0.070 g $g^{-1}day^{-1}$ by Nielsen and Sandjensen 1990) and not necessarily indicative of optimal growth. Under conditions of optimal growth, *Polysiphonia* may be expected to deplete nitrogen reserves earlier than demonstrated here, as it has a high SA:V ratio (surface area to volume). *Palmaria palmata* and *E. intestinalis* superficially have similar morphologies but *E. intestinalis* is commonly one cell in thickness and *Palmaria palmata* is at least 2-8 cells thick. The higher cell numbers may allow *P. palmata* to have better nitrogen reserves. However, this is not

necessarily reflected in the results here, with the two algae showing similar growth rates and rates of depletion of reserves.

Ability to store nitrogen by *Palmaria palmata* for periods of 7 to 21 days in a nutrient poor environment means that *P. palmata* requires only intermittent exposure to nutrients at these time scales to survive and grow. This may have implications for culture of the alga in the vicinity of fish farms. The regularity and duration of exposure of algae to nutrients at varying distances from the farm will be dependent on the prevailing currents and fish feeding regimes around the farm. Algae with the capacity for nutrient storage may benefit from nutrients originating from the farm for greater distances. Tidal currents vary at time scales of 6 to 12 hours. Meteorologically driven changes to currents operate in conjunction with tides and operate at time scales of weeks, months and years. The sum of these factors and how they interact with daily variations in fish feeding and ammonium input will largely determine how far from the farm algae will be exposed to nutrients and the duration and timing of the exposure. Dilution and rate of uptake of the nutrients by other organisms such as phytoplankton and bacteria will also affect the concentration of the nutrients and should be factored into models to indicate temporal availability.

6.4 Is ammonium taken up evenly over the thallus of *Palmaria palmata*? Is there translocation of nitrogen to new tissue?

Part of this thesis investigated the potential for using stable nitrogen isotope analysis of *P. palmata* in the vicinity of the fish farm cages to determine if farm-derived nitrogen is taken up by seaweeds and, if so, how far from the cages. To standardise the sampling of *P. palmata*, tips only of the plants were used for analysis (including tissue up to 0.75 cm from the apex). The sampling of the tips was also preferred because epiphytes tend to colonise older tissue towards the base. As we were sampling only the tips, it was desirable to know how representative of overall ^{15}N levels in the plants this sampling represented, whether recent nutrient history was reflected in samples from the tips and for how long.

As the plants grow from the apices, this is newer tissue, and is likely to consist principally of nitrogen taken up recently. At times of moderate to high growth this will be nitrogen incorporated over the previous one to two weeks based on growth rates determined in culture (Kakkonen 2004). If the supplied nitrogen varies in its isotopic ratio, then we may see variation in the isotopic ratio throught the thallus depending on the isotopic ratio of the supplied nitrogen at the time of growth.

There are two naturally occurring forms of nitrogen. There is the common form of nitrogen which contains seven protons and seven neutrons and is referred to as nitrogen 14 (^{14}N), and a heavier form that contains an extra neutron called nitrogen 15 (^{15}N).

Normally, the relative amount of ^{15}N to ^{14}N is determined relative to a standard and is described by the following:

$$\delta^{15}N\ (‰) = ((R_{sample}/R_{standard})-1)\ \times 10^3$$

where R_{sample} is the ratio of ^{15}N to ^{14}N of the sample and $R_{standard}$ is the ratio for a standard which for nitrogen is the ratio for atmospheric nitrogen. The accepted value for nitrogen isotope abundances in air is 0.0036765 (Junk and Svec 1958; Mariotti 1984). In this section, ammonium enriched with ^{15}N with an abundance of almost 1.0 (0.98) was used.

The aim of the section was to determine where in the *P. palmata* thallus ammonium is taken up. Algae with two different nutrient histories were used to see how this affected nutrient uptake. One week after exposure to the isotopically enriched ammonium, the algae were re-sampled to see if the enriched ^{15}N could be detected in new tissue. The results will show how representative sampling from the tips gives of distribution of ^{15}N in the thallus and movement of nitrogen within the thallus.

Methods

Palmaria palmata attached to *Laminaria hyperborea* stipes was gathered from Calbha Bay in north west Scotland (24/2/2005, see chapter 2 map). The algae were transferred to Dunstaffnage Marine Laboratories where they were held in cool aerated seawater until used a few days later. Plants chosen for use in the experiment were clean and healthy and those with obvious associated epifauna (e.g., bryozoans) were rejected.

The *Palmaria palmata* plants were rinsed with filtered (1 μm) seawater, padded dry with absorbent paper, weighed (10 g) and placed in 6 x 10-litre containers containing 5 litres of filtered (1 μm) seawater. Three of the containers had a full complement of f/2

nutrients ('Enriched' algae) and three containers had only trace metals, phosphate at f/2 concentrations i.e. with. no nitrogenous based nutrients ('Unenriched' algae).

The seawater in all buckets was aerated in a culture room maintained at 11^{o}C with fluorescent light providing 100 μmol photon $m^{-2}s^{-1}$ at the level of the cultures with a 16:8 hour light : dark cycle.

After one week, the *Palmaria palmata* samples were again wet-weighed and reduced to 12 g; the excess material was frozen for later analysis. The samples were added to 2.4 litre transparent plastic containers containing 1μm filtered seawater with 50 μM $^{15}NH_4Cl$ (98 atom % ^{15}N Sigma Aldrich, product No. 299251) and phosphate and trace metals at f/2 concentrations. A further three containers containing the same medium but no algae were run as controls to check for dissipation of ammonium over the time of the experiment. Seawater and nutrient media in all treatments had moderate aeration.

Seawater samples (50 ml) were taken at 0, 15 minutes, 1, 2, 4 and 6 hours after addition of the *P. palmata* to determine the amount of $^{15}NH_4Cl$ taken up and hence uptake rates. Seawater samples were also taken from controls, before and after the samples were exposed to media containing $^{15}NH_4Cl$. Samples (50 ml) were taken using a syringe and were passed through a 0.45 μM filter. Seawater samples were injected into acid washed plastic bottles that had been rinsed using 10 ml seawater from the container being sampled. Seawater samples were then stored in a freezer for later analysis by autoanalyser. Ammonium, ortho-phosphate, nitrate and nitrite in the seawater samples were measured on a Lachat Instruments autoanalyser (QuickChem 8000) following the method of Grasshoff (Grasshoff 1976). Analysis of the seawater samples was carried out in triplicate. In this instance, where high enrichment levels of ^{15}N were used, the results were expressed most conveniently in terms of atom percent (atom %).

By definition:

$$\text{atom \%} = \text{fractional abundance} \times 100$$

$$\text{ppm} = \text{fractional abundance} \times 10^6$$

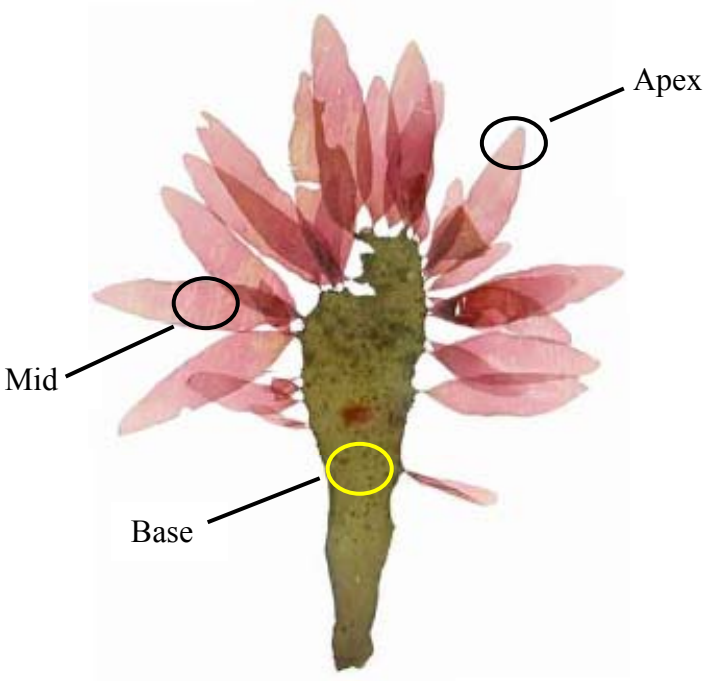


Figure 6.10 Diagram illustrating positions of different tissue types used for heavy isotope analysis of *Palmaria palmata*. Frond tissue samples were collected from the apical tip, mid-thallus, and older tissue as indicated. The illustrated plant is approximately 15 cm high. Picture source: www.cryptogamicbotany.com/ images/oa_rhodo/Pal.

At the end of six hours, the samples were wet-weighed and reduced to 10 g, with the excess (2 g) frozen for later analysis. The 10 g samples of *Palmaria palmata* were returned to the 6 x 10-litre containers each containing 5 l of filtered seawater with a full complement of f/2 nutrients (i.e. with nitrogenous nutrients).

One week later, final wet-weights were recorded and sub-sampled for analysis. All plant samples were rinsed in deionised water and duplicate 0.085 g wet-weight samples of the three different tissue types (apex, mid and base; see Figure 6.l0) were cut from *P. palmata* before exposure, directly after exposure and one week after the 6 hour exposure

to $^{15}NH_4Cl$ from each of the three replicates for the Enriched and Unenriched treatments and placed inside preweighed tin capsules. Samples were left in a drying oven for 48 h at 60^{o}C. On removal from the drying oven, the samples were cooled and weighed. Trays of samples were kept in an airtight jar containing a dessicant until analysed for nitrogen isotopes and total carbon and nitrogen with a ANCA-SL/GSL by PDZ Europa 20-20 mass spectrometer.

Relative growth rates (RGR: g $g^{-1}day^{-1}$) of *P. palmata* tissues were calculated as described previously, for the pre-conditioning treatment (2-9 March) and after the addition of $^{15}NH_4Cl$ (9-14 March 2005).

Where data met with the pre-conditions of normality, homogeneity of variance and independence, the difference between treatments was analysed by using Analysis of Variance (ANOVA) statistical analysis which was conducted using Minitab 14 statistical analysis programme.

Results

Growth rates of *P. palmata* are depicted in Figure 6.11. The results show that there was a significant difference between the growth rates of 'Enriched' and the 'Unenriched' algae in the preconditioning period. The growth of the algae with full f/2 nutrients was lower in the preconditioning period than after. Growth rates under full f/2 nutrients were comparable with previous results (i.e. RGRs of 0.05 to 0.15 g $g^{-1}d^{-1}$). The difference between the two growth periods may relate to an adaptation phase when the algae were first cultured under experimental conditions or the alga may have been at higher densities in the container for the earlier growth period limiting light availability.

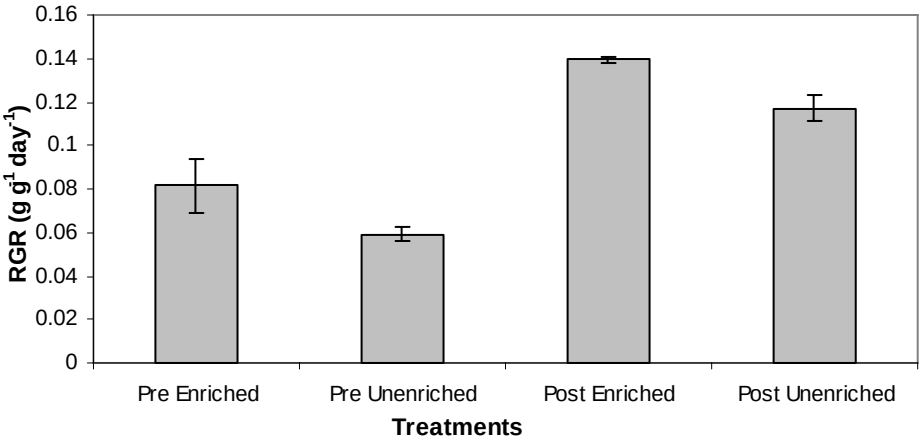


Figure 6.11 Mean relative growth rates calculated using wet-weights of *Palmaria palmata* samples after pre-conditioning treatment (2-9 March), and post exposure (9-14 March) to $^{15}NH_4Cl$ medium (vertical bars indicate 95% CI).

Mass spectrometer analysis

The bar graphs below (Figures 6.12) show the results for analysis of the heavy ^{15}N isotope in the three different tissue types for Enriched and Unenriched treatments for samples taken before exposure, directly after exposure and one week after exposure to $^{15}NH_4Cl$. A two-way ANOVA was conducted for differences in the amounts of ^{15}N, percentage nitrogen and carbon for tissue types (base, mid and apex) and between Enriched and Unenriched plants for each of the time periods, before, immediately after and one week after exposure to $^{15}NH_4Cl$ (see Table 6.2).

Before exposure

Before exposure to $^{15}NH_4Cl$, the mean value for At%^{15}N of the plant tissues was 0.38% with no significant difference between tissue types. For percentage nitrogen there was a highly significant difference between Enriched and Unenriched tissues, with means of 6.4 % and 3.8 % of dry weight respectively. There was no significant difference for percentage carbon and Carbon: Nitrogen ratios. The results for Carbon: Nitrogen ratios reflect percentage nitrogen results across all time periods.

Immediatley after exposure

Immediatley after six hours exposure to $^{15}NH_4Cl$, there were highly significant differences (log transformation of data) between At%^{15}N for tissue type and Enriched *versus* Unenriched plants. Apex and Mid parts of the plants had greater abundances than the basal parts (4.8, 3.4, 1.6 At%^{15}N, respectively). Unenriched plants had greater levels than Enriched (4.1, 2.5 At%^{15}N).

Percentage nitrogen remained significantly higher in Enriched plants than Unenriched (6.3, 4.1%). Percentage carbon showed a similar trend, being significantly higher in Enriched plants than Unenriched (44.6, 40.3%).

One week after exposure

For ^{15}N content, the apex and mid sections had greater abundances for Unenriched (2.5, 2.2%) versus Enriched (1.7, 1.7%) , but there was little difference between the two lots of plants for the base (1.2, 1.3%). For percent nitrogen, the apex had higher levels than mid or base (7.9, 5.7, 5.1 %) and Enriched remained significantly higher than Unenriched (7.1, 5.4%). There were significant differences for percentage carbon between tissue types and conditioning of plants. The base had higher percent carbon than the apex or mid (Apex: 36.0, Mid: 37.5, Base: 43.6) and Enriched plants had higher levels than Unenriched (42.1, 36.0%).

Table 6.2 ANOVA results for comparisons between tissue types, Unenriched and Enriched treatments and before exposure to $^{15}NH_4Cl$, directly after exposure and one week later; Means, F ratios and significance level (*p*).

	Mean values: Apex /Mid / Base	***F***	**p**	**En / Unenriched**	***F***	**p**	**Inter-action**	***F***	**p**
BEFORE									
Atom %	0.38		ns	0.38					ns
N %	5.6/5.0/4.8		ns	6.4/3.8	57.8	<.001		4.3	.04
C %	40.2/41.9/44.8		ns	42.3/42.4		ns			ns
AFTER									
Atom %	4.9/3.3/1.6	66.28	<.001	2.4/4.1	34.7	<.001			ns
N %	5.8/4.8/5.0	2.2	ns	6.3/4.1	25.6	<.001			ns
C %	42.9/40.4/43.9	2.4	Ns	40.3/44.6	10.1	<.01			ns
ONE WEEK									
Atom %	2.1/2.0/1.3	125.9	<.001	1.5/2.0	95.6	<.001		32.5	<.001
N %	7.9/5.7/5.2	15.48	<.001	7.1/5.4	18.25	<.001			ns
C %	36.0/37.5/43.6	5.06	.026	42.1/36.0	8.51	.013			ns

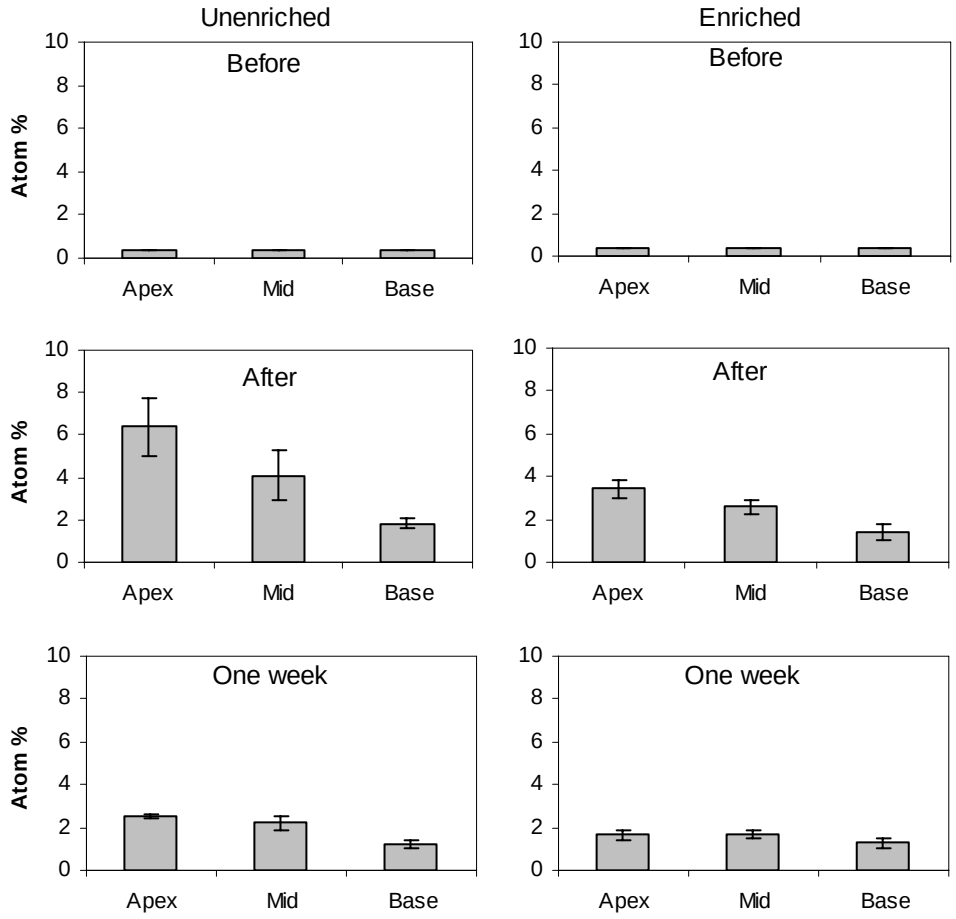


Figure 6.12 ^{15}N levels in conditioned plants before, directly after and one week after exposure to $^{15}NH_4^+$ for the three tissue types (vertical bars indicate 95% CI).

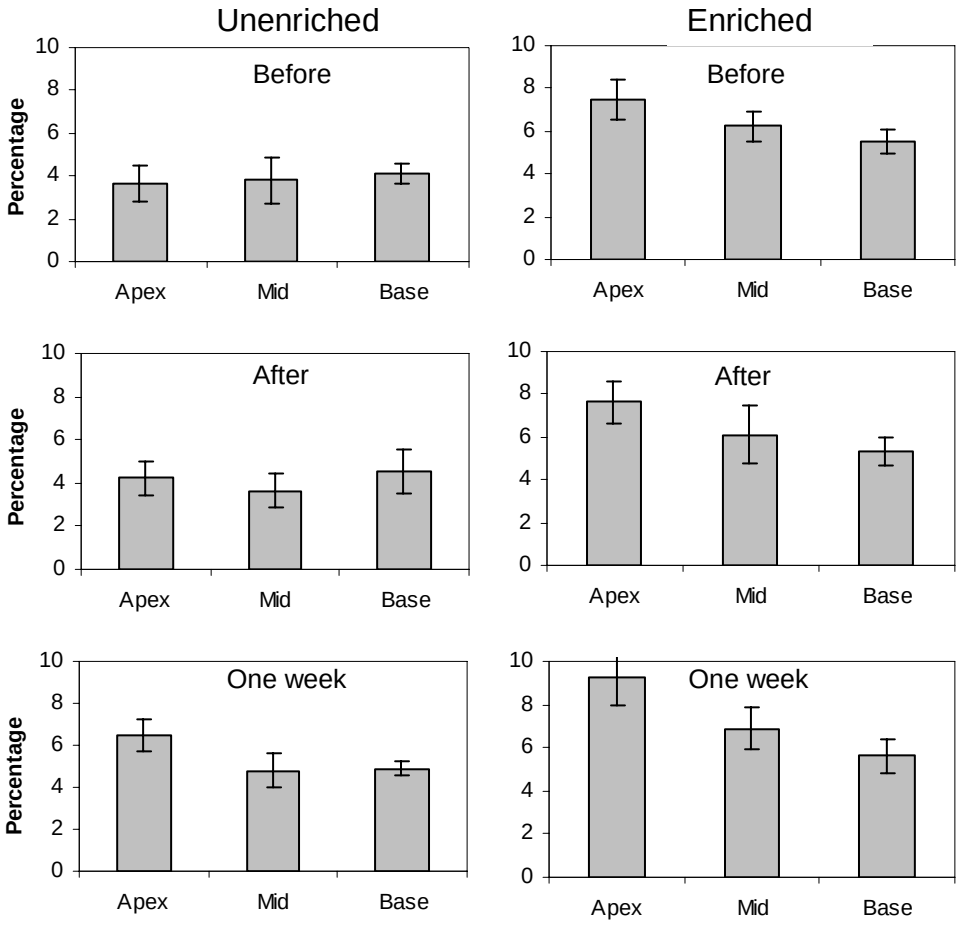


Figure 6.13 Percentage nitrogen levels in conditioned plants before, directly after and one week after exposure to $^{15}NH_4^+$ for the three distinguished tissue types (vertical bars indicate 95% CI).

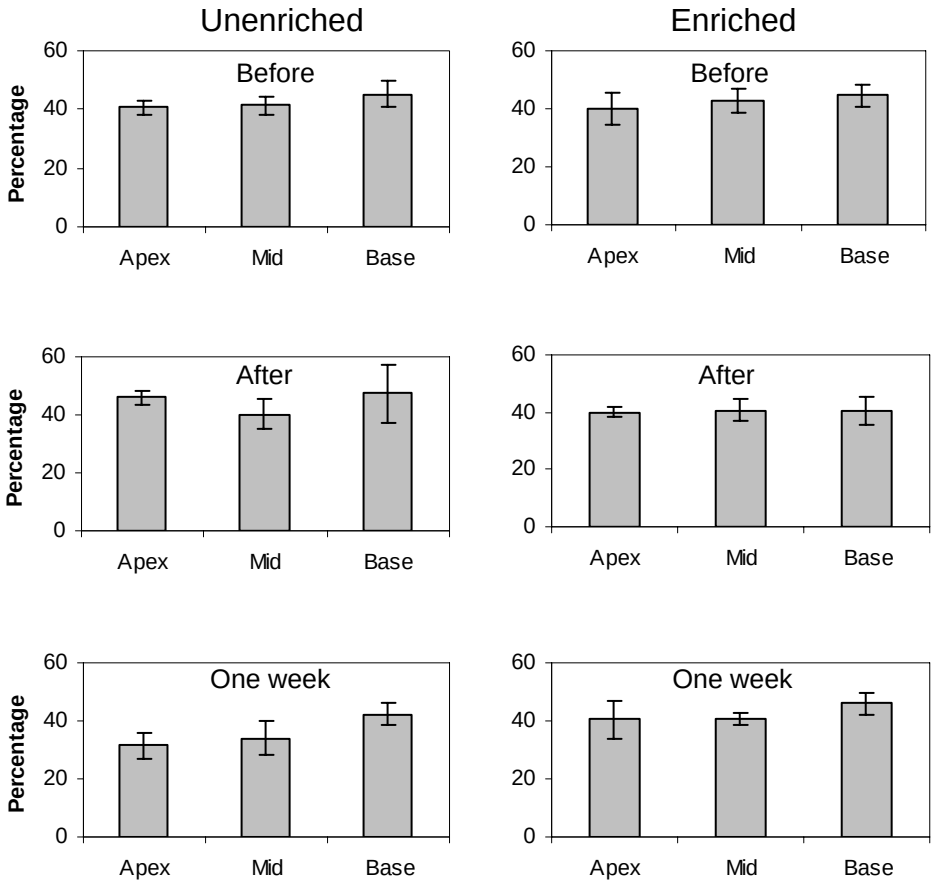


Figure 6.14 Percentage carbon levels in conditioned plants before, directly after and one week after exposure to $^{15}NH_4^+$ for the three distinct tissue types (vertical bars indicate 95% CI).

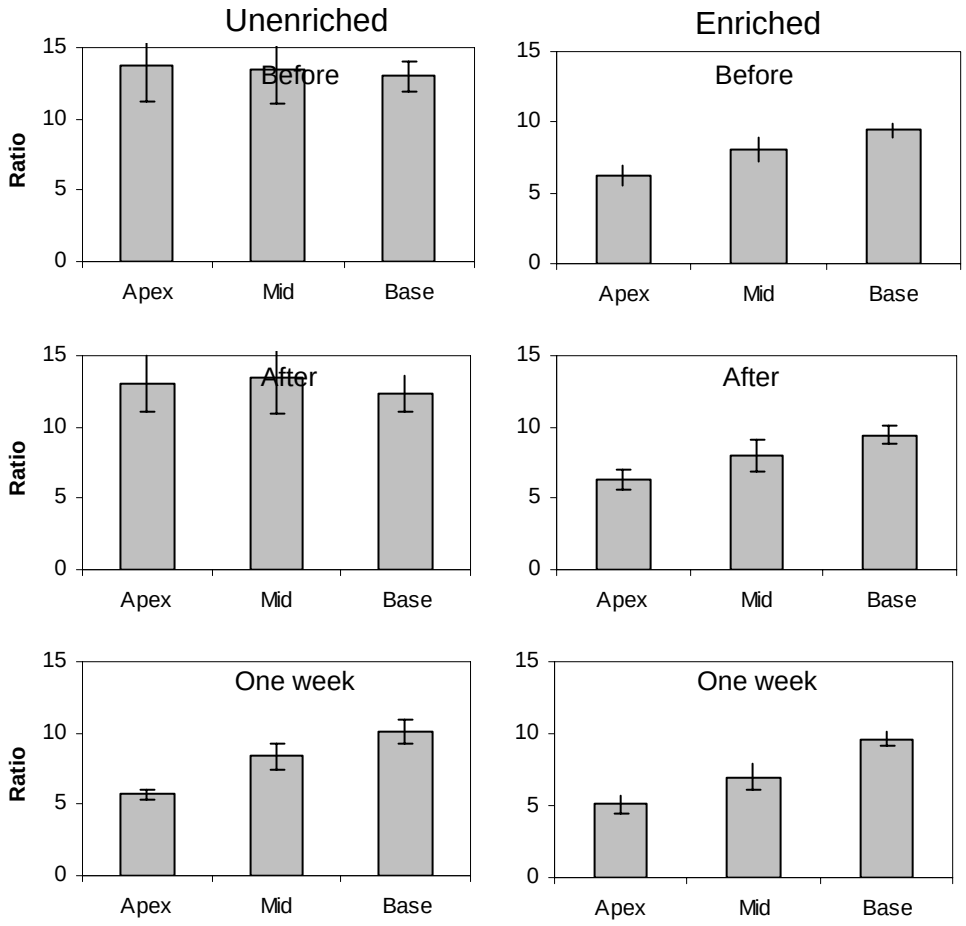


Figure 6.15 Carbon to nitrogen ratios in conditioned plants before, directly after and one week after exposure to $^{15}NH_4Cl$ for the three distinct tissue types (vertical bars indicate 95% CI).

Ammonium, nitrate, nitrite and phosphate concentrations for the duration of the uptake experiment are depicted in Figure 6.16. They show that uptake of ammonium by the Enriched algae was slower than by the Unenriched algae; this is also reflected in calculated uptake rates in Figure 6.17. Figure 6.17 also suggests that the principal reason for the difference may be an initial surge uptake by the Unenriched algae. Note that while there was a large apparent difference in uptake between the two different preconditioned algae, a t-test at 15 min did not give a significant difference, due to the high variability of the uptake by the Unenriched algae.

The pattern for nitrate is similar to, although not as marked as that for ammonium; i.e. uptake of ammonium by the Enriched algae is slower than by the Unenriched algae. The nitrite and phosphate concentrations show changes at approximately two hours, corresponding to when the ammonium and nitrate were completely absorbed by the algae. The bigger difference was for the phosphate with the solutions for the Enriched algae containing less phosphate at the end of the experiment.

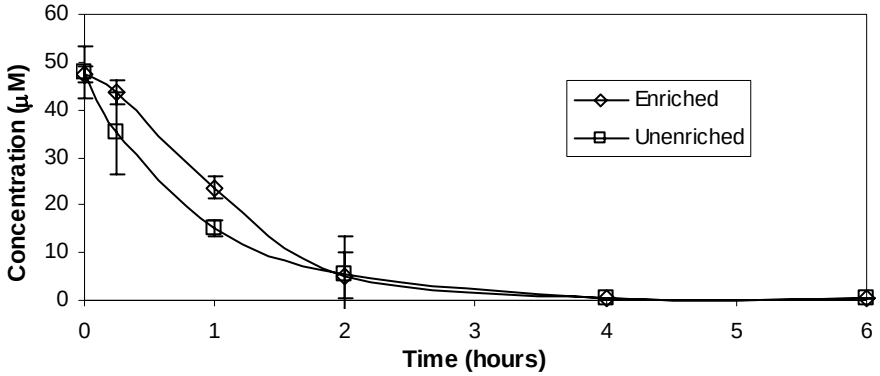


Figure 6.16 a) Changes in concentration over time of ammonium in the culture media (vertical bars indicate 95% CI).

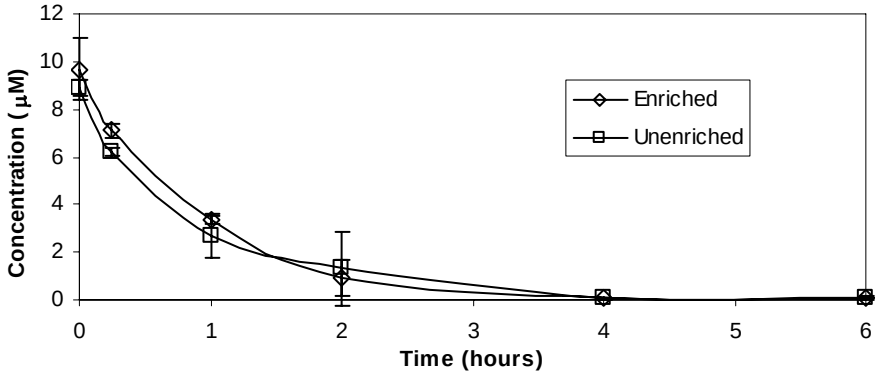


Figure 6.16 b) Changes in concentration over time of nitrate in the culture media (vertical bars indicate 95% CI).

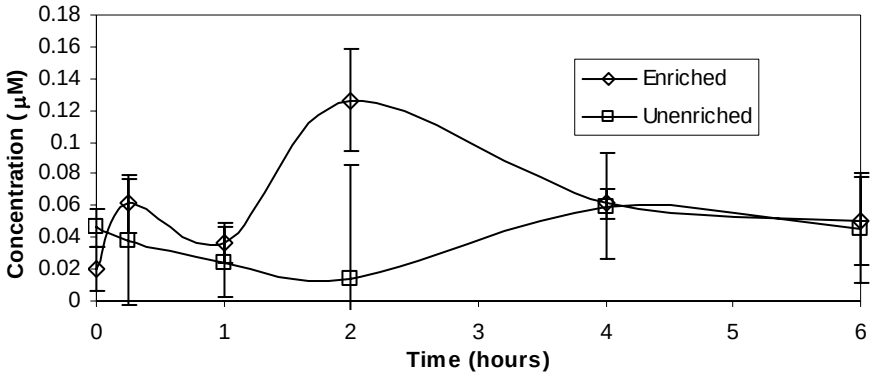


Figure 6.16 c) Changes in concentration over time of nitrite in the culture media (vertical bars indicate 95% CI).

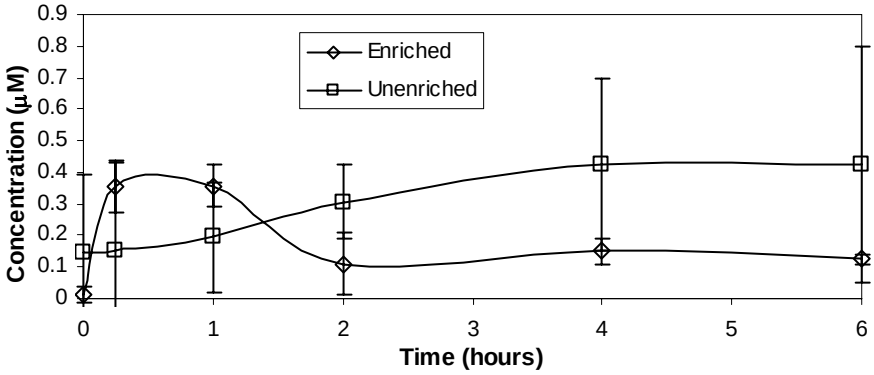


Figure 6.16 d) Changes in concentration over time of phosphate in the culture media (vertical bars indicate 95% CI).

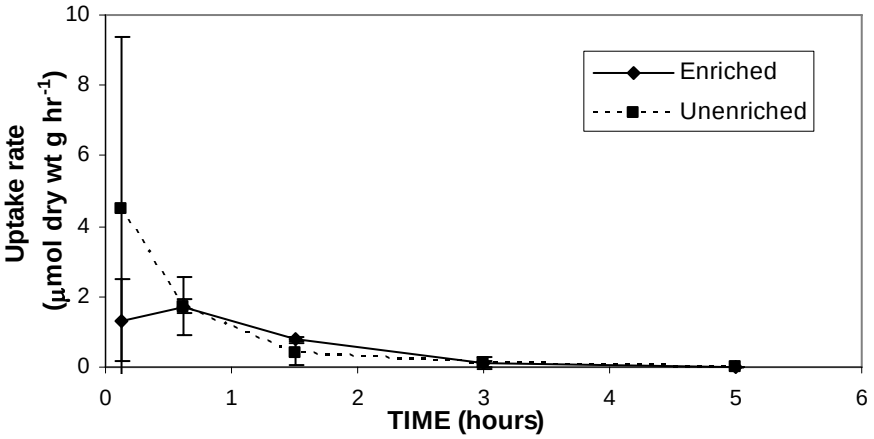


Figure 6.17 Variation in uptake rates with time of ammonium in the culture solutions (vertical bars indicate 95% CI).

Discussion

Nitrogen isotope abundances in air are used as the standard for ^{15}N and the accepted value is 0.0036765 (Junk and Svec 1958; Mariotti 1984) or 0.37 % which compares well with the levels found in the 'before' *P. palmata* of 0.38 % (Figure 6.12). Immediately after exposure in both pre-conditioning treatments, mid and upper parts of the plants had more than double the levels of those at the base. Within Unenriched plants there was more than three times as much in the apices as in the bases. This shows that there were large differences in uptake rates of ammonium across the thallus. There were also significant differences between the differently conditioned plants.

At an RGR of 0.1 g $g^{-1}day^{-1}$ the *Palmaria palmata* fronds may be expected to increase in length by 1.5 cm over a period of a week (Kakkonen 2004). This indicates that the majority of sampled apical tissue for this experiment is new tissue. Despite this, for the one-week post-conditioned algae, there was still a high Atom% ^{15}N in the apical tissue. This may have arisen by translocation of nitrogen within the tissue. Alternatively the cells storing the nitrogen may be expanding with the growing tip, taking the nitrogen with them. Although the apparent translocation to the tips, there is still a large difference in the Atom%^{15}N between the base and the upper parts of the thallus.

Evidence of long-distance transport in red algae includes the use of the tracer in *Delesseria sanguinea* and the seasonal growth of perennial algae where the gain in biomass in growing organs exceeds the local net carbon gain from photosynthesis *minus* respiration. Over short distances (less than, or equal to a few millimetres) there are well authenticated examples of symplasmic transport in red algae related to reproduction in the development of the carposporophyte and in red algal parasitic or other red algae (Raven 2003).

Under nitrogen limitation, intracellular nitrogen pools may be low and subsequent uptake rates may be greater due to a 'pool filling phase' that occurs in nitrogen limited algae (Lobban and Harrison 1996). The extent to which this occurs depends on factors such as the age of the tissue resulting in variation within the thallus. This experiment indicates that for samples taken that include all three tissue types (base, mid, apex), calculation of uptake rates or nitrogen contents may be influenced by the proportions of the different tissue types within the samples. Either all three tissue types and their proportions should be considered when calculating uptake rates or only one should be sampled consistently to obtain a true reflection of uptake rates.

Very high levels of nitrogen were recorded in the *P. palmata* tips with a maximum of 9.3% in the Enriched tips one week after exposure to the ammonium. This compares with values of 5% by Martinez and Rico (2002), 4% by Hagen Rødde *et al.* (2004) and 5.83% by Demetropoulos and Langdon (2004) for *Palmaria mollis* plant tissue. Using a standard conversion factor of 6.25 to estimate protein levels, this indicates protein levels may be as high as 58%. The elevated levels may be a reflection of the winter adapted algae used for the samples and the combination of ammonium and nitrate used for nutrient supply. The tips also may have tissues that are likely to have larger levels of free space for luxury nutrient uptake.

CONCLUSIONS

- Concentrations of ammonium greater than 100 - 500 μM in seawater with other nutrients in abundance, renewed weekly, at stocking densities of 1 g 10–litre^{-1} has a deleterious effect on the growth rate of *P. palmata*.

- Levels of ammonium of 3520 μM (nitrogen at f/1 rates as per Guillard and Rhyther 1962) in seawater with other nutrients in abundance, renewed weekly, immediately slows growth for *P. palmata* at stocking densities of 1 g 10–litre^{-1}.

- *P. palmata* is able to sustain the same level of growth when supplied with high concentrations of nitrate or with low concentrations of ammonium.

- *P. palmata* is able to take up both ammonium and nitrate simultaneously.

- Ammonium uptake does not inhibit nitrate uptake.

- The uptake rate of nitrate may be saturated at 10 – 20 μM whereas ammonium was not.

- *Palmaria palmata* with high initial nitrogen reserves and under optimal growth conditions can maintain growth for at least 15 days in a low nitrogen environment (other nutrients in excess).

- Levels of nitrogen percentage of dry weight of less than 2% or C:N ratios of greater than 25 are reflective of nitrogen starved *P. palmata* plants.

- Uptake of ^{15}N is higher in nitrogen starved plants.

- There are differences in uptake between apical, mid frond and basal tissue of *P. palmata* plants, the extent of difference dependent on the nutrient history of the plants and the time since exposure.

- There is transport of ^{15}N to newly growing apical tissue.

CHAPTER 7

VARIATION IN STABLE ISOTOPE ABUNDANCES IN MACROCALGAE GROWING IN THE VICINITY OF FISH FARMS.

Introduction

One of the premises of this study is that seaweed growth will be enhanced in close proximity to salmon farms and the seaweeds utilise will the excess nutrients. The nitrogen isotope composition of organisms can be used to determine how nitrogen is cycled in ecosystems. It can also be used as a tracer where there is a large difference in isotope composition between source and end nitrogen.

The aims of this section of the project were to answer the following questions through the use of variations in nitrogen isotopic composition:

1/ Is the nitrogen taken up by plants in the vicinity of the cages derived from salmon farm nutrients?

2/ How far from the salmon farm are the seaweeds taking up salmon farm-derived nitrogen?

3/ What proportion of nitrogen taken up is salmon farm-derived?

4/ What is the isotopic signature of the farm-derived ammonium?

Background information

The mass spectrometer used for this work analysed for carbon and nitrogen isotopes. Carbon isotope results have been collected and assessed incidentally as part of this project. The carbon isotope results can also assist in interpretation of environmental processes. Of all the farm-derived nutrients, the nitrogen budget is of most interest to this project, so I have concentrated principally on nitrogen isotope ratios.

The following background information for nitrogen isotopes in marine biological systems has been sourced largely from Owens (1987). Other review articles include those by Peterson and Fry (1987), Handley and Raven (1992), Raven (1992), Michener and Schell (1994), Peterson (1999), and Robinson (2001) and Bedard-Haughn *et al.* (2003).

There are two naturally occurring forms of nitrogen. There is the common form of nitrogen which contains seven protons and seven neutrons and is referred to as nitrogen 14 (^{14}N), and a heavier form that contains an extra neutron called nitrogen 15 (^{15}N). The relative amount of ^{15}N to ^{14}N is determined relative to a standard and is described by the following:

$$\delta^{15}N\ (‰) = ((R_{sample}/R_{standard})-1)\ \times 10^3$$

where R_{sample} is the ratio of ^{15}N to ^{14}N of the sample and $R_{standard}$ is the ratio for a standard which for nitrogen is the ratio for atmospheric nitrogen.

Isotope fractionation is the basis for the natural variation in ^{15}N in biological materials and the use of these variations as a tracer. There are two types of isotope effect leading to fractionation:

a) physical isotope effects and

b) chemical isotope effects.

Physical fractionation is the result of the lighter isotope moving more rapidly than the heavier isotope e.g. during diffusion. Chemical fractionation occurs because a chemical bond, which involves a heavy isotope, has a lower vibrational frequency and is stronger than the equivalent bond involving a light isotope.

The most important fractionation effects involved in the biological nitrogen cycle are those resulting from irreversible chemical reactions; these are termed kinetic isotope effects. For a given reaction, the degree of depletion in the instantaneous product is governed by the isotopic fractionation factor (α) which is a fundamental property of the reaction and is affected by environmental conditions, for example, temperature. In a closed system, the accumulated product becomes gradually less depleted in ^{15}N, so that when all of the substrate has reacted, the $\delta^{15}N$ of the product is identical to the $\delta^{15}N$ of the substrate at the start of the reaction. In the majority of kinetic fractionation effects the product becomes more depleted in ^{15}N relative to the substrate.

An important isotope fractionation effect is that associated with dinitrogen fixation. Hoering and Ford (1960) showed that the magnitude of the effect was small and in the majority of cases was indistinguishable within the limits of detection. The consequence of the small fractionation effect is that organic nitrogen derived from atmospheric dintrogen fixation should have $\delta^{15}N$ values close to atmospheric values. In contrast the largest fractionation effects are those associated with denitrification. This is considered to be due to the involvement in the reaction of the strong N-O bonds (Owens 1987).

One example of environmental effects on fractionation is the case of temperature. Temperature affects the rate of reaction which then affects the fractionation effect. Another example is the effect of light, Wada and Hattori (1978) found a strong relationship between growth rate and the isotope fractionation in light-limited cultures. There was also an increase in fractionation with increasing NO_3^- concentration. A similar relationship has been observed for NH_4^+ (Wada 1980).

The study by Wada and Hattori (1978) clearly established the relationship of the ^{15}N content of phytoplankton with its inorganic nitrogen supply and indicated that significant isotope fractionation can be expected. Furthermore, fractionation theory predicts that in nitrogen limited conditions no enrichment or depletion of the phytoplankton relative to the inorganic nitrogen supply will occur. Greatest fractionation occurs under non-nitrogen-limited conditions.

The range of $\delta^{15}N$ values found covers almost 100 δ units from -49‰ to +49‰. Generally however values fall within the fairly narrow range of -5‰ to +20‰. The average $\delta^{15}N$ values for each environment increases in the following order: atmosphere < terrestrial < freshwater < estuarine < marine (Owens 1987).

One of the most depleted values (-49‰) found so far has been in epibenthic algae in the Antarctic and was attributed to substantial fractionation associated with extremely slow growth rates in the low light and high nitrate environment. A highly enriched value (+30.7‰) also found in the Antarctic was from algal felt collected close to a penguin rookery. Wada *et al.* (1981) considered that this demonstrated an avian source of excreted ammonium, accompanied by a large fractionation effect during the volatilization of the ammonium as ammonia. Industrially-produced fertilizers have an

isotopic composition close to that of the atmosphere, reflecting their origin. Naturally occurring inorganic fertilizers have widely differing ^{15}N contents.

In food chain studies, $\delta^{15}N$ and the relationship between an organism and its diet has been used to track trophic levels. A review of a number of studies by Owens (1987) suggested that the average difference between $\delta^{15}N$ organism and $\delta^{15}N$ diet is 2.6‰ ± 2.1 (s.d.) which supports the earlier claim of Minagawa and Wada (1984) for a general enrichment between trophic levels of approximately 3‰. This relationship can be taken a bit further by considering the ^{15}N content of diet, individual tissues and/or excretory products. Some land based studies indicate that excretory products are consistently depleted in ^{15}N relative to diet, the average difference being -5.88‰ (mostly terrestrial invertebrates and mammals, Owens 1987). Because isotope mass balance must be maintained, the relationship between diet, tissue and excreted products can be represented in the form of a mass balance equation:

$$\delta^{15}N \text{ diet} = X.\delta^{15}N \text{ tissue} + Y.\delta^{15}N \text{ faeces} + (1-[x + y])\ \delta^{15}N \text{ excreted metabolite}$$

Where x & y are the fractions of the isotope incorporated into tissue and faeces respectively. It is likely that growth efficiency and assimilation efficiency account for the variability between the ^{15}N content of an organism and its diet.

The use of natural variations in ^{15}N as an indicator of the source of the nitrogen is based on three assumptions:

- materials of different origins or composition have detectably different ^{15}N contents;
- ^{15}N content of a particular material remains unique and

- the ^{15}N content remains unchanged or, if changes occur, the degree and direction (depletion or enrichment) of the change is known.

In reality, there is often overlap between different types of samples and their origins and the isotopic composition of a material may change through isotopic fractionation and the magnitude of the change may be variable. Despite this, natural variations in ^{15}N have been used to help determine the sources and origins of a variety of materials. For example, two source mixing models have been used by Grey *et al.* (2004) and Savage and Elmgren (2004). Grey *et al.* (2004) used a mixing model to estimate fish contributions to food webs using carbon and nitrogen isotopes at a trout farm in a freshwater lake in Esthwaite Water, UK, where marine feeds were being used. Percent contributions to the diets of zooplankton were estimated. Use of the model was facilitated by the large difference in stable isotope abundances between freshwater and marine entities.

For Savage and Elmgren (2004), percentage sewage contribution to total algal N uptake in algae near a sewage outfall was estimated before and after changes to sewage treatment which resulted in enhanced nitrogen removal. In 1989 this was estimated to be 40% and in 1999:12%. Similarly, temporal variation in individual plants estimate that the percentage sewage N contribution to algae within 1 km from the outfall declined from 40% in the mid-1990s to 20% in 2002. Nutrient budget calculations showed that *F. vesiculosus* is not an effective sink for N, assimilating only 3% of total annual N loads entering the bay.

In animal or sewage waste, nitrogen is excreted mainly in the form of ammonia and urea. Urea, when hydrolysed, produces a temporary rise in pH. The more basic conditions favour conversion to ammonia, which is easily lost by volatization to the

atmosphere. Fractionation during this process results in the ammonia, which is lost from the system, being depleted in ^{15}N. The remaining ammonium, now correspondingly enriched in ^{15}N, is subsequently converted to ^{15}N-enriched nitrate, which is more readily leached and dispersed by water (Heaton 1986; Costanzo *et al.* 2001). Although this is unlikley be an important factor in the ocean as it is relatively well buffered (Raven pers com).

Nitrogen isotope natural abundances have been used to determine the extent of effluent plumes from a variety of waste output producers ranging from sewage to industrial to aquaculture operations. In the marine environment, stable isotopes (primarily nitrogen) have been used to identify sewage effluent in marine ecosystems in America (Cabana and Rasmussen 1996; Kwak and Zedler 1997; Tucker *et al.* 1999), Australia (Costanzo *et al.* 2001; Gartner *et al.* 2002; Costanzo *et al.* 2003; Gaston and Suthers 2004; Carseldine and Tibbetts 2005), Denmark (Savage and Elmgren 2004; Savage 2005), Portugal (Machas *et al.* 2003), New Zealand (Rogers 2003), Indonesia (Heikoop *et al.* 2000; Risk and Erdmann 2000; Marion *et al.* 2005) and the United Kingdom (Thornton and McManus 1994; Waldron *et al.* 2001). In South Africa, levels of ^{15}N in *Gracilaria gracilis* were used to confirm nitrogen was sourced from a fish waste factory (Anderson *et al.* 1999). Stable isotopes have been used to track fish farm wastes in the Mediterranean, (Pantoja *et al.* 2002; Holmer *et al.* 2004; Vizzini and Mazzola 2004; Vizzini *et al.* 2005), in Australia (Ye *et al.* 1991; McGhie *et al.* 2000) and Denmark (Christensen *et al.* 2000) and for shrimp farms in Australia (Jones *et al.* 2001; Costanzo *et al.* 2005) and Thailand (Yokoyama *et al.* 2002).

Stable isotope signatures may be tracked in sediments, particulate matter or organisms such as macrophytes, fish and invertebrates depending on the waste component of

interest. Fish feed and faeces can be tracked directly in sediments and particulate matter or indirectly as prey items for fish and invertebrates. Soluble nitrogenous-based compounds can be monitored either indirectly through uptake by seagrasses or macroalgae or directly through extraction from the water column. Extraction from the water column is not a straightforward procedure, however, and concentrations are very variable. Monitoring through macrophytes has the advantage that they can integrate over extended periods of time. Owens (1987) highlighted the lack of determinations of dissolved pools conducted in conjunction with ecological studies. Recent methodological improvements to extraction of soluble nitrogenous compounds, such as ammonium (Johnston *et al.* 2003), should make this more practicable.

There are two stable isotopes of carbon, these are ^{13}C and ^{12}C. Determination of $\delta^{13}C$ is calculated in a similar manner to $\delta^{15}N$ and abundances are measured relative to the PDB standard (carbonate from the Cretaceous Pee-Dee formation). Organic matter in marine macroalgae has $\delta^{13}C$ values ranging from -3 to -35%. Seaweeds with very negative $\delta^{13}C$ (lower than -30‰) are mainly subtidal red algae; those with enriched $\delta^{13}C$ values (higher than -10‰) are mainly green macroalgae and seagrasses, with some red and brown macroalgae. Organisms with low $\delta^{13}C$ values rely on CO_2 diffusion for their carbon source while those more positive than -10 ‰ must involve HCO_3^- (Raven *et al.* 2002). The capacity to use HCO_3^- has been demonstrated for *P. palmata* (Colman and Cook 1985; Cook and Colman 1987; Maberly 1990; Maberly *et al.* 1992; Kubler and Raven 1994; Kubler and Raven 1995; Kubler and Raven 1996a). Light and water movement have been shown to affect fractionation of ^{13}C. Under low light there is greater fractionation resulting in lower $\delta^{13}C$ values (Kubler and Raven 1994; Kubler and Raven 1995; Kubler and Raven 1996b; Kubler and Raven 1996a). Higher water

movement lowers the diffusion boundary layer (Maberly *et al.* 1992; France and Holmquist 1997).

To determine the potential of using $\delta^{15}N$ to trace farm-derived nitrogen an understanding of the variation in background abundances at the sites was required necessitating investigations into seasonal and geographic variation. The following sections cover:

- Seasonal variation
- Geographic variation
- Variation for cultured seaweeds (*Laminaria saccharina* and *Palmaria palmata*)
- Gradient in $\delta^{15}N$ away from a farm
- Mass balance for nitrogen isotope abundances for cultured salmon.

7.1 Seasonal variation of nitrogen isotope abundances in *Palmaria palmata*.

The principal aim of this section was to determine the seasonality, if any, of heavy nitrogen isotope content of wild *Palmaria palmata* in the vicinity of Loch Duart fish farm sites. This information is valuable for elucidating patterns of the natural abundances of $\delta^{15}N$ found in seaweed when used for purposes such as tracer analysis and food web studies. Studies on seasonal variation of the stable isotopes of carbon and nitrogen in macroalgae are rare. They include studies on variation in nitrogen and carbon isotopes of epiphytes on seagrasses in a western Mediterranean lagoon by Vizzini and Mazzola (2003), seasonal variation in $\delta^{15}N$ of *Codium isthmocladum* and *Caulerpa brachypus var. parvifolia* on coral reefs of Florida by Lapointe *et al.* (2005) and in macrophytes in estuaries in Massachusetts by Cole *et al.* (2005) and carbon isotopes in two species of intertidal brown algae in eastern Scotland by Brenchley *et al.* (1997).

Vizzini and Mazzola (2003) found that, for algae associated with seagrass beds in the Mediterranean, $\delta^{13}C$ of the epiphytes showed the most enriched values and exhibited slight seasonal differences from -14.2 ± 0.6 ‰ in summer to -15.3 ± 0.8 ‰ (± s.d.) in winter. In contrast, *Chaetomorpha linum* exhibited a seasonal shift in $\delta^{13}C$ values of 4.8 ‰ from -19.0 ± 0.5 ‰ in summer to -14.2 ‰ in winter. Throughout the year *Cladophora* sp. ($\delta^{15}N$ = 0.7 ‰) and epiphytes ($\delta^{15}N$ = 0.9 ‰) showed fairly constant $\delta^{15}N$ values. Lepointe *et al.* (2005) found little seasonal change for the algae in Florida for $\delta^{15}N$ as did Cole *et al.* (2005) in Massachusetts estuaries. Cole *et al.* (2005) thought this may be due to low variation in available nitrogenous compounds in the water throughout the year. Greater seasonal differences may be found where there was a more

marked difference in concentration of available nutrients seasonally. For Brenchley *et al.* (1997) in a study on two brown algal intertidal species in eastern Scotland, the stable carbon-isotope ratio $\delta^{13}C$ did not differ between different tissue types in *Fucus serratus*, but values did vary seasonally, being less negative in the summer than in the winter: -13.5 ‰, compared to -18 ‰. The receptacle tissue of *Himanthalia elongata* also displayed a distinct seasonal variation in $\delta^{13}C$ values: -12 ‰ in summer and -16 ‰, in winter, whilst the $\delta^{13}C$ of the vegetative button did not vary seasonally.

A number of studies have been conducted on variation in $\delta^{15}N$ and $\delta^{13}C$ spatially and temporally for seagrass species. Anderson and Fourqurean (2003) found for *Thalassia testudinum* in the Florida Keys that both $\delta^{13}C$ and $\delta^{15}N$ values displayed seasonal enrichment-depletion patterns, with maximum enrichment occurring during the summer to early autumn. Enrichment in summer was thought to be caused by denitrification. Denitrification results in the loss of isotopically light ^{14}N, which enriches the remaining DIN pool with ^{15}N.

Methods

Three sites were selected at Calbha and were a subset of stations used for a concurrent study investigating the seasonality and variation in nitrogen content of *P. palmata* with distance from salmon cages (Cook *et al.* in prep.). In 2004-2005 there were three salmon cage groups located at Calbha (see Figure 7.1). Of the possible sample sites, two were chosen: 'Channel' and 'Point' were sited at maximal distances from salmon cages, minimising the influence of salmon farm-derived nutrients. A third site, 'Farm' was chosen because it was close to a set of salmon cages ('E', see Figure 7.1). The sample sites were sampled bimonthly for *P. palmata* beginning in May 2004 and finishing in

June 2005. A later sampling session planned for August 2005 was abandoned due to inclement weather.

In 2005 cultured fish at Calbha were second year fish of harvestable size, and biomass was at a maximum for the lease (see Figure 7.2). At operational peaks in 2004-2005, the cage groups each held three to four hundred tonnes of Atlantic Salmon (*Salmo salar*) and 70 to 90 tonnes of feed were put into each of the cage groups each month (Figure 7.2). In July 2004, harvesting of fish began for cage group 'E', and by February 2005 the cage group was empty of fish. Cage group C was emptied over a similar period to 'E' and 'A' was harvested approximately three months later.

On moderate to high wave-exposed rocky shores in the vicinity of Loch Duart sites, *P. palmata* is common on *L. hyperborea* stipes in the upper subtidal and in the lower intertidal on adjacent rocky reefs. To standardize sampling, *P. palmata* was sampled only from *L. hyperborea* stipes, during spring low tides. At each site, *P. palmata* was sampled from three randomly selected sub-sites each at least 10 m apart. At least five plants were collected at each sub-site. These were placed in plastic bags and kept at 4^{o}C until processing on return to the laboratory. From each sample of plants, tips of fronds (approximately 3 cm) from at least three plants were freeze dried. Only tips were used in order to minimise within plant variation because they do not have epiphytes that are often found, seasonally, closer to the basal parts of the fronds. Once freeze dried, samples were kept in the dark in a desiccator until analysed.

A PDZ Europa ANCA-SL/GSL 20-20 mass spectrometer was used to analyse samples. For optimal results when determining δ^{15}N and δ^{13}C, samples should contain approximately 100 mg of N or C. Where sample amounts fell outside acceptable ranges

for analysis of $\delta^{15}N$ or $\delta^{13}C$, samples were re-run with adjusted weights. Mass spectrometer results also enabled calculation of total carbon and nitrogen as a percentage of dry weight.

Statistics

Analysis of variance was used for between-sample comparisons. Data were checked for normality and homogeneity of variance (Bartlett's test). Post hoc tests were conducted using Tukey tests and all error bars presented on graphs are standard error. Statistics packages used were JMP IN (SAS Inst Inc) and Minitab.

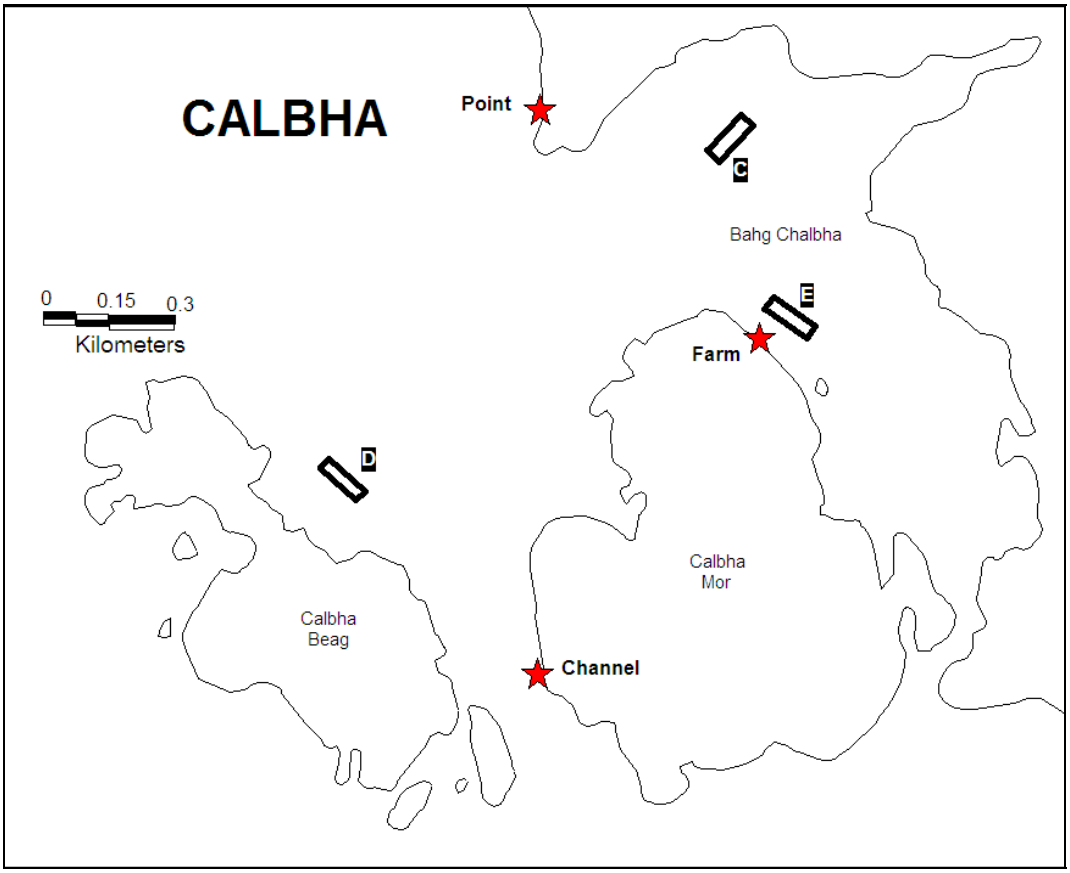


Figure 7.1 *Palmaria palmata* sample sites and salmon cage groups at Calbha Bay 2004-2005 (★).

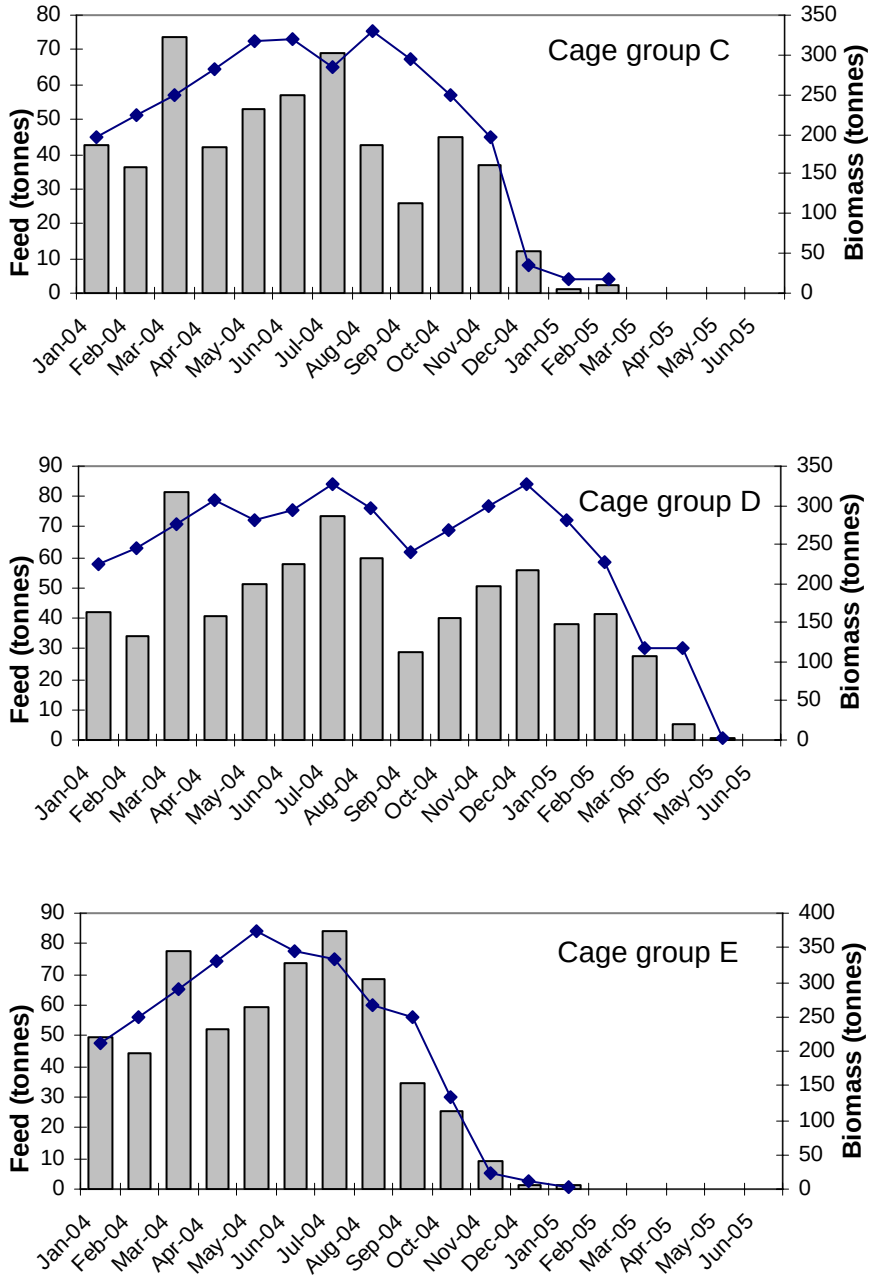


Figure 7.2 Feed input (bar) and biomass (line) of fish for each of the three cage groups (C, D & E) at Calbha, 2004-2005.

Results

For the three sites monitored, mean $\delta^{15}N$ values varied from 4 to 8.5 ‰ (Figure 7.3). The Channel site was consistently close to 8 ‰ for all sampling times except for the low February sample (4.1 ‰). The Point site was similar to the Channel site but lower by 1-2 ‰ for most samples. $\delta^{15}N$ for the Farm site plants decreased steadily for the

samples taken from July 2004 to February 2006 after which it increased to 8‰ in April before again decreasing. The initial decrease in values for the farm site closely follows the decrease in added feed to the adjacent cage group 'E'. Feed input decreased from a maximum in July 2005 until the finish of feeding with the finish in harvesting of these fish in January 2005 (Figure 7.2). There is no significant difference between sites for February (4-5‰) nor for the April 2005 (8‰) sampling sessions.

$\delta^{13}C$ values were at their lowest values at -25‰ for the winter samples (Figure 7.4) rising to a maximum of close to -20‰ for the spring and summer samples. At the beginning of sampling for $\delta^{13}C$ in July 2004 when feed was being added to the cages at the Farm site, $\delta^{13}C$ was at a maximum for this site and greater than the two other sites at -18.2 ‰ (t-test: < 0.05).

Percentage nitrogen (Figure 7.5) was similar across all sites with high levels from autumn to spring and a maximum of 7.8% with a low in early summer of 3.6%. Percentage carbon (Figure 7.6) peaks in the middle of winter at close to 50% and is at a minimum in late Spring early summer at around 40%.

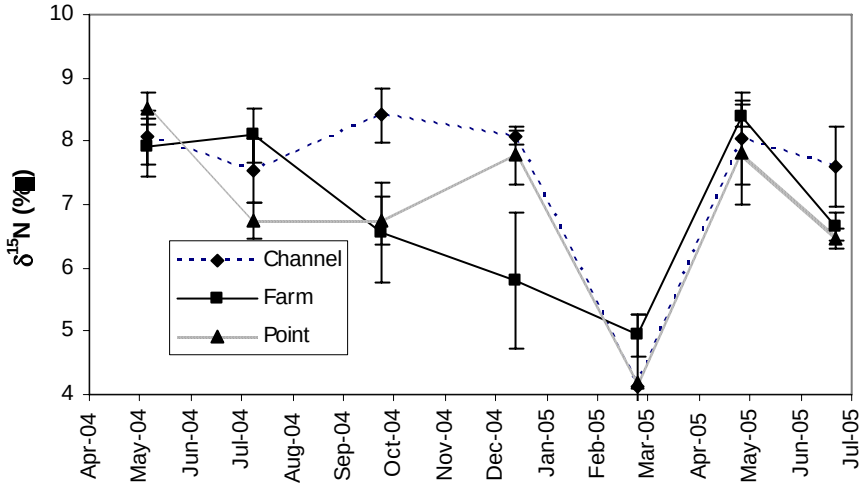


Figure 7.3 Change in $\delta^{15}N$ with time for *Palmaria palmata* frond tips at the three sample sites, Calbha Bay 2004-2005 (vertical bars indicate 95% CI).

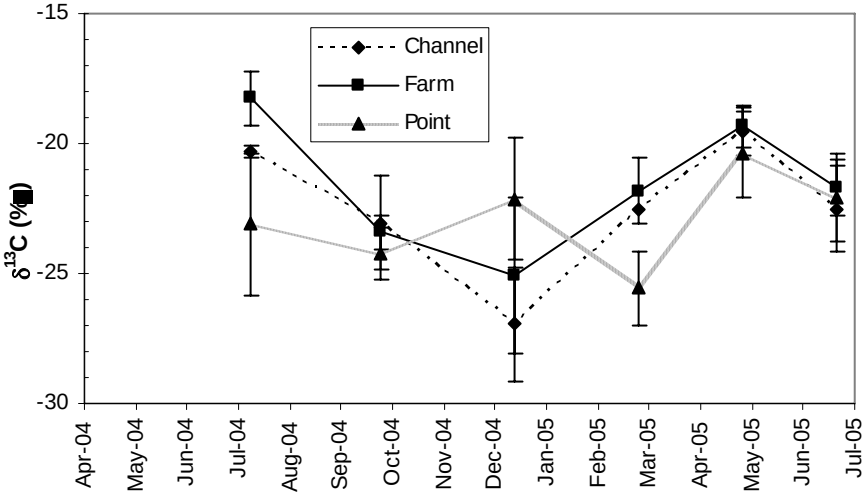


Figure 7.4 Change in $\delta^{13}C$ over time for *Palmaria palmata* frond tips sites at the three sample sites, Calbha Bay 2004-2005 (vertical bars indicate 95% CI). Note that $\delta^{13}C$ was not determined for samples collected in May 2004.

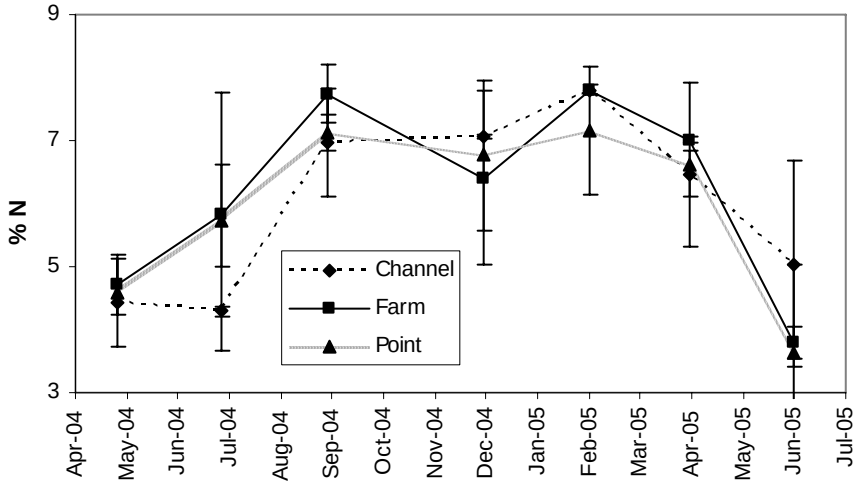


Figure 7.5 Change in nitrogen as percentage of dry weight in *Palmaria palmata* frond tips with time for the three *P. palmata* samples sites at Calbha Bay 2004-2005 (vertical bars indicate 95% CI).

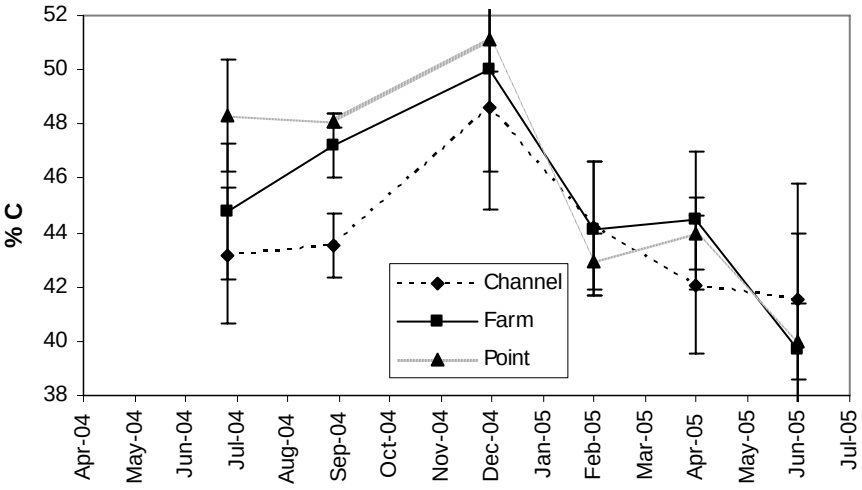


Figure 7.6 Change in carbon as percentage of dry weight in *Palmaria palmata* frond tips at the three samples sites, Calbha Bay 2004-2005 (vertical bars indicate 95% CI).

Discussion

The nitrogen stable isotope values of 4 to 9 ‰ fall within values reported by Handley *et al.* (2004) for *Palmaria palmata*. The upper range of values for $\delta^{13}C$ of -18 ‰ to -22 ‰ overlap with those quoted in Raven *et al.* (2002) for *Palmaria palmata* of -16.5‰ to -20.5‰ and the lower values are more depleted although Raven et al. (2002) quotes a value for *Palmaria decipiens* of -31.4‰.

Variation in $\delta^{15}N$ could be interpreted as seasonal with a peak in spring for all sites and a low in late winter. However, this study presents results for a single year and it is possible that the dip in $\delta^{15}N$ values in February is a one-off event, perhaps associated with the anomalous weather experienced in February 2005 rather than a seasonal cycle. The periodicity in the data is, however, consistent with Mariotti *et al.*'s (1984) findings regarding the ^{15}N content for suspended particulate matter in the North Sea. Immediately prior to the spring phytoplankton bloom, $\delta^{15}N$ values varied from ~ 4‰, increasing to ~10‰-12 ‰ during June and July at the height of the bloom, and declined during autumn (see also Cifuentes *et al.* 1988; De Brabandere *et al.* 2002). It was suggested that during the pre-bloom period the light limited and nitrogen sufficient condition of the phytoplankton would result in a significant isotope fractionation during nitrogen assimilation; the phytoplankton would be depleted in ^{15}N relative to the nitrogen source. During the nitrogen limited conditions prevailing at the height of the bloom, isotope fractionation would be low or would not occur and the phytoplankton would exhibit ^{15}N content similar to the nitrogen source. It is also possible that phytoplankton assimilate more enriched ^{15}N ammonium when ambient nitrate concentrations are low (Owens 1987). Either way, the data here show that there can be significant changes in nitrogen isotopic composition throughout.

The seasonal variability in carbon isotope is consistent with findings elsewhere for macroalgae (Brenchley *et al.* 1997; Vizzini *et al.* 2002) and seagrasses (Goering *et al.* 1990; Kang *et al.* 1999) with the most enriched $\delta^{13}C$ values obtained in summer and the most depleted in winter.

The seasonal differences observed in this study can be related to environmental factors. Freshwater run-off, irradiance levels, temperature, carbon and nitrogen sources and water movements are the main factors known to affect carbon and nitrogen isotopic composition in marine macrophytes (Vizzini *et al.* 2003). Here, there may be several causes of the observed seasonal differences in the isotopic composition of macrophytes and all the factors previously cited may be of influential to differing extents. Generally, however the depletion of both isotopes in winter and early spring months is likely to be due to fractionation at uptake by algae favouring the lighter isotopes when there are ample ambient nutrients and low light levels resulting in low productivity. Algae taking up the lighter isotopes are either advected away from site or sediment out onto the benthos.

Enrichment of $\delta^{15}N$ and $\delta^{13}C$ in summer occurs when the seaweeds are growing quickly. With the consequent increased uptake rates, fractionation is minimal, resulting in a higher uptake of the heavier isotope. Furthermore, in summer, the available ambient nitrates are enriched in ^{15}N as a result of preferential uptake of the lighter isotope by phytoplankton earlier in the season. In late summer, expansion of heterotrophic populations grazing on the phytoplankton can lead to a reduction in $\delta^{15}N$ as a result of NH_4^+ excretion. Excreted products from the zooplankton are isotopically lighter than the organism or its prey (Checkly and Miller 1989). The farm may also be influencing results.

The higher Farm site value for $\delta^{13}C$ in May 2004 possibly reflects the proximity of this site to cage group 'E' and may indicate slightly different carbon isotope sources. *Palmaria palmata* is believed to be able to take up carbon as either HCO_3^- or CO_2

(Johnston *et al.* 1992). Carbon from one of these two sources available to the algae may be more enriched as a result of proximity to the farm cages.

In May 2004, $\delta^{15}N$ was relatively high across sites. The Farm site values from July declined consistently until February 2005. These changes correlated well with reductions in added feed to the 'E' cage group (Figure 7.2). The Channel site with consistently high values of at least 8‰, except for February 2005, may be taking up isotopically enriched nitrogen originating from cage group 'D' despite a separation of nearly 600 m. The Point sampling site also may have received a mixture of both farm-derived and naturally available ^{15}N giving intermediary values for $\delta^{15}N$. While it was believed that the 'Point' and 'Channel' sites had minimal exposure to farm-derived wastes, and thus nutrients, a significant proportion of farm-derived nitrogen in the thallus which may have been derived from re-mineralisation of processed nitrogen.

The seasonal pattern of $\delta^{15}N$ abundances for the sites may be indicative of current patterns for Calbha Bay as depicted in Figure 7.7. These proposed current patterns were also supported by current meter measurements conducted by Stirling University for the three cage group sites at Calbha, measured as part of regulatory requirements to SEPA (Scottish Environmental Protection Agency) for the farm lease sites in 1999. Cage groups 'D' and 'E' have residual currents in a southerly direction, while the 'C' cage group has a residual north to south westerly component (see appendix 1). A model of current movements in the bay, based only on tidal movements and current meter results, suggests similar current patterns (SAMS, Gillibrand pers com). If the effects of the prevailing south-westerly winds are incorporated, overall water movement through the bay towards the northeast would be emphasized. The proposed current patterns support 'new' water to the area being received on to the area of coast closest to the Farm site.

When farm feed inputs are decreased, the Farm site $\delta^{15}N$ values also decrease relatively quickly as farm-derived nitrogen becomes less important and more nitrogen is taken up from the 'new' water by the plants. 'New' water, is sourced from offshore, and is likely to have higher nitrate levels and lower abundances of ^{15}N.

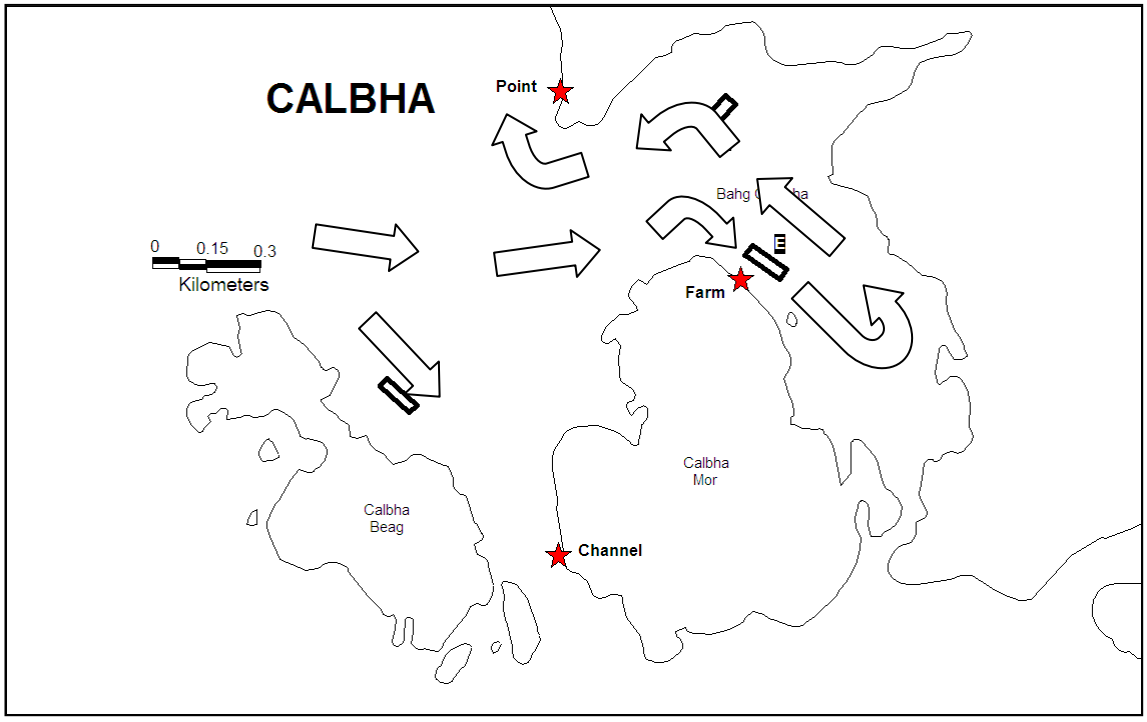


Figure 7.7 Proposed principal water movement patterns at Calbha that would explain the seasonal change in ^{15}N abundances in *Palmaria palmata* as determined for the three monitored sites. They are also consistent with current meter measurements taken at each of three cage sites.

Similarly, the decrease across all sites in the $\delta^{15}N$ values for the February 2005 sampling session agrees with the replenishment of coastal waters with well mixed offshore waters with high nitrate concentrations that are commonly associated with this area of the northeast Atlantic in winter, with values of 4 to 6 ‰ for $\delta^{15}N$ (e.g. Altabet 1996; Voss *et al.* 1996; Rolff 2000; Antia *et al.* 2001; Savoye *et al.* 2003). These low values followed the extraordinary hurricane event that occurred on the west coast of Scotland in January 2005. Winds in excess of 100 mph were experienced over a number of days and would have resulted in a flushing of all inshore coastal waters with well

mixed offshore waters. This was reflected in the low $\delta^{15}N$ abundances measured at all three sites in February 2005. The ready availability of the nitrate with respect to ammonium nitrogen from the farms would have swamped the $\delta^{15}N$ signal. The sharp increase in $\delta^{15}N$ for the following sampling session in April 2005 agreed with a general increase experienced in late spring after lighter ^{14}N nitrate is preferentially taken up by phytoplankton in early spring (Altabet *et al.* 1991; Nakatsuka *et al.* 1992).

There may also be some enhancement of $\delta^{15}N$ levels from sediments under the sites of former cage groups. At a trout farm in Sweden, Hall *et al.* (1992) found 23% of feed ended up on the bottom as particulate matter from excess feed and faeces. 11% of the nitrogen input to the farm sediment was released back into the overlying water on a seasonal basis. This correlates well with estimates of benthic recovery of weeks to nearly three years, following movement of cages when presence of sulphides or biota is taken as a measure of recovery (Holmer and Kristensen 1996; Karakassis *et al.* 1999; Brooks *et al.* 2003; Macleod *et al.* 2004). This, however, does not indicate how much or at what rate nitrogenous material is being released. McGhie *et al.* (2000) found that benthic oxic conditions were comparable to 30 m from the cages after 12 months but there was evidence that nitrogenous material of fish farm origin remained in the sediment. In Calbha Bay for the 2003-2005 season (April 2003 to February 2005), 1581 tonnes of feed was added (fish cages C & E). At the finish of harvesting, as much as 364 tonnes may have found its way to the sea bottom underneath the cages. If we assume, as a worst case scenario, that most of the nitrogen (29 tonnes) is dissolved into the water column from the finish of harvesting (as it is likely that there is minimal denitrification, e.g. Christensen *et al.* 2000), then over a period of a minimum of 2 months to a maximum of 12 months, there could be a release of 78-485 kg of nitrogen into the water column each day throughout the bay, or the equivalent of 12-77 μM

(Calbha Bay volume of approximately 6.3 million cubic meters seawater). Absolute concentrations within Calbha Bay would vary depending on the flushing of the Bay, and certain parts of the Bay would concentrate the dissolved nitrogen more than others. These figures indicate a considerable of pool of nitrogen available for macroalgal uptake.

For $\delta^{15}N$, the data suggested a close agreement between added feed and elevated abundances in the seaweeds of $\delta^{15}N$ of approximately 8.5‰. The elevated abundances determined at the Channel and Point sites indicated that the influence of fish farm-derived nitrogen on $\delta^{15}N$ levels may be experienced at greater distances from the fish farm cages than anticipated. The Channel site is approximately 600 m from the 'E' cages and the Point site is 500 m from the 'C' cage group. However the picture is further complicated by the possibility of the presence of reprocessed nitrogen in the bay. This project would have benefited from regular sampling for ^{15}N in dissolved fractions such as ammonia, nitrate and DON and sampling of *P. palmata* at greater distances from the farm cage groups.

Percentage nitrogen in *P. palmata* ranged from 3.6 to 7.8%. The upper values are relatively high values for *P. palmata* and are likely to be reflective of generally higher nutrient availabilities in the water. Lower light values experienced in these higher latitudes may enhance nitrogen storage levels.

7.2 Geographic variation in nitrogen isotope abundances in *Palmaria palmata*.

The aim of this section was to look at variation in nitrogen isotope values in *Palmaria palmata* at a broader scale across a number of Lochs that contain fish farms, comparing different lochs and inside lochs with outside. This would give some indication of the extent of influence of farm-derived nitrogen geographically and indicate other possible sources of nitrogen enriched with ^{15}N.

Scottish Lochs suitable for salmon farming have been categorised by the Scottish Executive, based on predicted relative levels of nutrient enhancement and percentage areas of seabed degraded by organic carbon deposition. They are scaled from 0–5 for nutrient enhancement and for seabed degradation, and the two scaled values are added together to provide a single combined index. On the basis of this combined index, areas are designated as Category 1, 2 or 3, where Category 1 areas are considered to be the most environmentally sensitive to further fish farming development due to high predicted levels of nutrient enhancement and / or benthic impact (Gillibrand *et al.* 2002; FRS 2006). For the Loch Duart Farm Sites, Calbha is a Category '1', Badcall is '2' and Loch Laxford is '3'.

Gubbins *et al.* (2003), in a eutrophication assessment of Scottish Lochs, found that data regarding the possible impact on composition of macroalgal communities in areas surveyed were not available. The researchers sampled fucoid macroalgae for nitrogen isotope ratios to determine where the nitrogen they were assimilating originated. No trends in the nitrogen isotope composition of fucoid algae with increasing distance from individual salmon farms were evident. However, preliminary comparison of loch-

averaged values with predicted nitrogen enhancement suggested a possible relationship between these parameters.

Nitrogen isotope evidence for dispersion of nitrogen at distance from the input source has been used in a number of studies. Vizzini and Mazzola (2004) found the nitrogen isotope signal from fish farm waste was readily detectable in primary producers, including algae, and impacts were detected 500 m from the effluent source. Vizzini *et al.* (2005) found elevated $\delta^{15}N$ in seagrass leaves greater than 500 m from the effluent pipe of a land-based fish farm in the Mediteranean. Savage and Elmgren (2004) found elevated levels of $\delta^{15}N$ in *Fucus vesiculosus* up to 24 km from a sewage effluent point in a Baltic Sea coastal embayment. Seagrass has been used more often in analysing $\delta^{15}N$ abundance variations as a result of anthropogenic input.

Ye (1991) found influence to 30 m distance in sediments using the $\delta^{15}N$ signature under salmon cages in Tasmania. Sara *et al.* (2004) found elevated $\delta^{15}N$ in sediments up to 300 m from cages in the Mediterranean.

Savage and Elmgren (2004), looking at variation in $\delta^{15}N$ in sediments with distance from a sewage pipeline in the Baltic Sea, found that $\delta^{15}N$ values decreased significantly with distance (9 ‰ down to 4 ‰) and approached background levels within 10-12 km of the outfall after a sewage treatment upgrade. Elevated $\delta^{15}N$ signals were found in macroalgae from sewage derived nitrogen 10-15 km from the outlet in Moreton Bay, Queensland (Costanzo *et al.* 2001). The ^{15}N sewage signal originating from Pirovac Bay in the Central Adriatic was evident up to nearly 8 km NW of the Bay as detected in a sea anemone (*Anemonia sulcata*), though it decreased more quickly with distance from the shore (Dolenec *et al.* 2005).

Cole *et al.* (2005) measured $\delta^{15}N$ signatures of macrophytes and particulate organic matter (POM) in six estuaries and three freshwater ponds of Massachusetts. They found that the $\delta^{15}N$ values of macrophytes and POM increased as water-column-dissolved inorganic nitrogen concentrations increased. They found that $\delta^{15}N$ of macrophytes, but not of POM, increased as N load increased. The $\delta^{15}N$ values of macrophytes and groundwater NO_3^- tracked the percent of wastewater contribution linearly.

Mapping of $\delta^{15}N$ has thus been shown to be a useful tool in tracking the impact of effluent nitrogen from a variety of sources. It can be measured in particles in the water column or in sediments or primary producers or consumers. The volume of the input and the dispersion characteristics of the area, determines how far from the source the nitrogen is detected. A number of studies (e.g. Savage *et al.* 2004; Cole *et al.* 2005) have shown the capacity of marine coastal systems to incorporate and retain nitrogen. A report by Gubbins *et al.* (2003) indicated that Scottish Lochs may incorporate and amass nitrogen from anthropogenic sources, including fish farms, and this will be reflected in the $\delta^{15}N$ signal of those lochs. This could be expected to be most pronounced in lochs with lower flushing rates.

In the area of the northwest Scottish coast where the sites in this study were located, possible sources of plant available nitrogen are limited. This is a relatively pristine coastal area of the British Isles and anthropogenic inputs are minimal (Rydberg *et al.* 2003). Plant available nitrogen comes principally from offshore sites as nitrates which peak in concentration in the winter (Slesser and Turrell 2003). In summer, ammonium is the largest source of nitrogen and arises principally from heterotrophic organisms in the water column and remineralisation of organisms such as algae.

Rhydberg *et al.* (2003) reviewed nutrient loads arising from riverine inputs for the UK based on SEPA data. They found that river nutrient concentrations varied considerably, depending on the type of land use, and to lesser extent, ON the type of soil. Rivers in rural areas, e.g. Dee, Ayr and particularly Annan and Tweed, had high nitrate concentrations (1 mg l^{-1} or more), whereas rivers such as Carron and Lochy featured mean values of 0.07 and 0.09 mg l^{-1}. Although data from north of the Caledonian Canal were limited, nitrate input on the north coast was assessed to be small compared to the rest of the UK coast (see Table 7.1).

While there are freshwater inputs adjacent to the Loch Duart Ltd. sites at Lochs Laxford, Badcall and Calbha, these are assumed to be contributing minimal nitrates for plant growth. However, Rhydberg *et al.* (2003) noted that in Scottish streams, while the nitrate concentrations are low, total nitrogen is high due to organic compounds. For the purposes of this project, inputs from land are assumed to be minimal, impacting little on nitrogen stable isotope abundances.

Table 7.1 Average river runoff, concentrations and nitrate fluxes in different regions of Scotland. Most uncertainty for the north, due to few observation sites (from Rhydberg *et al.* 2003).

District	Runoff (m^3s^{-1})	Nitrate concentration (mg l^{-1})	Nitrate flux (t y^{-1})
NNW	720	0.07	1,600
NNE	490	0.2	3,200
NE	360	0.7	8,000
E	468	1	14,900
W	714	1.2	27,000

Methods

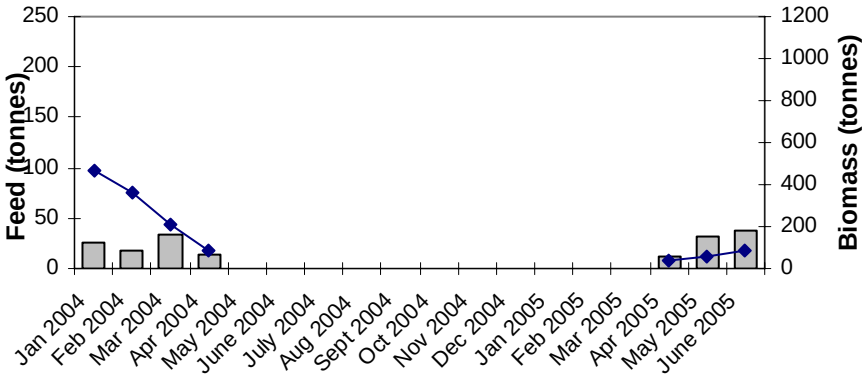
Palmaria palmata was sampled at farm lease areas in Loch Laxford, Badcall Bay and Calbha on 22nd and 23rd June 2005. At the time of sampling, there were first year fish at Loch Laxford, fish of harvestable size in Badcall (second year fish), and at Calbha fish had just been harvested (see Figure 7.8). Three sites were sampled in the lease areas that contained fish (Badcall Bay and Loch Laxford) and one site at Calbha in close proximity to salmon cages (within 100 m, see Figure 7.9 - 7.11) and at varying numbers of sites at distance from cages at all three farm lease sites.

The aim was to sample at even greater distances from the farm sites, but on the day of sampling, weather and swell precluded sampling at more remote locations including at the mouth of Loch Laxford.

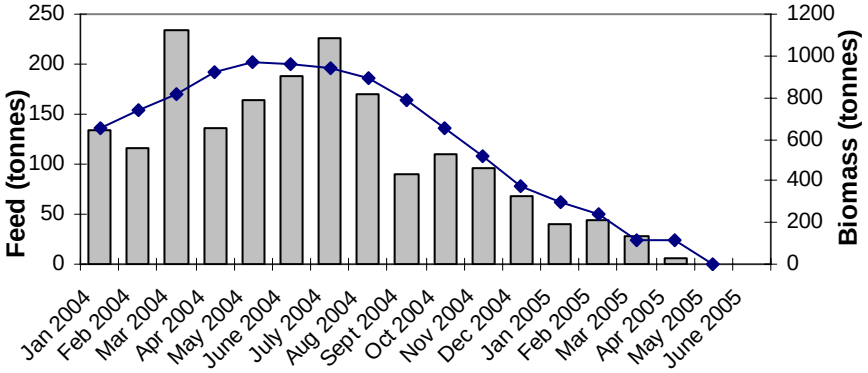
At each site, *P. palmata* was sampled from *Laminaria hyperborea* stipes at low tide at three sub-sites, each approximately 10 m apart. At least five *P. palmata* plants were collected at each sub-site. These were placed in plastic bags and kept at 4^{o}C until processing on return to the laboratory. Plants were analysed as described previously.

To investigate the possible differences of sampling *P. palmata* from adjacent rocky reef surfaces as opposed to on *L. hyperborea* stipes; at one site: LaxOut1, three samples were sampled from both habitat types.

Loch Laxford



Calbha



Badcall

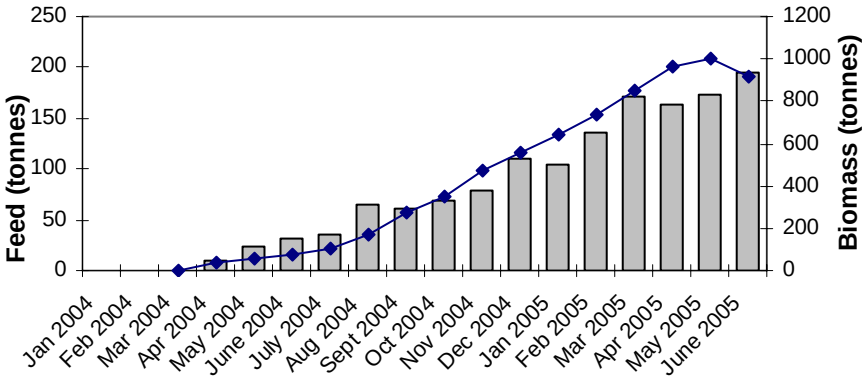


Figure 7.8 Feed added (bars) and biomass of fish (line) for months January 2004 to June 2005 for the three Loch Duart sites.

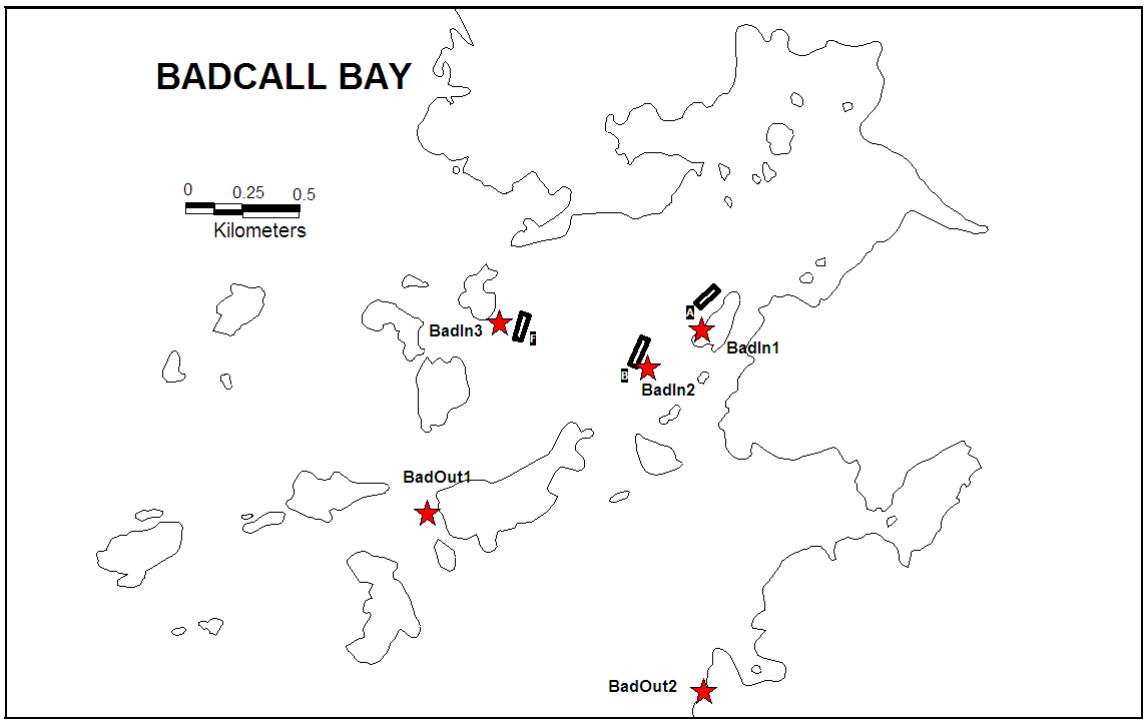


Figure 7.9 Location of sampling sites (stars) at Badcall Bay.

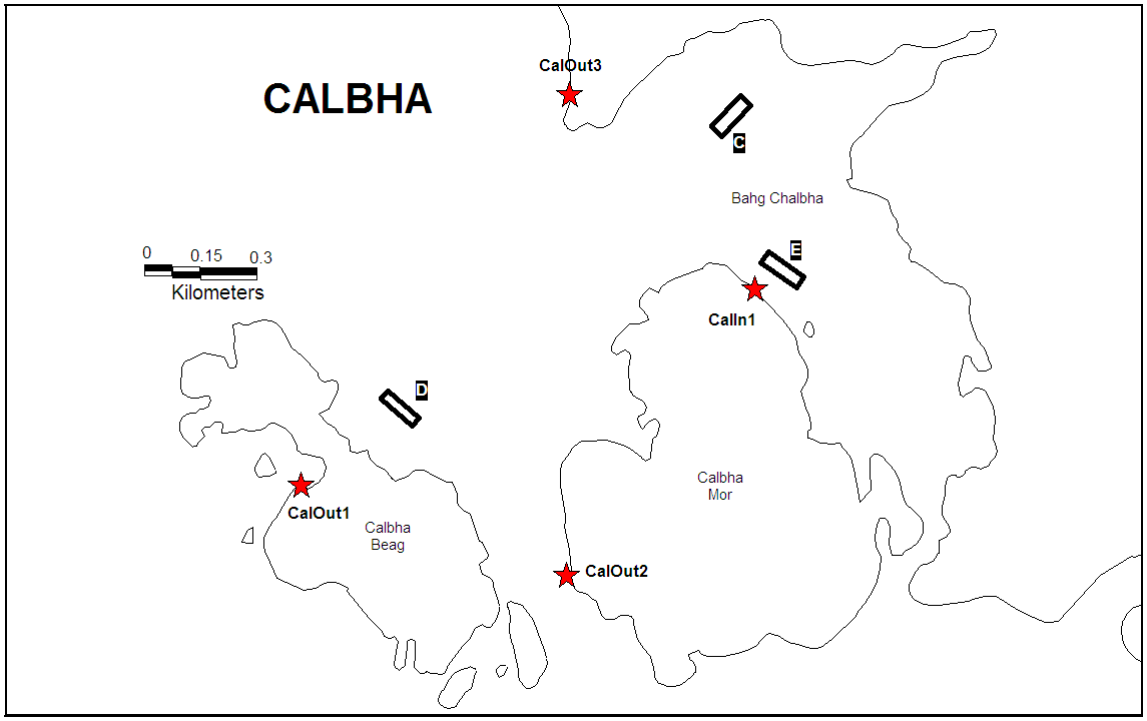


Figure 7.10 Location of sampling sites (stars) at Calbha Bay.

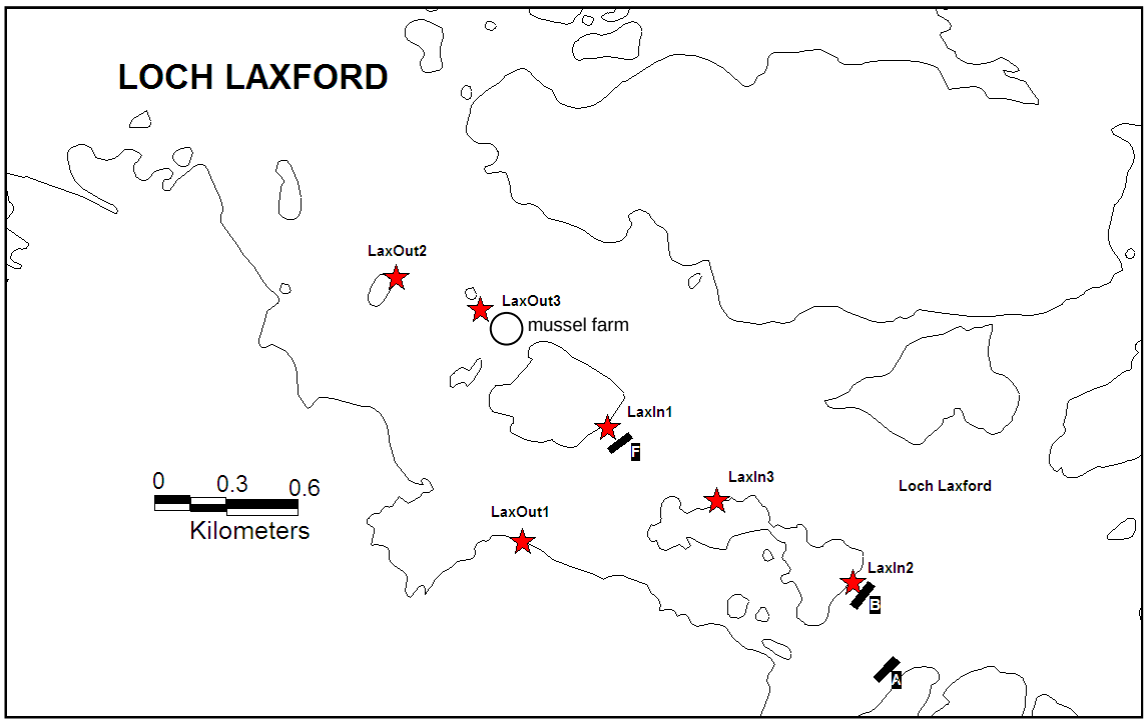


Figure 7.11 Location of sampling sites (stars) at Loch Laxford.

Results

A comparison of sites adjacent to the farm cages across all farm lease sites (Figure 7.12, one way ANOVA, site nested, without LaxOutT1) showed that the levels of $\delta^{15}N$ in the plants were significantly greater at the farm lease sites with fish, Loch Laxford and Badcall, than at Calbha (df: 2, F = 21.5, p <0.001). The mean $\delta^{15}N$ value for sites adjacent to the farm cages was 9.2‰ (at Badcall and Laxford) and 6.6‰ at Calbha. At Badcall, there was a significant difference between cage sites (9.2‰) and sites away from the farm cages (7.9‰, df: 1, F = 19.44, p < .001) but no significant difference for Loch Laxford when close to cage sites were compared with those away. High abundances were also detected away from cages.

Values for $\delta^{13}C$ showed no significant differences for farm leases across all sites (Figure 7.13). Percentage nitrogen in the plants was higher at the site with the most fish (Figure 7.14). Badcall plants had a mean N content of 5.5%, Laxford 4.6%, and Calbha 4.1%

(df: 2, F = 6.94, p < .001). There are no significant differences in percentage C in plants between lease sites.

The intertidal rocky reef sourced plants were significantly different from immediately adjacent plants collected from *L. hyperborea* stipes (t-test, < 0.05) for $\delta^{15}N$, $\delta^{13}C$ and percentage carbon; $\delta^{15}N$ was greater for the intertidal plants (9.1; 7.5‰), $\delta^{13}C$ is lower (-24.4; -20.3‰) and percentage carbon was greater (42.6; 38.6‰, no significant difference between individual sites).

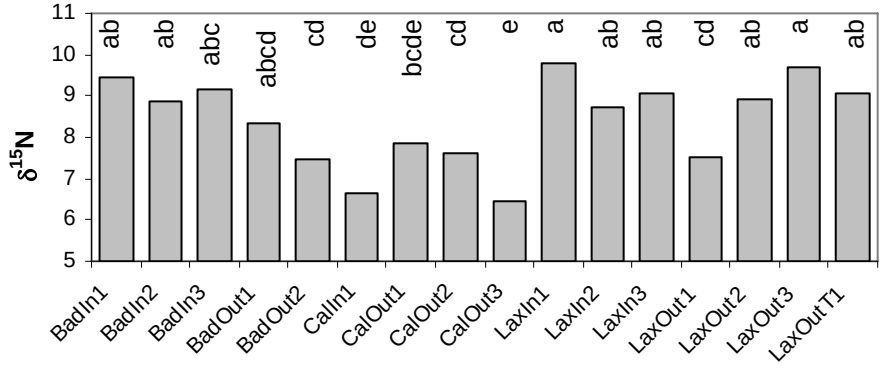


Figure 7.12 Mean $\delta^{15}N$ of *Palmaria palmata* determined for all sites. Each value is the average of three samples taken within 20 m of the coastline. Sites that share the same letter are not significantly different at the $p = 0.05$ level.

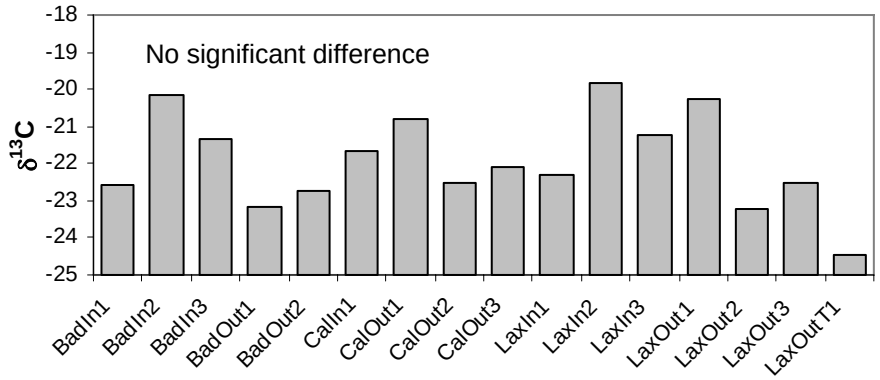


Figure 7.13 Mean δ13C determined for all sites.

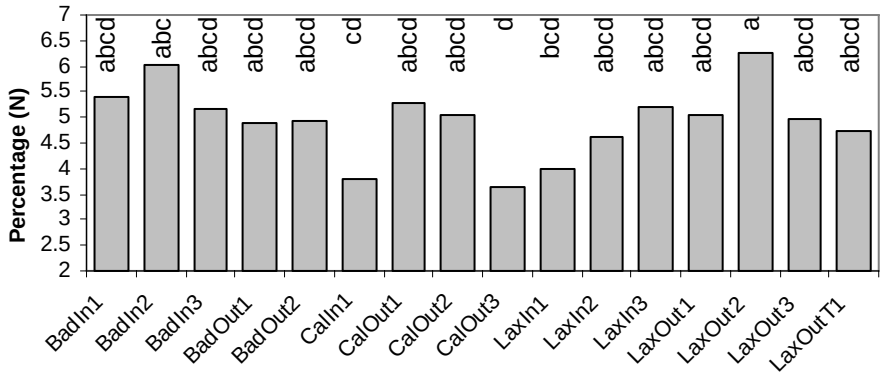


Figure 7.14 Percentage nitrogen for *Palmaria palmata* from all sites. Sites that share the same letter are not significantly different at the $p = 0.05$ level.

Discussion

At the lease site with the highest biomass of fish at the time of sampling (Badcall), there were higher $\delta^{15}N$ values for the plants obtained close to the cages (mean of 9.2‰) relative to far away (7.9‰). This included the BadOut1 site, where the levels were relatively high at 8.3‰. Conceivably, currents may have been transporting nitrogenous materials from the vicinity of the farms to this outer site, a distance of 840 m.

While the Laxford site had fish at the time of sampling, there was a low biomass because they were first year to sea fish. LaxIn1 was adjacent to the fish farm cages that had held fish for the greatest period of time (two months) and the plants from here had greater $\delta^{15}N$ values (9.2‰) than the other two sites adjacent to the other cages at Laxford. The elevated abundances of ^{15}N values from plants from LaxOut2 and LaxOut3 are more difficult to account for. These may be receiving wastes on the outgoing tide from the 'F' group fish farm cages. Previous studies (see chapter 2 and section 4 of this chapter) have shown that there is a strong tide running in the main channel at Loch Laxford. This may be responsible for disseminating wastes to the outer two sites. If the elevated $\delta^{15}N$ detected at LaxOut2 and LaxOut3 originates from wastes

from the fish farm cages, then wastes are being incorporated at distances of up to 1.3 km from the fish farm cages.

There are other possibilities, however, which include:

1/ The nutrients with enhanced levels of ^{15}N may have come from the mussel farm that is located between LaxOut3 and the island Eilean Ard. Recent research has indicated that the contribution of mussel farms to nitrogen and phosphorus in seawater has been underestimated (Nizzoli *et al.* 2005). Mussel rope communities have been shown to be an enormous sink for oxygen and particulate organic matter, and a large source of dissolved inorganic nitrogen and phosphate to the water column. Mussel farming also induced intense biodeposition of organic matter to the underlying sediments, which stimulated sediment oxygen demand, and inorganic nitrogen and phosphorus regeneration rates compared to a nearby control station.

2/ There are other sources of enhanced abundances of ^{15}N in the Loch such as those that result from denitrification.

3/ Within the Loch, there are areas where preferential uptake of the lighter nitrogen isotope during uptake of nitrate by phytoplankton has resulted in a higher concentration of the heavier isotope in the remaining nitrate.

4/ There are elevated abundances of ^{15}N as a result of the activity of heterotrophs in the water column of the Loch.

5/ Residual elevated abundances of ^{15}N remain in the Loch from previous farming.

As already noted, on the day of sampling, weather hampered collection of *P. palmata* so that algae could not be collected at even greater distances from the Laxford fish farm cages.

A comparison of the $\delta^{15}N$ abundances for the plants from L. *hyperborea* stipes with those from the rocky shore immediately adjacent shows significant differences in $\delta^{15}N$, $\delta^{13}C$ and percentage carbon. For $\delta^{15}N$, the differences for the intertidal rock-bound plants arise from either from sediment or microbial activity associated with the plants' habit (i.e. recumbent on the rocks). They may also source nitrogen from some of the animals on the seashore such as limpets and barnacles. The difference in the $\delta^{13}C$ may reflect either productivity differences between the two differing habitats, differing light climates (Raven *et al.* 2002), uptake of $C0_2$ from the air (Surif and Raven 1990) or a combination.

This section has shown that habitat is important for the nutrition and subsequent biochemistry of the plants with potentially wide variation in stable isotope abundances on small spatial scales and underlies the importance of properly stratifying the sampling design. This concurs with the findings of Savage *et al.* (2002) in Denmark where, although sewage-derived nutrient impacts were evident even 20 km distant from the point source, comparisons among local basins showed that local physical features (e.g. sill depth) can greatly ameliorate or exacerbate impacts and highlighted the need for spatially explicit studies to detect impacts.

There is evidence from this section that $\delta^{15}N$ may be enhanced as a result of uptake of fish farm nitrogen at distances from 0.8 to 1.3 km from source.

7.3 Variation in $\delta^{15}N$ for cultured seaweeds.

The aim was to determine how $\delta^{15}N$ abundances for cultured seaweeds of known age and similar backgrounds are affected by outplanting to sites of varying environmental conditions, including those close to and at distance from salmon farm cages. The factor of depth was also considered.

Percentage nitrogen of the plants was determined so that nitrogen budgets could be estimated for any cultured seaweeds grown in the vicinity of the fish farms. In a similar study investigating the bioremediation potential of growing *Gracilaria chilensis* adjacent to a salmon farm in Chile, results showed that cultivation of a 1 hectare plot of the algae close to the fish cages had the potential to remove at least 5% of dissolved inorganic nitrogen released from a fish farm producing annually around 227 metric tons of salmon *(Oncorhynchus mykiss* and *0. kisutch*). Data obtained from this section are to be used for similar calculations for Loch Duart farms (Chapter 8).

Methods

Frames supporting cultures of *Laminaria saccharina* and *Palmaria palmata* were deployed at a number of sites close to and distant from fish farm cages, three frames per site, at sites differing principally in exposure to wave action (see maps in chapter 6 for location). Nitrogen isotope levels were determined in conjunction with carbon isotope levels, percentage nitrogen and carbon content. The variation of these elements was also examined in the cultured seaweed. The advantage of using cultured plants was that they were of a known age and similar background, reducing the variables that needed to be

considered when comparing sites. Samples were also taken from seaweeds grown on longlines sited close to and at distance from a cage group at Badcall, and the fallow site in 2004-2005 (i.e. Calbha). Each longline had three groups of five droppers of each of the two species. The algae from longlines were used to look at differences in measured factors with depth. Seaweed were cultured as detailed in chapter 4 and put out onto the sites on longlines and frames as described in chapter 6.

Palmaria palmata sampled from the frames consisted of plants outplanted in either January or February 2005. *Palmaria palmata* plants harvested from the longlines consisted of plants taken from the January outplant. Individual samples from longlines were taken at 1, 2, 3 and 5 m depth. Plants were harvested in mid-late June 2005. Individual samples consisted of at least five plants. Plants were kept in plastic bags at 4^{o}C until sub-samples were taken as described previously for freeze drying and subsequent analysis by mass spectrometer. For the farm longline, all three groups of *P. palmata* droppers were sampled. Two groups were sampled from the Badcall and one group from the Calbha reference longlines.

Each sample of *L. saccharina* taken from the frames consisted of 5 cm diameter discs cut from the mid-point approximately 10 cm above the stipe blade intersection from three plants. Samples to be analysed consisted of freeze dried fragments from each of the three plants. At each site there were three frames and thus three samples. *Laminaria saccharina* was also grown in three groups of five droppers at each of the three longlines. On longlines, groups were sampled at 1, 2, 3 and 5 m depth with each sample consisting of sections cut from three plants in the same manner as from the frames. All three groups were sampled from the Farm and Badcall-reference longlines and one from the Calbha longline.

Samples were prepared and statistical analysis conducted as previously described.

Results

$\delta^{15}N$ values for the frames when comparing farm and non farm sites showed a significant difference (Table 7.2), with farm sites having slightly higher values. Most of the frame sites had high abundances of ^{15}N ($\delta^{15}N$ > 9‰) for *P. palmata* and *L. saccharina* within the bays when compared to outside, the significant exceptions being the south eastern farm site (FSE; *P. palmata*; 7.5 ‰ and for *L. saccharina*; 7 ‰) and CalbhaLL (CLL) for *L. saccharina* frames (7.5 ‰).

Except for these anomalous results there were similar trends across sites between the species of alga. For both frames and longlines, the algae of both species have higher nitrogen contents close to the farm than those further away.

For the frame grown algae, $\delta^{13}C$ was higher in the algae grown adjacent to the farms, but longline-grown algae were not significantly different. The frame-grown *P. palmata* had significantly higher carbon contents adjacent to farm cages than further away.

There were no readily apparent trends or significant results for $\delta^{15}N$ or $\delta^{13}C$ with depth (not presented) although the algae grown at depth appeared to have a higher nitrogen content.

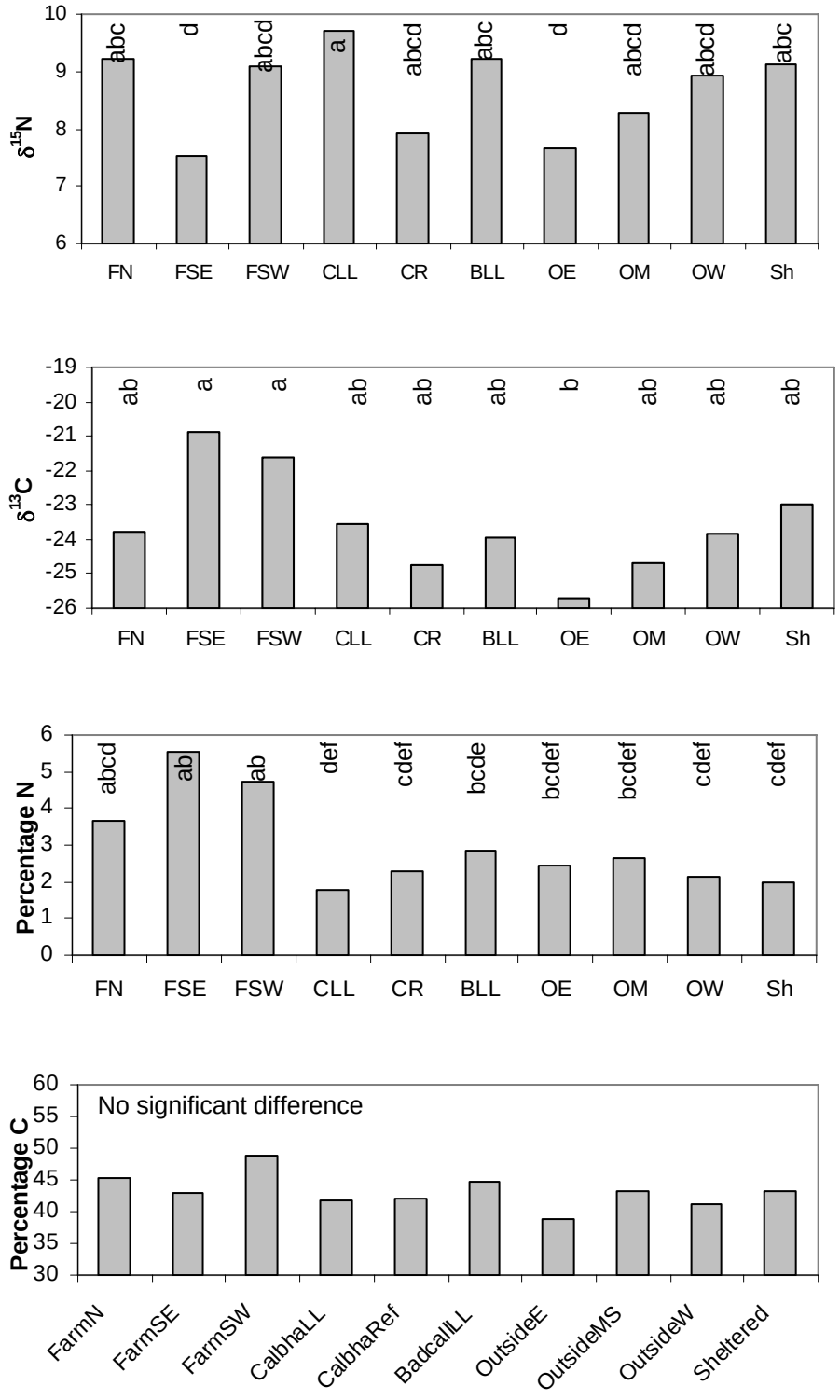


Figure 7.15. Mean values of $\delta^{15}N$, $\delta^{13}C$, percentage N and percentage C for *Palmaria palmata* grown on the frames. Sites that share the same letter were not significantly different at the 0.05 level. See lower graph for x-axis legend.

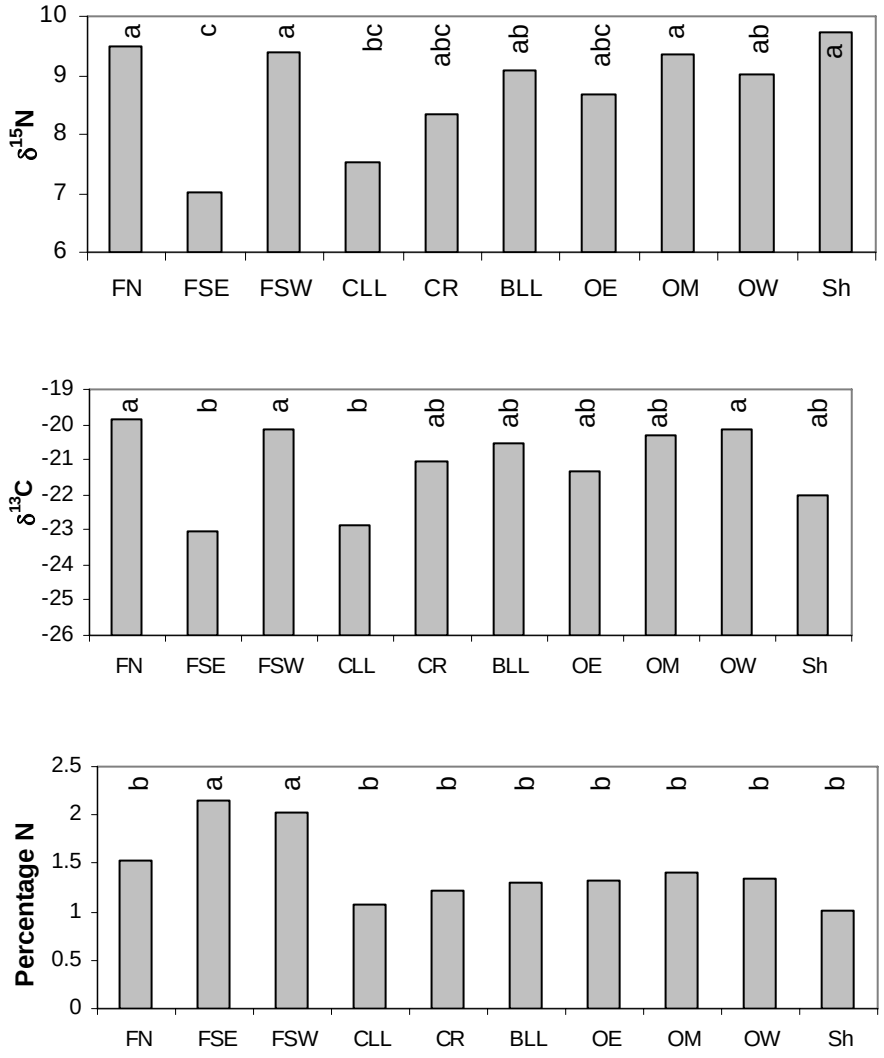


Figure 7.16. Mean values of $\delta^{15}N$, $\delta^{13}C$, and percentage N for *Laminaria saccharina* grown on the frames. Sites that share the same letter are not significantly different at the 0.05 level. FN- Farm north, FSE- Farm south east, FSW- Farm south west, CLL- Calbha longline, CR- Calbha reference, BLL- Badcall longline, OE- Outside east, OM- Outside middle, OW- Outside west and Sh- Sheltered Badcall.

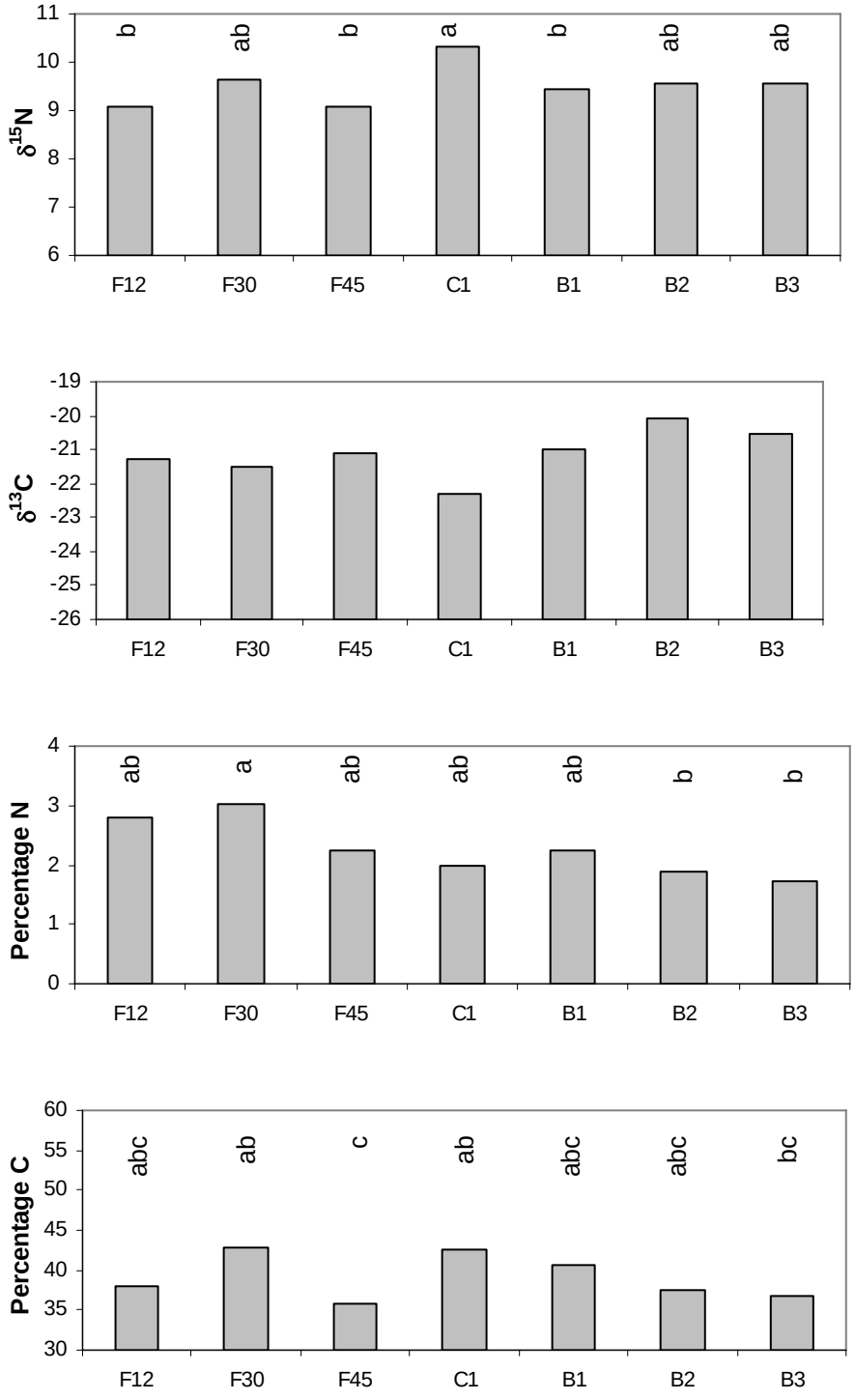


Figure 7.17 Mean values of $\delta^{15}N$, $\delta^{13}C$, percentage C and percentage N for *Palmaria palmata* grown on longlines. Sites that share the same letter are not significantly different at the 0.05 level. F12, F30 and F45 were 12, 30 and 45 m from farm cages, C1- Calbha longline group, B1, B2 & B3- Badcall longline groups.

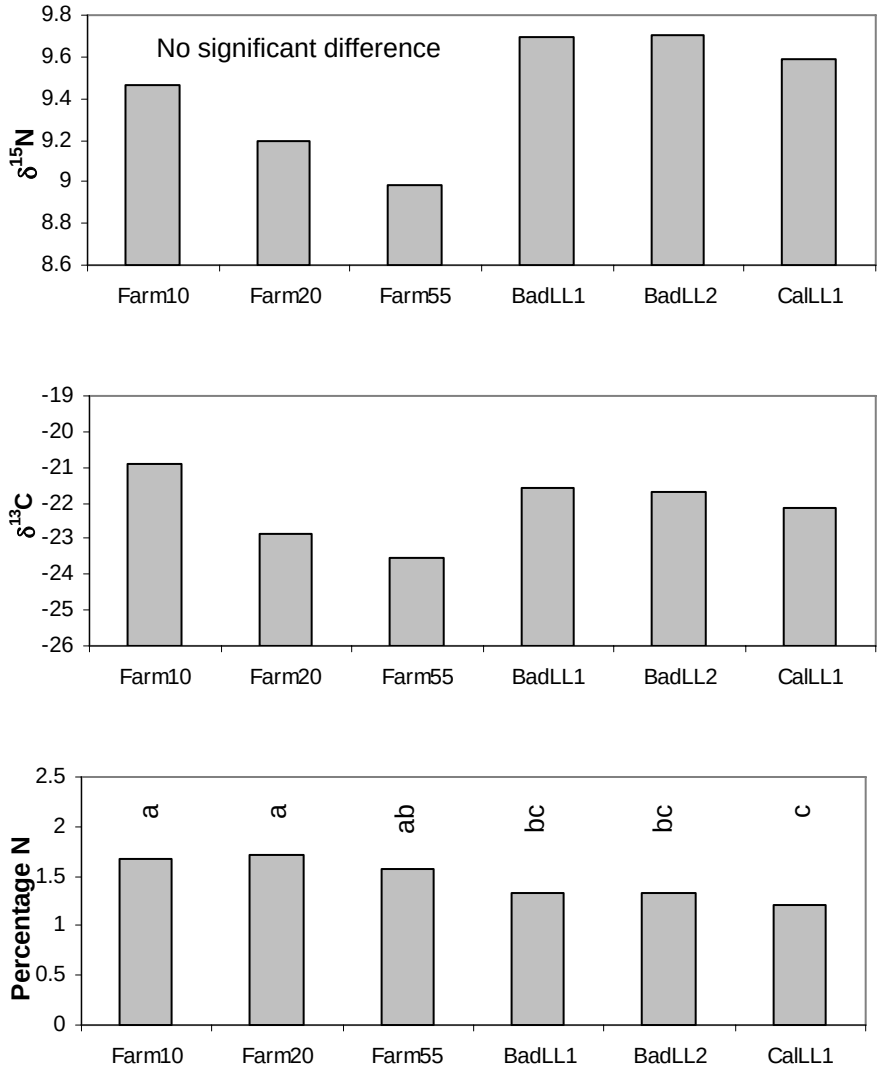


Figure 7.18 Mean values of δ15N, δ13C, and percentage N for *Laminaria saccharina* grown on longlines. Sites that share the same letter are not significantly different at the 0.05 level. F10, F20 and F55 were 10, 20 and 55 m from farm cages, BadLL1, BadLL2C1- Badcall longline groups, Calbha longline group.

Table 7.2 Summary of ANOVA results comparing δ15N, %N, δ13N and %C for Farm versus non-farm sites. Site nested within farm; degrees of freedom, F ratio and significance level. Significant farm *versus* non-farm values are in bold.

	$\delta^{15}N$				%N			
FRAMES								
L. saccharina								
	Means	df	F	p	Means	df	F	p
Farm/Non-farm	9.4/8.8	1,23*	5.85	**0.029**	1.9/1.2	1,25	113.13	**<.0001**
Site (Farm)		7,23*	3.95	0.012		8,25	5.44	0.002
P. palmata								
Farm/Non-farm	9.2/8.7	1,25*	5.33	**0.03**	4.6/2.3	1,29	160.9	**<.0001**
Site (Farm)		7,25*	6.58	0.001		8,29	4.69	0.002
LONGLINES								
L. saccharina								
Farm/Non-farm	9.2/9.7	1,23	4.98	**0.039**	1.7/1.3	1,23	41.91	**<.0001**
Site (Farm)		4,23	0.52	0.725		5,23	1.22	0.339
P. palmata								
Farm/Non-farm	9.3/9.7	1,27	11.47	**0.003**	2.7/2.0	1,27	14.46	**0.001**
Site (Farm)		5,27	4.4	0.007		5,27	1.47	0.241
	δ13C				%C			
FRAMES								
L. saccharina								
Farm/Non-farm	-20/-21.2	1,23	10.27	**0.006**	29.1/29.7	1,23	0.13	0.725
Site (Farm) *		7,23	3.9	0.013		7,23	1.42	0.269
P. palmata								
Farm/Non-farm	-22.7/-24.2	1,27	7.4	**0.014**	47.0/42.2	1,27	8.74	**0.008**
Site (Farm)		7,27	2.11	0.092		7,27	0.85	0.564
LONGLINES								
L. saccharina								
Farm/Non-farm	-22.4/-21.8	1,23	1.26	0.277	31.7/29.4	1,23	3	0.101
Site (Farm)		4,23	1.95	0.145		4,23	2.53	0.076
P. palmata								
Farm/Non-farm	-21.3/-21	1,20	0.46	0.508	38.8/39.4	1,27	0.33	0.572
Site (Farm)		5,20	1.54	0.241		5,27	6.04	0.001

* Analyses did not include FarmSE.

Discussion

Within the bays, the $\delta^{15}N$ results that are most at odds with expected were those for FarmSE (FSE) which were low for *Palmaria palmata* and *Laminaria saccharina* despite being close to farm cages with the highest measured ammonium levels (see chapter 2). Care had been taken to set frames at all farm cages at similar orientations with respect to light in particular (all on the southern side of cages or not shaded), to

minimise differences between frames other than exposure to farm effluent. However, farm management moved spare cages over the frames at FarmSE between February and March 2005. These appear to have limited light availability to the frames which is reflected in low growth of the plants (see chapter 5). Despite higher measured nitrogen availability, as reflected in highest seawater ammonia concentrations measured at this site (see chapter 2) and in the plant tissue, growth rates were low and $\delta^{15}N$ was also low. Phytoplankton have been found to discriminate against uptake of ^{15}N when nitrogen is abundant and the alga is exposed to low light conditions (Wada and Hattori 1978). This may explain the anomalous results for this cage group.

Low $\delta^{15}N$ and percentage nitrogen values for the sites in the area between Badcall Bay and Calbha Bay, particularly CalbhaRef (CR) and OutsideE (OE) suggest wide dispersion of farm-derived nitrogen within the bays where $\delta^{15}N$ values are consistently higher.

When the anomalous FarmSE results were excluded and comparisons made between farm and non farm for *P. palmata* and *L. saccharina* for the frames and the longlines. I found consistent results indicating that the farm grown algae (see Table 7.2) were taking up farm-derived nitrogen.

Within the bays, the $\delta^{15}N$ for the plants on frames near cages were low relative to those on frames away from the cages, in particular in the more sheltered parts of the bays such as Calbha Bay (CLL, except for *Laminaria saccharina* $\delta^{15}N$), the bay with the Badcall reference longline (BLL), and the sheltered site (Sh). This was due to either one or more of the following:

- utilisation of re-processed farm-derived nitrogen, either through zooplankton or bacteria (heterotrophs) which were further fractionating the nitrogen that then became available to the plants.
- Utilisation of re-mineralized nitrogen from sediments or
- Nitrogen uptake may have been restricted to nitrates with heavier ^{15}N as a result of previous preferential uptake of the lighter isotope by phytoplankton (i.e. the higher abundances of ^{15}N might not always necessarily have been originating from fish farm cages).

When compared to the multiple reference sites away from the farms, farm grown algae were found to have higher percentage nitrogen and higher $\delta^{15}N$.

The *L. saccharina* $\delta^{15}N$ for CLL (frame) is lower than the result for the *L. saccharina* from the longline and the results for the *P. palmata* for the longline and frames. The reason for this is not clear, but may relate to reasons as outline previously. It should be noted that the frames were not located directly over the farm cage site. Perhaps, they were not using nitrogen released from the sediments below the cages. They were also closer to the creek at the eastern part of Calbha Bay and nitrogen from the burn may be influencing results although the nitrogen available is believed to be small from the creeks (Rhydberg *et. al.* 2003).

Within the areas where the farms were sited, farm based nitrogen was widely distributed and may have been concentrated in quieter areas of the bay where reprocessing of the nitrogen was occurring, further concentrating ^{15}N. For example, urea, when hydrolysed to ammonia is easily lost by volatilisation to the atmosphere. Fractionation during this process results in the ammonia, which is lost from the system, being depleted in ^{15}N.

The remaining ammonium, now correspondingly enriched in ^{15}N, is converted to ^{15}N-enriched nitrate, which is readily leached and dispersed by water (Heaton 1986; Costanzo *et al.* 2001). Elevated levels of $\delta^{15}N$ in plants away from the farms cages suggested that farm based nitrogen may be taken up by plants at least one kilometre from farm cages.

Plants close to the cages had elevated $\delta^{13}C$ when compared to plants from sites further away. This may have related to differing origins of carbon taken up by the plants. A significant proportion of the carbon in the plants close to the cages may have originated as a result of fish culture.

Nitrogen and carbon isotope abundances for *P. palmata* were found to be correlated with water movement as measured by plaster loss (Figures 7.19). Water movement has been shown to be a determinant of $\delta^{13}C$ for periphyton (MacLeod and Barton 1998; Trudeau and Rasmussen 2003; Singer *et al.* 2005) and in seagrass and epiphytes of seagrass (France and Holmquist 1997). These differences have been related to boundary layer differences and the diffusion of nutrients. MacLeod and Barton (1998) suggested differences may be due to the intensity of metabolic activity.

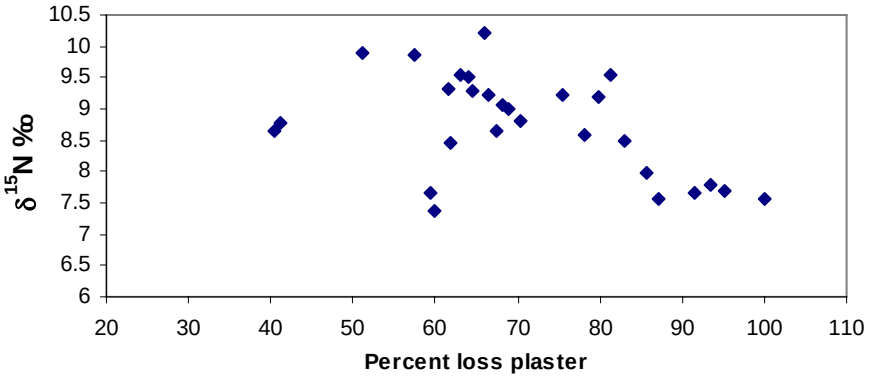


Figure 7.19 a). Correlation of water movement as indicted by plaster loss from cylinders for each of the frames versus $\delta^{15}N$: r = 0.48, df = 28, p < 0.01 (Pearson's correlation coefficient).

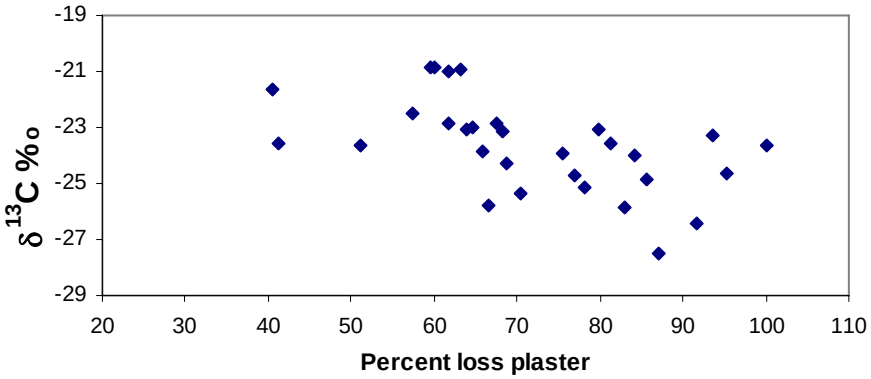


Figure 7.19 b). Correlation of water movement as indicted by plaster loss from cylinders for each of the frames versus $\delta^{13}C$: r = 0.54, df = 28, p < 0.01 (Pearson's correlation coefficient).

Isotope analysis and percentage nitrogen of the marine algae indicated that nitrogen in plants close to the cages was of fish farm origin. Determining how far from the fish farm, fish-farm-derived nitrogen forms a significant proportion of *P. palmata* nitrogen is restricted by the variability in isotope abundances. There is inadequate knowledge of all the processes involved in isotopic fractionation. Discrimination of farm-derived nitrogen is further compounded by the small difference between farm and background $\delta^{15}N$ (and $\delta^{13}C$). This is particularly the case in summer when ambient abundances of ^{15}N are more enriched.

7.4 Gradient in stable nitrogen isotope abundance away from a fish farm cage group

This section was part of an initial pilot study to determine the potential for using $\delta^{15}N$ values to investigate the fate of fish farm-derived nitrogen and can now be considered in the light of the former sections. The aim of this section was to look at finer scale changes in $\delta^{15}N$ in natural populations of *Palmaria palmata* close to the salmon cages to see how feasible it was to determine how far fish farm-derived nitrogen may be taken up by the plants using ^{15}N abundances.

Methods

The study was conducted at Loch Laxford in 2003 when fish biomass was at a maximum for the farm lease site (see Chapter 1 for biomass of fish on site). The project was conducted at the western most cage system adjacent to Eileen Ard (Figure 7.12). Two plant types were considered for analysis; these were wild plants and cultured tethered plants.

Wild plants

To examine variation in nitrogen isotopes with distance from the cages, plants were sampled from wild populations growing on the pontoons around the salmon cages and from adjacent rocky reef surfaces at low tide at successively greater distances away from the farm up to 600 m (Figure 1.3). The principal direction of sampling was to the north west, avoiding the confounding influence of farm-derived nitrogen originating from salmon cages further up the loch (to the south east) and to take advantage of

relatively consistent rocky reef surfaces from which the algae were sampled. Samples were all collected on 31 July 2003. Some ancillary samples were also collected at sites randomly chosen within the loch.

Tethered plants

As part of the complementary study into growth of tethered *P. palmata* with varying distance from the fish cages (see chapter 3), 10 mm weighted polypropylene lines (droppers) were hung from a header line at distances of 0, 5, 25 and 50 m from the cages. Some plants were also maintained at a reference site, approximately 700 m to the west on a buoyed line. The droppers and buoyed line had *P. palmata* plants attached at depths of 1, 2, 3, 5 and 6m. The plants were in the water for a period of 29 days. After pictures of the plants had been taken for growth analysis, plants from 3 m depth were kept for nitrogen isotope analysis. Samples were all collected on 30 July 2003.

Sample preparation and analyses

The plants were sampled from near the apices of actively growing plants to ensure that the analysis was reflecting uptake of nitrogen from that site (as distinct from nitrogen that may already have been incorporated in the plant from its previous location for the tethered algae) for the time they were in the water. Actively growing tissue could be distinguished as it had a relatively clean surface and the shape of the apices changed when growing and adapting to the new environment.

Initial analyses were conducted at SAMS using a Europa Mass spectrometer. Algae were dipped in double distilled water to clean off surface contaminants before cutting

tissue for analysis and drying at 60^{o}C overnight. Samples were weighed to give an expected weight of 100 μg nitrogen.

After experiencing some problems with the SAMS Mass Spectrometer some samples were analysed at the Scottish Crop Research Institute in Dundee (SCRI). Isotope analysis at SCRI was carried out using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser (Europa Scientific, Crewe, UK). At SCRI, samples analysed in single isotope mode for δ^{15}N (precision ~0.3 ‰). Working standards were a 1:4 leucine/citric acid mixture.

Results

Results show elevated levels of nitrogen isotope close to the cages with decreasing abundances with distance from the cages. This was most apparent in the transplanted tethered algae where all samples from the lines close to cages (< 50 m) were greater than the samples from the reference site (500 m, Figure 7.20-7.22). Samples taken from sites outside of this principal area of interest, further up the loch (east), had higher δ^{15}N values.

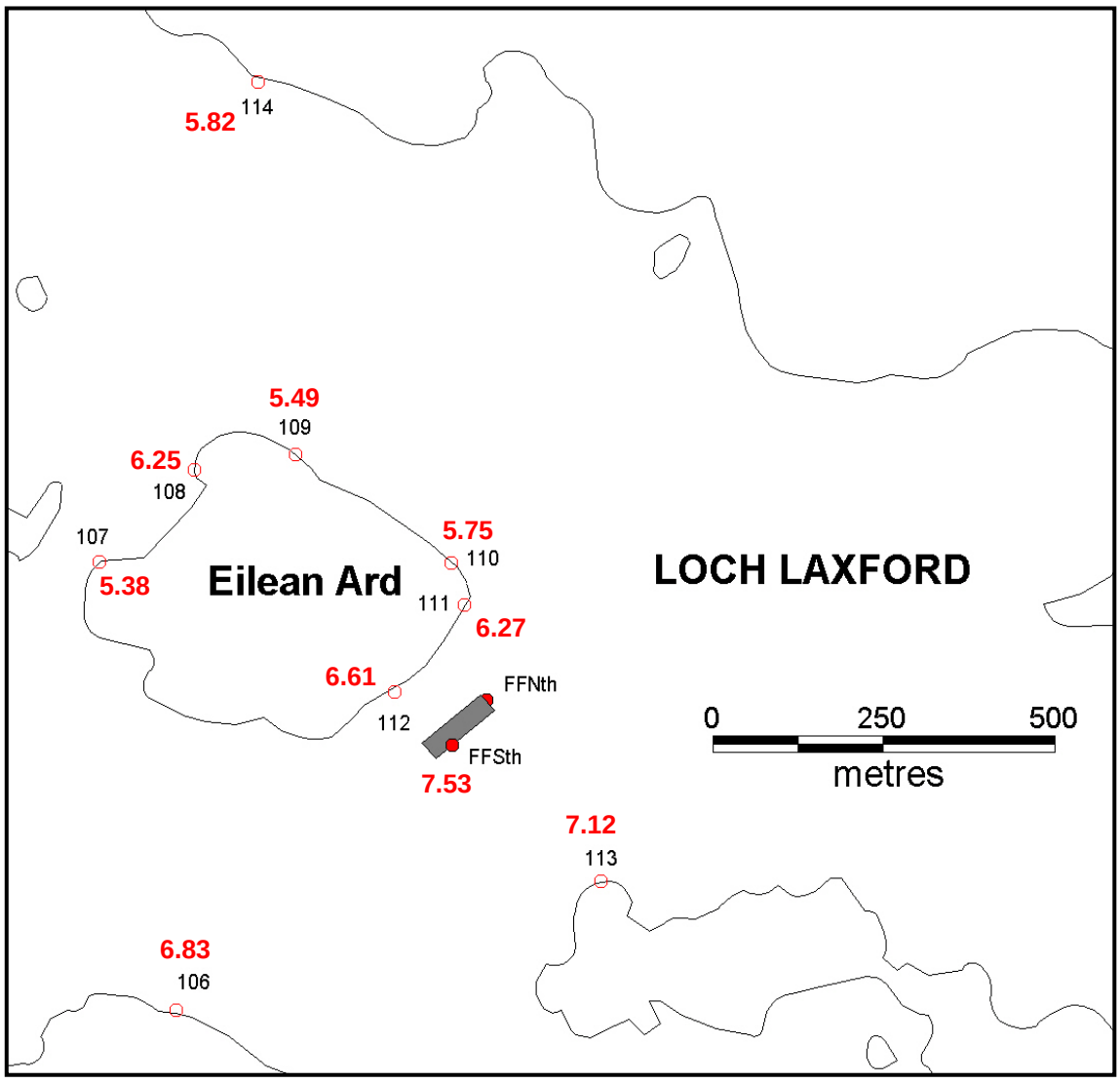


Figure 7.20 Site location for samples taken around the Laxford salmon farm cage group. Red values indicate the mean values of $\delta^{15}N$ for three plants.

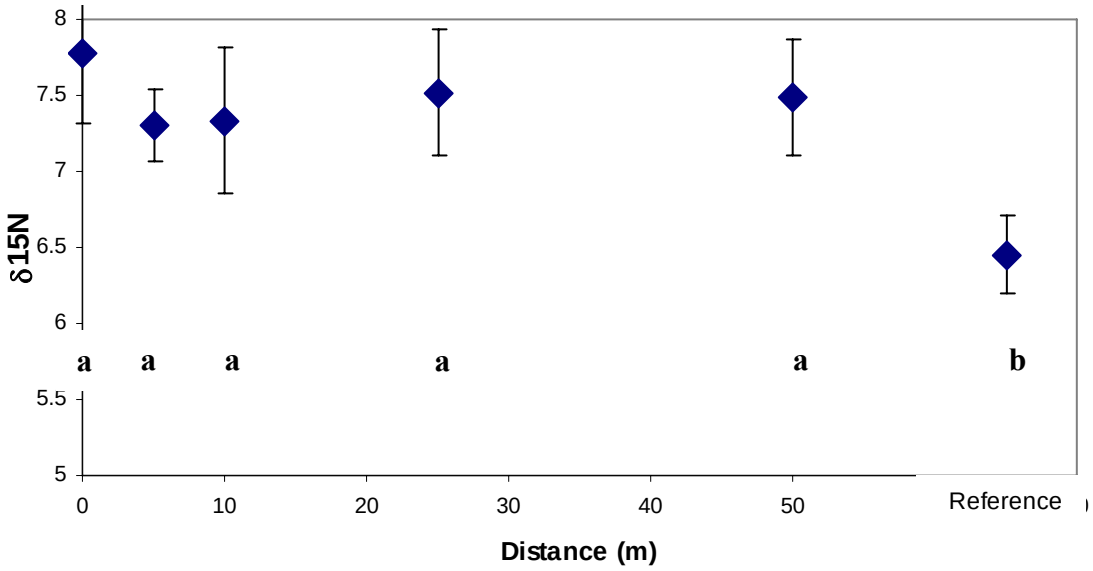


Figure 7.21 Nitrogen isotope abundance values for tethered *Palmaria palmata* plants against distance from the farm cages.
Each value is a mean of six plants. Sites that share the same letter are not significantly different at the 0.05 level. Horizontal bars are the 95% confidence intervals.

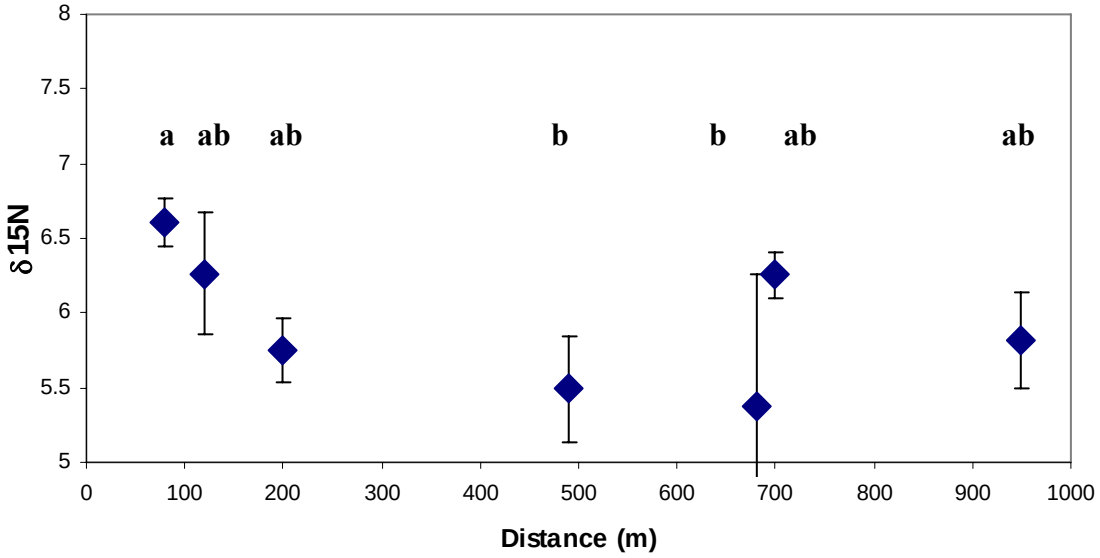


Figure 7.22 Nitrogen isotope abundance values for wild *Palmaria palmata* plants against distance from the farm cages. Each value represents a mean of three plants (horizontal bars are the 95% confidence intervals). Sites that share the same letter are not significantly different at the 0.05 level.

Discussion

Declining $\delta^{15}N$ trends in plants with distance from the salmon farm cages indicate decreasing amounts of farm-derived nitrogen being taken up. Samples from sites outside of the principal area of interest, further up the loch (east) were enriched in ^{15}N, consistent with greater availability of farm-derived nitrogen likely to be originating from cages further up the loch and some build up of farm-derived nitrogen within the loch.

The results were in agreement with those obtained in the later geographic (section 7.3) and cultured seaweed (section 7.4) studies. However, the full extent of distribution of fish farm-derived nitrogen is still poorly defined. At the extent of distances from fish farms where plants were sampled, ^{15}N abundances were still relatively high and there is some doubt as to whether or not this was as a result of nitrogen of fish farm origin.

More studies are required including comparisons with lochs without fish farm inputs. Similarly, more sampling of plants needs to be done outside the lochs, further away from fish farms, to better assess natural ambient variations in nitrogen isotopes. Sampling needs to be stratified temporally and with habitat type to gauge more confidently the impact of fish farm nitrogen. Studies would be assisted by monitoring nitrogen isotopes abundances in the water column and relating this to the abundances measured in the plants.

7.5 Mass Balance for Nitrogen Isotope abundances for cultured salmon.

The use in this project of nitrogen isotope abundances for tracing the fate of salmon farm-derived nitrogen assumes that the nitrogen isotope abundances of salmon derived and ambient nitrogen have detectably different ^{15}N contents. To test this assumption, $\delta^{15}N$ for soluble salmon wastes was determined. As the abundance of ^{15}N of the ammonium in seawater is not easy to measure, the $\delta^{15}N$ for nitrogenous based soluble waste products has been estimated here by mass balance.

The projected fate of nitrogen in salmon farm open ocean aquaculture as proposed by Black (2001) was used as the basis for calculating the mass balance (Figure 1.3). It is recognised that in reality there is some variation around this estimate. Actual amounts of soluble N released are dependent on many factors. When farms started operating in the early eighties, feeding efficiencies were low and feed conversion rates may have been as low as 2:1 reflecting the loss of much feed to the environment. With refinement of farming practises, some farms now claim better than 1.1:1 conversion rates (Naylor and Burke 2005). There are other factors determining feeding efficiencies including feed formulation. Trials are under way to use plant materials in feeds this will affect the amount of nitrogen passing into through the system into seawater (Francis *et al.* 2001). Loch Duart salmon feed is sourced (2003-2006) from a Norwegian based company, Havsbrun. Havsbrun sources fish for their fish meal from the North Sea and the company's web site claims it consists primarily of Blue whiting, herring, mackerel, and caplin. The amount of each of constituent fish species is dependent on season and would affect feeding efficiencies and release of nitrogen to the environment. Black's (2001) estimate is used as it represents a median estimate of the nitrogen budget.

Methods

Abundances of ^{15}N were measured in feed, fish flesh and faeces for salmon collected at Badcall Bay on 20 June 2005. Five fish (3-5 kg) from two different pens were culled and muscle tissue sampled. The fish were also 'milked' of faeces using a method similar to 'milking' for roe. Some of these were harvest fish and had not been fed and had no faeces in their intestines resulting in samples from only four fish. Samples of feed were obtained from five different feed-lot bags.

Samples were kept cool and freeze dried on return to the laboratory. Samples were then ground using a mortar and pestle and sub-samples weighed to give 100 mg of nitrogen for analysis in the mass spectrometer.

The following equation was used to calculate the $\delta^{15}N$ of soluble waste nitrogen:

$$\delta^{15}N \text{ diet} = X.\delta^{15}N \text{ tissue} + Y.\delta^{15}N \text{ faeces} + (1-[x + y])\ \delta^{15}N \text{ excreted metabolite}$$

Where x & y are the fractions of the isotope incorporated into tissue and faeces, respectively.

Samples of fish feed had also been sampled twice previously in May and August 2003 and these results have been included for comparison. For each of these samples, a salmon farm pellet was taken from three different bags of feed on a salmon walkway (Laxford).

Results

Calculation of $\delta^{15}N$ for soluble fish waste for 20 June 2005 using a mass balance based on values of $\delta^{15}N$ determined for fish feed and flesh indicates a value of 9.5 ‰ (see Table 7.3).

Two earlier measurements had been conducted previously for $\delta^{15}N$ of fish feed. The value for fish feed of 11.3 ‰ compares with values of 9.1 ± 0.5 ‰ and 9.5 ± 0.5 ‰ determined for May and August 2003. The differences indicate some variation in 15N abundances in fish feed over time.

Table 7.3 Values used for calculation of $\delta^{15}N$ for salmon soluble wastes. Numbers in brackets indicate pen number from which they were sampled.

Source	Mean $\delta^{15}N$	n	se	
Salmon Flesh (2)	13.92	5	0.19	Av. flesh
Salmon Flesh (1)	13.76	5	0.17	13.85
Salmon Feed:	11.26	5	0.16	
Salmon Faeces (2)	11.74	4	0.40	
Soluble wastes	**9.47**			

Discussion

The difference in $\delta^{15}N$ between fish flesh and feed of 2.7 ‰ agreed well with general enrichment estimates between an organism and its prey of 3.4 ‰ (Minagawa and Wada 1984) and was in accordance with findings of 2.3% (± 0.3‰) in a study on the effect of growth rate on tissue-diet isotopic spacing for Atlantic salmon (Trueman *et al.* 2005).

The estimated value of 9.5 ‰ for the $\delta^{15}N$ of soluble nitrogen waste compared well with nitrogen isotope abundances measured in algae sampled in June 2005 from cultured macroalgae grown next to the salmon cages. These were 9.2 ‰ and 9.4 ‰ for frame grown *Palmaria palmata* and *Laminaria saccharina* and 9.3 ‰ and 9.2 ‰ for longline grown *P. palmata* and *L. saccharina* (see section 7.3). It was also very similar to values obtained for wild *Palmaria* sampled from stipes of *Laminaria hyperborea* adjacent to fish farm cages in late June 2005 in Badcall Bay (9.2‰) and Loch Laxford (also 9.2‰ , see section 7.2). The close agreement between the derived $\delta^{15}N$ value for the soluble wastes and algae sampled at a similar time strongly suggests that the nitrogen in the algae samples close to the farms is likely to be farm-derived.

There was a difference of 2.2 ‰ between fish feed samples taken in May-August 2003 and the June 2005 samples. The variability in values concurred with the findings of Handley *et al.* (2004) for samples taken from a salmon farmer's sites in Lochs Linnhe and Sunart. Tests of $\delta^{15}N$ for 14 fish feed samples taken over October, September and February 2002/2003 by Handley *et al.* (2004) showed an average of 10.4 (± 0.5 se) ‰ with a range of 8.4 – 14.6 ‰. This variability suggests that the $\delta^{15}N$ of the soluble nitrogenous wastes may vary with season but perhaps also in relation to the particular fish stocks that make up the fish meal at the time it is processed.

Values of $\delta^{15}N$ found for *Palmaria palmata* as part of this study varied from 4 to 10 ‰ with the variation being seasonally related. Lower values can be expected in late winter – early spring when ambient nitrates are in abundance and higher values are found in summer after the nitrate nitrogen isotope pool has become enriched as found by Mariotti *et al.* (1984) for suspended particulate matter in the North Sea. The value for $\delta^{15}N$ of 9.5 ‰ in nitrogenous wastes suggests that calculation of percentage uptake by the algae of the different nitrogenous components may be possible in winter when there is a larger difference between isotope abundances for source and sink. In summer, however, the difference between source and end $\delta^{15}N$ is low and the chances of reliably tracing the effluent are low.

Conclusions

- There is temporal variation in naturally occurring carbon and nitrogen isotopes of *P. palmata* at Calbha in north-west Scotland. Values range from 4 ‰ to 9+ ‰ for $\delta^{15}N$ and -27 ‰ to -19 ‰ for $\delta^{13}C$ with the more enriched values occurring in the summer.

- Farm-derived nitrogen is widely dispersed throughout the lochs containing Loch Duart salmon farms. Farm-derived products may be dispersed for distances of greater than a kilometre from the cages as evidenced by $\delta^{15}N$ values for wild and cultured algae.

- Interpretation of isotope signals is confounded by factors affecting fractionation such as water movement and light. Supply of nitrogen and isotope composition is also potentially influenced by denitrification, uptake of lighter isotopes by phytoplankton enriching the remaining nitrate pool, mineralisation of sediments and reprocessing of nitrogen in the water column by heterotrophs. There may also be some freshwater inputs of nitrogen with its own isotope composition.

- Flushing time of the lochs appears to affect overall $\delta^{15}N$ values.

- Algae sampled within metres show differences in nitrogen isotope composition that may be attributed to habitat differences e.g. stipe versus reef bound *P. palmata*.

- There seems to be some evidence that water motion may be influencing $\delta^{15}N$ and $\delta^{13}C$ abundances in *Palmaria palmata*.

- The use of $\delta^{15}N$ to track salmon farm nitrogen in algae is likely to give a clearer picture in winter when there is a bigger difference in $\delta^{15}N$ between source and sink values.

- These studies need to be done in conjunction with broader scale (> 2km from any fish farms) geographic distributions of $\delta^{15}N$ under a variety of wave exposures to better determine natural ambient variation.

CHAPTER 8

NITROGEN AND FINANCIAL BUDGETS FOR GROWING *PALMARIA PALMATA* AND *LAMINARIA SACCHARINA* ADJACENT TO SALMON FARM CAGES.

Introduction

One of the original premises for this project was that seaweeds could be grown for their bioremediation potential adjacent to salmon cages. Concern has been expressed that the nutrients released as a result of salmon farming contribute to eutrophication of coastal areas possibly leading to greater prevalence of harmful algal blooms (HABs). The principal limiting nutrient for the growth of seaweeds in coastal waters is nitrogen (Lobban and Harrison 1996). In temperate waters, nitrates are renewed from deeper waters in the winter, often assisted by winter winds and swells. In winter, solar radiation is in decline, so the nutrients that are renewed are not taken up as quickly. In spring, when nutrients are plentiful and the days become longer, seaweed growth is at a maximum. As the days lengthen, and seas moderate, available nitrates are used up and from early summer, nitrogen again becomes limiting.

Growing seaweeds adjacent to salmon farms can lead to the uptake of nitrogen-based nutrients, particularly ammonium, that originate from the farm, thus extending the growing season for the seaweed. The excess ammonium, available also during winter and spring may contribute to overall plant productivity, as ammonium is taken up in preference to nitrate by some species of seaweed. As part of this project, it has been

demonstrated that yields of *P. palmata* and *L. saccharina* have been enhanced when grown in the vicinity of fish farm cages. Sampling of plant tissues in summer has shown elevated nitrogen in plants sampled closer to the cages. Plants sampled close to the cages have also shown greater abundances of ^{15}N which can be traced to ammonium originating from fish farm cages. So, for the fish farms investigated as part of this project, nitrogen originating from the cages has led to enhanced yields of seaweed and the nitrogen is at least partially fish farm-derived.

Elevated ammonium levels were found in the water column 200-300 m from fish farm cages indicating that the influence of fish farm-derived nitrogen is not confined to areas close to the cages. Elevated levels of ^{15}N were also found in plants at distances of 500+ m from fish farm cages. Sampling of wild *P. palmata* from coasts adjacent to the fish farms in this project suggests that fish farm nitrogen remains in the vicinity of the fish farm cages for durations that depend on the exchange rates of the seawater in the area. Enclosed water bodies with limited exchange are likely to lead to a build up of farm-derived nitrogen and show elevated levels of soluble nitrogen in the water compared to sites with greater water exchange. As nitrogen stays in the vicinity of the cages for extended periods of time, the nitrogen budget here is not concerned so much with nitrogen uptake directly from farm wastes, but with nitrogen that is taken out of the system as a whole by the cultured seaweed.

When calculating the nitrogen budget, I will consider the nitrogen for the system over the life cycle of a cultured salmon of two years. Two crops of *P. palmata* and *L. saccharina* are possible over two years. The following sections will look at the amounts of nitrogen taken up by the seaweed as a proportion of the total nitrogen that is put into

the system through fish farm operations and the commercial viability of growing *P. palmata* and *L. saccharina*.

8.1 Nitrogen budget

When formulating possible nitrogen budgets for growing the seaweeds there are constraints set by the amount of available data. As yet, little *P. palmata* or *L. saccharina* has been cultured in the sea in Scotland, none commercially. There has to be some speculation as to possible values for commercial size crops but based on the evidence that is available so far the variables to be considered are:

- Optimal culture methodology including:
    - Weight of seaweed cultured per metre of longline
    - Number of longlines per unit area
- Wet: dry weight of mass cultured seaweed
- Percent nitrogen of dry weight for harvested seaweed.

Browne (pers com.) has achieved yields for *P. palmata* of 1.85 kg wet weight m^{-1} of dropper (7 m long droppers, multiple harvests over a seven month period) from longlines. Browne (pers com.) claims droppers can be attached to the longlines at 15 cm intervals which would result in wet weight values of 86.3 kg m^{-1} of longline. These values contrast with the results suggested in this project of one harvest per year of 1 kg m^{-1}. The logistics of maintaining droppers every 15 cm may not be realistic due to tangling issues. However, if nets could be seeded rather than lines for the droppers, then these yields may be attainable.

The highest mean yields for a longline of *L. saccharina* achieved as part of this project were 28 kg m^{-1}. This compares well with results obtained for other members of the Laminariales. The value is not as high as for a particularly high yielding strain of *Alaria*

esculenta which Kraan and Guiry (2001) claim yielded 45 kg m^{-1} with other strains of *Alararia esculenta* ranging from 5 – 14 kg m^{-1}. Edding and Tala (2003) have reported 10 kg m^{-1} for *Lessonia trabeculata* in Chile. Holt (1984) for cultures of *L. saccharina* quotes a yield of 2.8 kg dry weight, m^{-1} of rope on the Isle of Man which equates to 20-30 kg m^{-1} at wet: dry ratios of: 7 to 10:1. In Japan, yields of 10-30 kg m^{-1} wet weight are generally accepted for *L. japonica* (Tegner 1989). It should be noted also that yields may be dependent on the desired end product. If the alga is being grown as fodder or fertiliser, where quality may not be so much of an issue, maximal yields may be sought. If however, the alga is being grown for human consumption or chemical extraction, the alga would be grown to maximise desired properties rather than yield of the alga.

There is little information on the recommended distance between longlines or the number that can be set out per unit area. In China, longlines with *L. japonica* appear to be less than 2 m apart. For this project, we will assume that 40 longlines each 100 m in length can be fitted into a hectare (approximately 2.5 m between each).

Wet weights of seaweeds can be variable. This is not solely a function of the internal water content of the seaweed; it is also a function of the amount of water that is caught up in the alga when it is harvested. For instance, as part of this project the wet to dry weight ratio of *P. palmata* varied from 6 to 9:1. Seaweed fronds that were hand blotted dry had the lowest wet:dry ratio and the algae that were directly harvested lying loose had much entrained water. For this exercise, wet to dry weight ratios used are 7:1 for *P. palmata* and 9:1 for *L. saccharina*.

Nitrogen contents of algae vary depending on species and the alga's physiological history which is often determined by environmental conditions. For this project, percent

nitrogen for *P. palmata* varied from less than 2 % to more than 7 % while percent nitrogen for *L. saccharina* varied from 1 to 3 % of dry weight. These values are within the range for these species described in the literature. Normally, when harvested for commercial purposes, particularly when used for human consumption, the alga is harvested when in optimal condition and percentage nitrogen for the alga would be expected to be at least 3.5 % for *P. palmata* and 1.5 % for *L. saccharina*.

As presented in the introduction of this thesis, for every 1000 g of salmon produced on 1200 g of salmon feed, 46 g of dissolved nitrogen is lost to the environment mainly through excretion from the fish but including dissolution resulting from feed oversupply and breakdown of benthic waste. These figures vary depending on many factors such as water temperature, feeding efficiency of the farm and feed composition. Much research is currently being conducted on feed composition and on finding substitutes for fish meal. The inclusion of these alternatives will likely alter the nitrogen content of the feed and the resulting waste products. Fish farms have become a lot more efficient in their feeding practices so less feed is wasted. The percentage of nitrogen released to the environment may decline in the longer term as a result.

The following extrapolations have been calculated for nitrogen made available from a 500 tonne farm over the time the fish are in the water to when they are harvested. A feed conversion ratio of 1.2:1 is assumed, so for 500 tonnes of fish at harvest, 600 tonnes of feed would have been used. For these 600 tonnes, 46 g in every 1200 g of feed will end up as dissolved nitrogen or the equivalent of 23 tonnes (see Figure 1.3). This reaches the sea over a period of two years and, during this time, two crops of seaweed are possible. See Tables 8.1 to 8.5 for extrapolated algal harvest possibilities and the potential resulting nitrogen uptake.

Palmaria palmata

Table 8.1 Yield (kg wet weight) per metre of longline for *Palmaria palmata* with varying yields per metre of dropper and distances between droppers which are 7 m long.

	Yield m^{-1} dropper					
	1 kg m^{-1}			**2 kg m^{-1}**		
Distance between droppers on longline cm	**45.0**	**30.0**	**15.0**	**45.0**	**30.0**	**15.0**
Yield per metre longline Kg m^{-1}	15.6	23.3	46.7	31.1	46.7	93.3

Table 8.2 Yield (kg wet weight) per longline and hectare, given 40 longlines per hectare and varying yields per metre of longline.

Yield per metre longline (kg m^{-1})	**Yield per longline tonnes**	**Yield per hectare tonnes**
10	1.0	40
15	1.5	60
25	2.5	100
35	3.5	140
45	4.5	180
55	5.5	220
65	6.5	260
75	7.5	300
85	8.5	340
90	9.5	380

Table 8.3 Nitrogen taken up by a one hectare seaweed farm over two years (two crops, for varying yields per metre of longline and nitrogen dry weight content for *Palmaria palmata*) as a percentage of dissolved nitrogen generated from a 500 tonne salmon farm over that period of time. Lighter shaded values represent the range of values encountered, the darker shaded are the most likely encountered values.

	%N dry wt					
Yield per metre longline (kg m^{-1})	**2**	**3**	**4**	**5**	**6**	**7**
10	1.0	1.5	2.0	2.5	3.0	3.5
15	1.5	2.2	**3.0**	**3.7**	**4.5**	5.2
25	2.5	3.7	**5.0**	**6.2**	**7.5**	8.7
35	3.5	5.2	**7.0**	**8.7**	**10.4**	12.2
45	4.5	6.7	**8.9**	**11.2**	**13.4**	15.7
55	5.5	8.2	10.9	13.7	16.4	19.1
65	6.5	9.7	12.9	16.1	19.4	22.6
75	7.5	11.2	14.9	18.6	22.4	26.1
85	8.4	12.7	16.9	21.1	25.3	29.6

Laminaria saccharina

Table 8.4 Yield (kg wet weight) per metre longline with varying yield per dropper and distances between droppers for *Laminaria saccharina* .

	Yield per dropper					
	25 kg			50 kg		
Distance between droppers on longline (cm)	**30**	**70.0**	**110.0**	**30**	**70.0**	**110.0**
Yield per metre longline (kg m^{-1})	83.3	35.7	22.7	333.3	166.7	111.1

Table 8.5 Nitrogen taken up by a one hectare seaweed farm over two years (two crops, for varying yields per metre of longline and nitrogen dry weight content for *Laminaria saccharina*) as a percentage of dissolved nitrogen generated from a 500 tonne salmon farm over that period of time. Lighter shaded values represent the range of values encountered, the darker shaded are the most likely encountered values.

	Percentage N dry weight of alga				
Laminaria longline (kg m-1)	**1**	**1.5**	**2**	**2.5**	**3**
10	0.4	0.6	0.8	1.0	1.2
15	0.6	**0.9**	**1.2**	**1.4**	1.7
25	1.0	**1.4**	**1.9**	**2.4**	2.9
35	1.4	**2.0**	**2.7**	**3.4**	4.1
45	1.7	**2.6**	**3.5**	**4.3**	5.2
55	2.1	**3.2**	**4.3**	**5.3**	6.4
65	2.5	3.8	5.0	6.3	7.5
75	2.9	4.3	5.8	7.2	8.7
85	3.3	4.9	6.6	8.2	9.9

8.2 Financial viability

Incentives for farmers to grow seaweed next to salmon farms might include allowances for growing more salmon than is currently allowed on lease sites as some of the nitrogen released by the farms is being taken out through harvesting seaweed. It might be a part of the accreditation process for organic certification as growing salmon in conjunction with seaweed would make the operation more environmentally sound. A true incentive would be if the seaweed grown was at worst cost neutral or, better,

returned a profit. *Palmaria palmata* was chosen for trials as there is a known market for the alga that is currently undersupplied. *Laminaria saccharina* was chosen not only because it grows well next to salmon farms but it is very similar to *L. japonica* which is grown in Japan for the edible market (Kombu) and there is currently research into extractives from *L. saccharina* which may have biomedical applications. These two market areas promise to show the best return for weight of product. Other more low value markets are as a source of alginates, for fertilizer and as fodder to animals such as abalone and urchin.

A search of web prices for *P. palmata* products has revealed a diverse range including Dulse flakes, granules, tablets, liquid and powder principally for use as a dietary supplement and just dried or smoked for edible purposes or as a tea! There are also cosmetic products including soap, face cream, face mask, body cream, body lotion, shower gel and a foaming bath. *Laminaria saccharina* shows a similar variety of products including eye gel, body balm, shampoo, conditioner and edible products. A cross section of prices indicates possible gross returns for these algae varying from £400 to £9000 a wet tonne (see Table 8.6). This contrasts with the price paid by one of the manufacturers for raw product of 50 p per wet kilo of *P. palmata* : £500 per wet tonne (Heath, pers com).

Table 8.6 Prices obtained from the world wide web for *Palmaria palmaria* and *Laminaria saccharina* products (May 2006).

Product	Weight	Weight Units	UK pounds	Price/dry tonne	Price / wet tonne	Producer (or supplier)
Palmaria palmata						
flakes	4	oz	3.02	£26,622	£3,803	Maine Coastal Sea Vegetables
granules	1.5	oz	1.57	£36,906	£5,272	Maine Coastal Sea Vegetables
Fluid	1	fl oz	0.74			Bernard Jensen Products
Tablets	0.55	g	2.45	£44,514	£6,359	Bernard Jensen Products
flakes	4	oz	3.97	£34,976	£4,997	Now Foods
granules	16	oz	9.76	£21,514	£3,073	Blessed Herbs
Whole dried	16	oz	12.10	£26,673	£3,810	Blessed Herbs
tea	8	oz	12.72	£56,063	£8,009	TerraVita
Whole dried & smoked	17	g	0.97	£57,069	£8,153	Dolphin Sea Vegetables
Whole dried	17	g	0.87	£51,027	£7,290	Dolphin Sea Vegetables
Laminaria saccharina						
Whole, dried	25	g	1.94	£77,614	£8,624	Dolphin Sea Vegetables
Whole, dried	40	g	2.75	£68,626	£7,625	Quality Sea Vegetables
Alginates	1000	tonne	400	£400	£44.4	Nutrasweet

The cost of cultivating the algae includes estimates for infrastructure such as materials for longlines, seeding the lines (see Table 8.7) and labour. It should also include administration components, insurance etc. As many costs as possible have been included in the cost estimates for the longline and seaweed farm presented here (Tables 8.7 to 8.9) however, if the seaweed farm were to be run by a salmon farming company, then there may be savings brought about by sharing the use of equipment such as boats, anchors, administration and facilities such as laboratories etc.

Table 8.7. The cost of materials required to construct a 100 m longline (2006).

Costs for a 100 m longline			
Product	**Number required**	**Cost per item**	**Total**
Grey surface barrels	20 per 100m line	£ 69.91	£ 1398.20
Headline & anchor rope- 32mm polypropylene rope	Bundle 220m	£347.57	£ 347.57
Stainless steel swivels	8 swivels	£ 7.09	£ 56.72
Thimbles for splicing rope	10 thimbles	£ 6.00	£ 60.00
Shackles	12 shackles	£ 10.00	£ 120.00
Mooring blocks	2 blocks	£ 200.00	£ 400.00
		TOTAL	**£ 2382.49**

Two cost estimates are given below – an estimate per longline and an estimate per hectare (Tables 8.8 & 8.9). Two full time personnel would be required to set up and run a one hectare farm. One skilled worker would be paid at a rate of £20000 per annum and the other unskilled at £15000 per annum. They would seed the lines (hatchery phase, August to December), put the longlines in place (September – December), attach the seeded lines to the longlines (September – December), maintain them and then harvest (April – July). We will assume a relatively conservative harvest yield of 25 kg per metre of longline (equivalent to 2.5 tonne per longline and 100 tonne per hectare). Return for the alga is 50p per kilogram based on current returns for suppliers to Dolphin Seafoods (Heath pers com).

Table 8.8 Costs involved in managing one 100-m longline for culture of *Palmaria palmata*.

Item	Details	Capital cost	Write off period yrs	Total cost
Longlines	One	£2382.49	5	£476.498
Labour-Seed line	2 personnel 2 week			£1346.154
Labour-Maintain longline	2 personal part time 4 weeks equivalent			£2692.308
Boat-shared/hired		£20000	10	£2000
Shed, air, tanks, power drying facilities etc	one off cost	£20000	10	£2000
Admin incl insurance, licences, marketing etc				£1000
Consumables				£400
Maintenance				£200
			TOTAL	**£10114.96**
Revenue	2.5 tonne @ 50p kg fresh weight		**INCOME**	**£12500.00**

Table 8.9 Costs involved in managing a one hectare seaweed farm for culture of *Palmaria palmata*.

Item	Details	Capital cost	Write off period	Total cost
Longlines	40	£95299.60	5	£19060
Labour	2 full time personnel			£35000
Boat		£20000	5	£4000
Seed line	100000 m @ 10 per km			£1000
Shed, air, tanks, power drying facilities etc		£20000	10	£2000
Consumables				£1000
Admin incl insurance, licences, marketing etc				£2000
Maintenance costs				£1000
			TOTAL	**£65060**
Revenue	100 tonne @ 50p kg		**INCOME**	**£50000**

The figures show that at these current income rate estimates, anticipated revenue may match costs of production. Worst case scenario suggests that revenue would have to be 50% greater to meet costs. To ensure profitability, revenue per wet kilogram, on the basis of these figures needs to be in the vicinity of £0.75 – £1.00 per kg wet.

The figures presented here are for *P. palmata*. *Laminaria saccharina* would be a lot easier to produce. Ten metres of *L. saccharina* seeded string has the potential to give 10,000 kilograms of *L. saccharina* whereas 10 m of *P. palmaria* seeded string will produce 10-20 kg of product. The spores of *L. saccharina* are motile making seeding the line a lot easier. *Laminaria saccharina* brood stock is also a lot easier to collect than *P. palmata*. *Laminaria saccharina* is also more robust and thus more likely to succeed. Savings in producing *L. saccharina* are likely to equate to at least 10% of the above costs.

Discussion

While the cost differential between farm gate price and retail would seem to be considerable (ranging from 6:1 to 18:1), there is also a considerable price differential between various product types. *Laminaria saccharina* is a promising product based on the above prices either as an edible product or for biomedical or pharmaceutical applications. However ISP (Internationally Specialised Products), Girvan, Scotland is offering only ~£400 per dry tonne of *Ascophyllum nodosum* and *L. hyperborea* for alginates (~£50 per wet tonne, Rodger pers com, 2005). Similar price differentials may be expected for fertilizer and fodder applications.

Productivity rates for cultivated red algae depend on the culture type, whether longline, benthic, tank and either intertidal or subtidal. There are currently no red algae grown commercially on subtidal longlines. In Chile recently however, Avila *et al.* (1999) presented results of studies where frames with nylon and polyfilament of different diameters were seeded in the laboratory with *Sarcothalia crispata* and out-planted to the sea. These authors indicated that a total output of 140 g dry weight m^{-1} can be obtained over the growth period November–May. In Ireland a farm cultivating *Asparagopsis* in

the late 1990s, using a longline system, yielded one tonne wet weight of the plant per hectare (Kraan and Guiry 2006). For subtidal areas in southern Chile, it has been established that *Gracilaria* production can reach 91–149 tons ha^{-1} $year^{-1}$ (Westermeier *et al.* 1991). In contrast, intertidal systems established at the same latitude are less productive, with biomass levels never exceeding 72 tonnes ha^{-1} $year^{-1}$ (Buschmann *et al.* 1995). In China, *Gracilaria* is also grown intertidally and yields for this alga are quoted as 3 tonne dry weight per hectare (Wu and Pang 2006). *Eucheuma* is cultivated on lines in the intertidal. Productivity for *Eucheuma* in the Phillipines and Malaysia has been quoted at up 80 tonnes wet weight or 5 – 20 tonnes dry weight per hectare (Anon 2006; Neish 2006; Trono and Montano 2006). *Eucheuma* yields per hectare in India are 10 tonne dry weight per hectare (Anon 2006). For tanks on land Neori *et al.* (2006) claim 500 tonne wet weight per hectare for *Ulva*, and Molloy (2006) 24 tonne dry weight per hectare for *Gracilaria* in Namibia.

Quoted rates for longline productivity for Laminariales include 40-70 tonnes for *L. saccharina* in Russia and 70-80 tonnes wet weight per hectare in the Sea of Japan for *Costaria* (Selivanova *et al.* 2006). Chopin and Ugarte (2006) claim 1.5 tonne dry weight per hectare for *L. saccharina* and *Macrocystis integrifolia* at L. Druehl's seaweed farm in British Columbia.

Productivity levels obtained here for *P. palmata* and *L. saccharina* are within quoted yields for seaweeds found elsewhere for seaweed culture systems. Subtidal yields for seaweed culture appear to be greater than intertidal but not as high as for tank based culture. A maximum of 100 tonnes wet weight per hectare for *P. palmata* or *L. saccharina* would seem to be achievable. At these maximal extrapolated yields, a hectare of *P. palmata* may absorb as much as 30% of the nitrogen output from a 500

tonne salmon farm (340 tonnes wet weight *P. palmata* per hectare, 7 % dry weight nitrogen). More conservative estimates range from 3.2 to 11.2 % (up to 180 tonnes wet weight per hectare, 5 % dry weight nitrogen). *Laminaria saccharina* might absorb as much as 10 % (340 tonnes wet weight, 3% nitrogen dry weight) but more conservative estimates are up to 4.3 % (220 tonnes wet weight, 2 % nitrogen dry weight).

At yields of 100 tonnes wet weight per hectare or 2.5 tonnes wet weight per longline, growing seaweed approaches cost neutrality at current prices if the algae are to be used for edible or biomedical applications. Increasing seaweed yields through growing them adjacent to salmon farms also enhances profitability while utilising what would otherwise be a waste product (salmon cage nutrient stream) and a potential environmental problem.

CHAPTER 9

CONCLUSIONS

9.1 Nutrients in the vicinity of salmon fish farm cages.

The greater proportion of nitrogen excreted by teleost fish such as Atlantic salmon (*Salmo salar*) is as ammonium (approximately 90%). The remainder consists principally of urea. Analysis for excess nutrients in this project therefore concentrated on ammonium as most of the DIN originating from salmon cages is found in this form (Petrell *et al.* 1993). The results of this study showed that in the vicinity of fish cages holding approximately 300 tonnes of salmon, ammonium levels are consistently elevated by 2-3 μM over ambient at distances of at least 50 m from the cages in the direction of currents and leeward side of fish cages. Surveys further away from the cages showed elevated levels of ammonium of approximately 1 μM at 200-300 m in the direction of currents. These levels are low relative to ambient plant available DIN (mainly NO_3^-) from November to March of 6-10 μM, but are likely to be the principal source of nitrogen for the remainder of the year and are elevated for most of the daylight hours from 2-4 h after the initiation of daily feeding to 2-4 h after the finish.

Seasonal monitoring of nutrient concentrations of seawater adjacent to the salmon cages showed only a small percentage of readings that exceeded Ecological Quality Objectives related to Eutrophication (EcoQOs) set by OSPAR: 'Convention for the Protection of the Marine Environment of the North east Atlantic'(OSPAR 2005). These specifications include:

1/ Winter DIN and/or DIP should remain below a justified salinity-related and/or area-specific % deviation from background not exceeding 50%.

2/ The Redfield ratio (N:P ratio) should not exceed 25.

The readings that exceeded guidelines were either very close to the cages or were taken in summer. This is in accordance with the findings of recent reviews regarding enhanced nutrient levels arising from aquaculture in Scotland (Tett and Edwards 2002; Rydberg *et al.* 2003; Smayda 2006).

Nitrogen isotope evidence indicates that fish farm-derived nitrogen is found in algae growing adjacent to cages and may be found in plants more than 1 km from source. These findings agree with a similar study by Gubbins (2003) and confirms concerns expressed in a review regarding nutrient loadings in Scottish waters from fish farms by Rydberg *et al.* (2003). Rydberg *et al.* (2003) questioned the capacity of current regulatory requirements regarding fish farms to determine problem areas. The current regulations primarily address benthic impacts. There is also consideration regarding the levels of nutrients going into the lochs from fish farms, but there are still shortcomings regarding the longer term effects of fish farms and their contribution to eutrophication of the lochs as a whole.

Whereas local effects of fish farming, i.e. sedimentation and recovery underneath cages, have been subject to comprehensive studies, expected broader scale long term effects in water bodies with fish farms, such as changing nutrient and oxygen fluxes, have yet to be followed up. Larger fish farms are carrying out monitoring programmes which are centred on the site, rather than the wider environment, and do not cover the long-term impacts.

This project has also demonstrated the detectable enhancement of nutrients in the water column at distances of more than 200-300 m from the farm cages in the direction of currents. Water movements within bays can also lead to the concentration of nutrients in more quiescent areas of the bays. Even on more exposed coasts, concentrations of nutrients can occur in quieter sections of the coast (supported from modelling of coastal water movement patterns; Gillibrand pers com). Evidence for build-up of nutrients in such areas was found in this project, at the sheltered site at the head of Badcall Bay where elevated ammonium concentrations were recorded regularly.

Many reports from European coastal areas, particularly in the Baltic Sea, have demonstrated that zoobenthos (an important food for fish) have been eliminated as a result of oxygen deficiency resulting from eutrophication (anthropogenic in origin but not necessarily fish farms). In those areas, areas of 'dead bottom' are often encountered. Despite great efforts that have been made to halt the eutrophication process (Ambio 1990; Ambio 2000), 'dead bottom' continues to increase deeper waters and also in many archipelagos (Gyllenhammar and Hakanson 2005). Monitoring for such impacted areas should be considered in the broader vicinity of fish farm lease areas.

Rydberg *et al.* (2003) have suggested that the Scottish ECE (Environmental Concentration Enhancements) model, used for predicting hyper-nutrification and for salmon farming consents, must be improved to include internal mixing. Taking internal mixing into account will result in longer, in some cases much longer, flushing times for water bodies. In many cases, it will take several years before a balance between input and output is established at the sediment-water interface, presuming that the farming is going on elsewhere. Parts of the inorganic nutrient waste from the farms will be stored in the sediments**,** but the rest is returned to the water column, eventually showing up as

larger internal nutrient recirculation and potentially as excess winter nutrient concentrations (Rydberg *et al.* 2003, Stigebrandt 1999).

9.2 Algae cultured adjacent to salmon farm cages

This study has shown that it is possible to grow seaweeds adjacent to fish farms and that yields of two crops from one hectare may absorb the equivalent of up to 30% of the soluble waste emissions from culturing 300 tonnes of salmon. Yields of *Palmaria palmata* and *Laminaria saccharina* were enhanced when grown adjacent to salmon cages. If these algae are to be grown for the edible market, however, questions must be asked about the possible impact of chemicals that are added for salmon culture or particulate matter, such as that resulting from faeces, on the quality of the algae. Preliminary research has indicated that Slice (emamectin benzoate) used for sea lice treatments, cannot be detected in populations of *Palmaria palmata* grown adjacent to cages where treatments have been taking place (pers. com. Julie Graham, PhD Student, ERI Environmental Research Institute, Thurso). At Loch Duart farms, antifoulants are not used on nets so heavy metals are unlikely to be an issue, but they may be an issue at farms where nets are so treated. It was often noted in this project that seaweeds very close (<20 m) to the cages, *Palmaria* more than *Laminaria*, were covered in particulate matter and 'goo' probably consisting of feed and faecal particles. The effect of these on the suitability of the product if used for edible purposes would have to be addressed.

The results of the nitrogen isotope study showed that the influence of fish farms may be loch wide. While obvious impacts arising from a fish farm are limited to the benthos to a maximum of 50 m from the centre of deposition around the cages, and nutrients may be detected to 200 m from the cages, longer term impacts are not as obvious and may be

further reaching. Nutrients and organic matter arising from enhanced production may be accumulating in more sheltered areas of bays with fish farms. Culturing algae would lead to a net extraction of nitrogen from the system and algae would not have to be grown in the immediate vicinity of the fish farms to have a bioremediation effect. The farms could be located in areas that are more suitable for the growth of the alga. In the case of *Palmaria palmata*, for instance, this might be where there is more water motion such as in tidal current areas. A further advantage would be that the algae would not be exposed directly to chemicals and wastes that might originate from the farm, thus making them more suitable as food. There is also the possibility of growing the algae on fallow sites, thereby enhancing the utilisation of fish farm sites whilst providing a bioremediation function.

Palmaria palmata was trialled in this project as there is a market for this alga in Northern Ireland and demand cannot currently be met by supply (Browne 2001). Cultivation of this alga has received much recent interest for this reason (Browne 2001; Le Gall *et al.* 2004; Pang and Lüning 2004; Matos *et al.* 2006; Pang and Lüning 2006). *Laminaria saccharina* was also trialled as it grows quickly, has been shown to grow well adjacent to salmon farm cages in other studies (Subandar *et al.* 1993; Ahn *et al.* 1998), is easy to cultivate, has already been cultivated in the United Kingdom (Holt 1984; Dawes 1987), has a large biomass and has some potential (although as yet untested) economic value (Cumashi *et al.* unpubl.).

Commercial scale culturing trials of *Palmaria palmata* were conducted and in the process, advances were made in production efficiencies, particularly in seeding lines at the hatchery stage to be deployed at sea. Improving production efficiencies enhances the prospects of making culturing of the algae economically viable. Multiples of 100 m

lengths of line were seeded in spawning sessions as distinct from multiples of 10 m as had been reported previously (Browne 2001). Avenues for further improvement were suggested including investigating the analogous Japanese nori culture and adapting some of the methodology including the use of nets for seeding rather than lines. Using the 'tumble' process for spore release as originally developed by Pang and Lüning (2006) meant that greater control was achieved over spore settlement densities, spore hygiene and viability with potentially longer term release and greater numbers of spores per unit area of *P. palmata* frond possible.

Taking the concept of growing seaweed into the field, and trialling culture of the algae adjacent to fish farm cages, brought up issues that must be confronted before cultivation of algae in Scottish coastal waters can be successful. Of primary concern, was the occurrence of epiphytes. These included algal epiphytes that bloom in spring and early summer, particularly close to the cages, and larval epiphytic fauna such as mussels and bryozoans that become most evident in late summer, fouling crops. These epiphytes contaminate the crops, compromising quality and are an issue for edible crops and algae for extractives where quality and presentation are often critical. Most of the salmon farms were in relatively sheltered waters where these problems are exacerbated. Areas where there is more water motion may not be as subject to colonisation by these contaminants. In the more sheltered waters, to avoid the problem of epiphytes, one crop only would be possible per year. In order to do this, seeded lines should be in the water as soon as possible after the summer settlement of epifauna. The seeded crops will then be at maximal size before harvest in late spring of the following year. Harvest may also be possible over a period of time extending supply of fresh product to the markets.

A further issue for quality of the crops was found in bleaching of the *Palmaria palmata* around June. Ideally, unless a solution is found for this problem, crops should be harvested before the onset of bleaching. Possible solutions may be lowering the seeded lines in the water column thus exposing the crop to cooler waters with higher nutrient levels at depth, but also taking them out of the range of the high light environment in the shallows. Another possible solution may be to shade the plants. This could be done with a shade material but it may also be possible to have a multiple seaweed crop and grow an alga such as *Laminaria* spp. or *Alaria esculenta* above the *Palmaria* thus shading it.

While the project has advanced the methodology for cultivating *Palmaria palmata* on a commercial scale, growing this species close to the cages is not always optimal, especially when water flow is low. On occasion, discoloration was noted for plants grown close to the cages, where they can also be subject to colonisation by epiphytic algae. While increase in yield of the plants was demonstrated, this was only in the immediate vicinity of cages (< 50 m). Although algae grown at greater distances from cages are likely to take up farm-derived nitrogen, given the lower amounts relative to ambient it would seem unlikely that the extra nutrients would be sufficient to make a significant difference to yields.

In contrast, *Laminaria saccharina* grows very well close to salmon cages and appears to benefit from uptake of farm nutrients. Questions have been raised, however, concerning the potential for commercialisation of *Laminaria saccharina* for the edible market. *Laminaria saccharina* is known to have high levels of iodine (2789-5277 µg/g, CEVA 2004: Centre d'Etude et de Valorisation des Algues, www.ceva.fr, Teas unpubl. report). The Recommended Dietary Allowance (RDA) for adult men and women is 150 µg d^{-1}.

The median intake of iodine from food in the United States is approximately 240 to 300 μg d^{-1} for men and 190 to 210 μg d^{-1} for women. The Tolerable Upper Intake Level (UL) for adults is 1,100 μg d^{-1} (1.1 mg d^{-1}), a value based on serum thyroptropin concentration in response to varying levels of ingested iodine. (US Institute of Medicine 2001) .

In France the use of *L. saccharina* as a foodstuff is restricted to a condiment (CEVA pers com). *Alaria esculenta* is closely related to *Laminaria saccharina*, is known as an edible alga (Kraan and Guiry 2006), grows well on Loch Duart salmon cage structures (Robinson pers com) and thus may be a better option for cultivation next to salmon cages (166 μg g^{-1} iodine, Teas unpubl report, MCSV 2005: http://www.seaveg.com/chart.html). *Alaria esculenta* has been the subject of research for longline culture in Ireland with good results (Kraan and Guiry 2001). Other algae for consideration are *Himanthalia elongata* (edible, Kraan and Guiry 2006), *Porphyra* spp. (edible, Chopin *et al.* 1999; edible, Carmona *et al.* 2006) and the *Falkenbergia* phase of *Asparagopsis armata* (chemical extractives ,Schuenhoff *et al.* 2006).

Possible seaweed species for growing in Scotland can be divided into those for: 1/ human food; 2/ chemical extractives (including cosmetics and thalassotherapy); 3/ fertilizer; and 4/ animal fodder. An economic evaluation as part of this project suggested that, for *Palmaria palmata*, a price of £1 per kilogram wet weight rather than the current price of 50p per kilogram would make the alga a more attractive option for cultivation.

Many macroalgal species used for human food and chemical extractives are obscure species with unusual life cycles that often require specific environmental condition for optimal quality either for taste and appearance or for maximal return of the required

chemical. Growing these algae is often more costly; often, however, the return is greater.

The Scottish seaweed industry has been briefly reviewed recently by Milliken and Bridgewater (2001) and worldwide usage of seaweeds has been reviewed by Mc Hugh (2003). Some edible algae that are suitable for culture in Scotland have been covered already in this chapter and other species include: *Ulva*, *Laurencia obtusa* and *Chorda filum* (Kenicer *et al.* 2000). Reviews of chemical extractives from seaweeds have been conducted recently by Smit (2004) and Fitton (2003). Smit (2004) claims that despite the intense research effort by academic and corporate institutions, very few products with real potential have been identified or developed. Substances that currently receive most attention from pharmaceutical companies for use in drug development, or from researchers in the field of medicine-related research include: sulphated polysaccharides as antiviral substances, halogenated furanones from *Delisea pulchra* as antifouling compounds, and kahalalide F from a species of *Bryopsis* as a possible treatment for lung cancer, tumours and AIDS. Other substances such as macroalgal lectins, fucoidans, kainoids and aplysiatoxins are routinely used in biomedical research and a multitude of other substances have known biological activities. As part of this project, *Laminaria saccharina* was investigated for fucoidans (Cumashi *et al.* unpubl.). Fucoidans are a group of highly sulphated polysaccharides of brown seaweeds and echinoderms. They are characterized by different types of physiological activities including anticoagulant, antiviral, anti-inflammatory and others. The properties of these products have been reviewed by Berteau and Mulloy (2003).

Some algae are easier and thus cheaper to grow per kilogram. The larger brown kelps fall into this category. Algae used for fertiliser and as feed for animals such as sea

urchins and abalone (Cook *et al.* 1998; Cook *et al.* 2000; Kelly *et al.* 2001) would need to be cheap to produce as their viability as a commercial option is dependent on producing them at a very low price. One advantage in producing algae for fertiliser and feed for animals is that the quality in terms of content and appearance is not so critical making their production more cost effective. The larger brown algae such as *Laminaria* spp. are particularly well suited in this regard. In Japan and Korea, samples of algae that are not of sufficient quality for edible purposes, such as *Undaria pinnatifida*, are used for feeding urchin and abalone. Growing the algae for both purposes (edible ad animal feed) optimises economic returns.

Integrated aquaculture or polyculture, consisting of growing sea urchins or oysters, scallops, seaweed and salmon, is currently being investigated at SAMS (Scottish Association) as part of the MERMAIDS (Multi-trophic culture for Environmental Remediation) and the AAAG (Atlantic Arc Aquaculture Group) projects. By growing sea urchins (or even abalone) in conjunction with the salmon and seaweeds, there are multiple benefits in terms of the costs. The sea urchins feed on excess feed and faeces from the salmon cages, the seaweed benefits from the excess nutrients also emanating from the cages and the seaweed can be used to feed the sea urchins and abalone.

At present, there are no seaweeds that are being cultured for commercial purposes in Scotland. To be considered for cultivation adjacent to fish cages, seaweeds must be economically viable in their own right. This would suggest that, without further incentives, farmers are unlikely to take up growing seaweeds solely for the purpose of taking up excess nutrients. Further research must be directed at developing a commercially viable seaweed crop which should be profitable without other incentives.

This would include improving marketing opportunities for current commercial species and developing markets for other prospective species.

If research into larger scale, longer term impacts of salmon farms in Scottish waters does indicate deleterious effects which have knock-on impacts to fisheries and the natural environment (perhaps for example, by impacting on biodiversity in quiescent areas of lochs), then perhaps the regulatory authorities should consider giving incentives to salmon farmers to grow extractive species that have some bioremediation potential such as sea urchins and seaweed. As there do seem to be some uncertainty about the potential impacts of the fish farms, perhaps the precautionary principle should be applied. Incentives to consider integrated aquaculture may be to keep current fish biomass consents or risk further controls such as lower biomass limits in the longer term if the salmon farmers cannot demonstrate that they are extracting farm wastes from the environment. This would be irrespective of any changes that may come along in regard to feed technology that lessen the release of soluble nutrients to the environment.

One disincentive for salmon farmers becoming involved in growing other crops is that it is a lot easier and more cost effective to concentrate on one species. While costs for growing seaweed or sea urchins may be saved due to shared infrastructure, there are extra costs in terms of extra expertise and time required. There is also the matter of prioritization of species for maximising quality and market supply of both. For *Palmaria palmata*, this is not so much of a problem as it is likely that the demand for the alga (optimal harvest time) occurs after the busy harvest time for salmon. One way around this for other species of seaweed, may be to have a central seaweed (or/and urchin) based company (-ies) that is contracted to grow seaweed at salmon farm sites across a number of different fish farming companies. This would make seed production

much more cost effective and would centralise expertisewhich in turn would accelerate advancements in technology.

From a regulatory point of view, if there is a commercially viable seaweed, then it is a lot easier to grant lease areas for the crop within the currently defined salmon farm lease areas than the current situation where sperate areas would have to be considered further locking away and affecting coastal waters. This would concentrate aquaculture activities within currently accepted areas. However, if it is considered that growing the crop somewhere in the vicinity of the fish farms, such as within same loch, would have bioremediation advantages, then some extension to leases, only for seaweed crops would have to be planned for.

Currently, organic certification for salmon farms is in a process of constant review due to the current levels of interest by the general public and the very recent application of the organic movement's principles to the marine environment. Both of the UK-based organisations that certify aquaculture businesses as organic, the Soil Association and the Organic Food Federation, are attracted to the idea of integrated aquaculture as it helps meet their ideals by adding an element of nutrient recycling. In fact one certifier, the Soil Association, is even considering that demonstrating an element of nutrient recycling should become a prerequisite of an organic salmon farming operation. Given the public interest in this area, adoption of the concept of integrated aquaculture and organic certification has a double benefit in terms of care for the environment and marketing advantages.

On the broader scale, fish farms are not the only contributors to nutrient enhancement in the Scottish coastal environment, although they do make up a significant proportion. In

the year 2000, the nitrogen contribution from fish farms possibly matched the nitrogen arising from the total sewage input from the population of Scotland (MacGarvin 2000). In north west Scotland, where most of the fish farms are concentrated, this is likely to have occurred as long as 20 years ago (Tett and Edwards 2002). Perhaps growing seaweed should be considered and encouraged on a larger scale to ameliorate sewage impacts? This would be more suitable for seaweed grown for fertilizers or perhaps for biofuel production (for example see Horn *et al.* 2000; Raiko *et al.* 2003).

The most economical means of culturing seaweed is to rely on natural settlement. To obtain high tonnages of seaweed easily, longlines can be deployed over great areas in late summer – autumn. These pick up natural settlement of spores of multiple species from the water column as the season progresses. By hanging suitable fertile seaweeds such as Laminariales at regular intervals on the longlines or by deploying lines/strings to be seeded over suitable seaweed beds to maximise settlement of desired species at times of maximum fertility before deploying on longlines, the settlement of desired species can be encouraged. This reduces the cost of seeding lines. However, it is only suitable when the species required for culturing is not critical such as for fertilisers, for biofuel production or for feed to animal species.

In summary, this research has shown that seaweeds have the potential to remove nitrogen from the environment in the vicinity of fish farm cages thus having a bioremediation value. However, until the technology and/or their commercial value/pricing changes, this may not be economically viable.The main avenues of future research should focus on developing, marketing and promoting seaweed products to establish prices that would qualify culturing seaweeds to be financially viable in their own right. If an environmental cost can be attributed to the impact of the farm wastes

and if these are high or even potentially high, then incentives could be offered to salmon farmers and potential seaweed growers to further develop seaweed crops. The current returns from seaweed crops are close to making a seaweed farm financially viable. Regulatory authorities might consider encouraging a pilot scale operation that would produce commercial seaweeds and lead to increased demand, better returns and efficiencies from scale.

Scotland has a long history of seaweed utilisation going back to the seventeenth and eighteenth centuries (Thomson 1983; Kain and Dawes 1998; Kenicer *et al.* 2000; Milliken and Bridgewater 2001). Perhaps, by stimulating the beginning of a new industry that provides new products and is of benefit to the environment, the tradition of innovation in the area of seaweed utilisation will be upheld in Scotland.

APPENDIX 1

ENVIRONMENTAL DATA MEASURED ON SITE FOR THE LOCH DUART LTD. LEASE SITES.

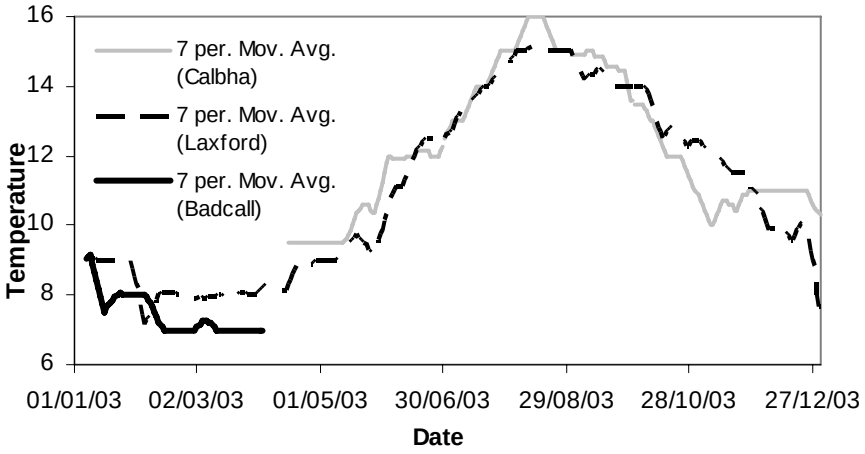


Figure A1.1 Temperature variation at the three Loch Duart Ltd. lease sites, Calbha, Laxford and Badcall for 2003. Temperature measurements taken by staff on walkways at 4 m depth approximately daily. Lines follow seven day averages.

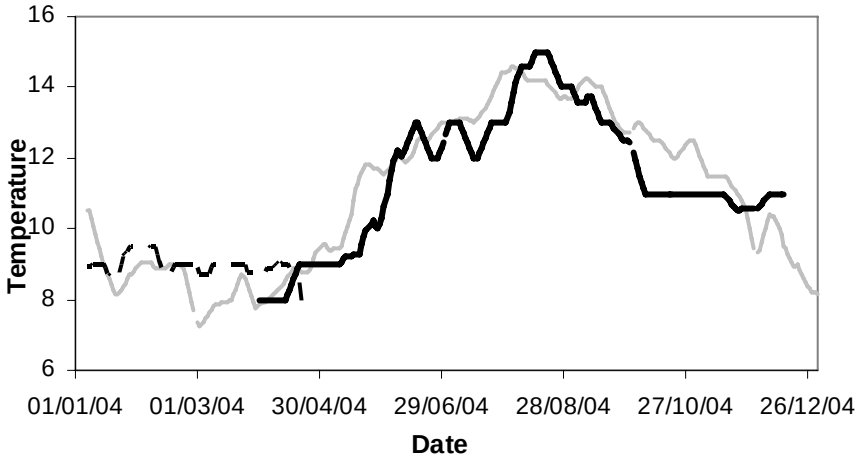


Figure A1.2 Temperature variation at the three Loch Duart Ltd. lease sites, Calbha, Laxford and Badcall for 2004. Temperature measurements taken by staff on walkways at 4 m depth approximately daily. Lines follow seven day averages.

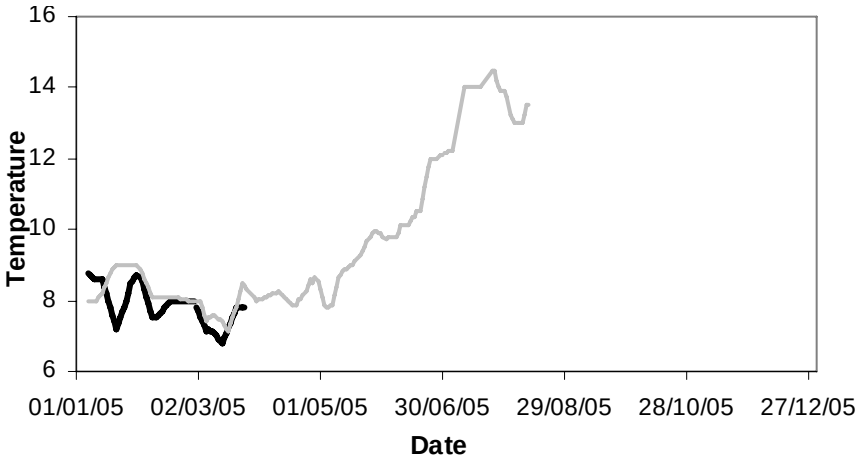


Figure A1.3 Temperature variation at the three Loch Duart Ltd. lease sites, Calbha, Laxford and Badcall for 2005. Temperature measurements taken by staff on walkways at 4 m depth approximately daily. Lines follow seven day averages.

CURRENTS
Laxford

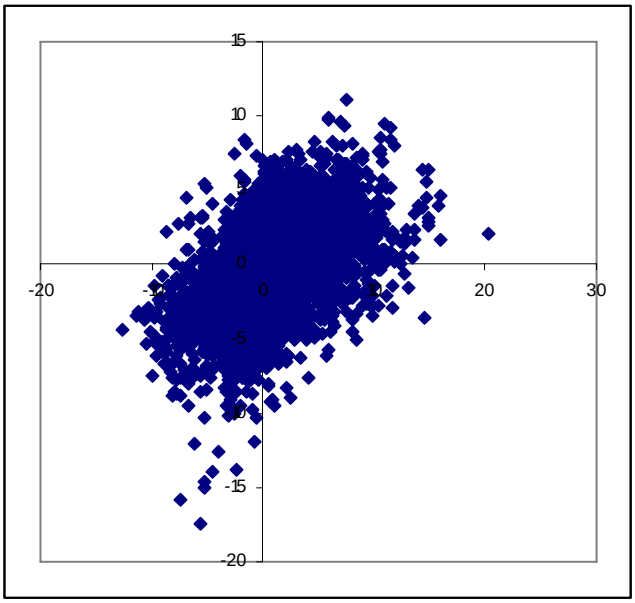


Figure A1.4 Near surface current speed 130 m SE of 'F' walkway, 3/7/2003 to 27/8/2003, ,. max 20.4 av 3.1 km/h.

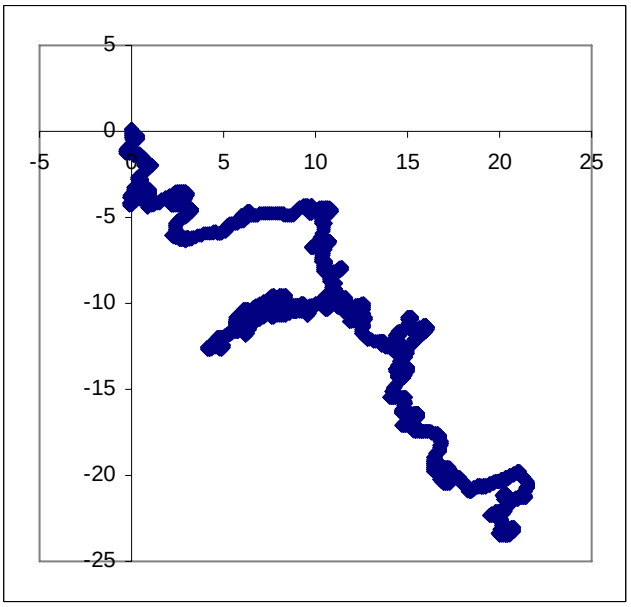


Figure A1.5 Near surface cumulative distance. 3/7/2003 to 27/8/2003. Axis units are kilometres.

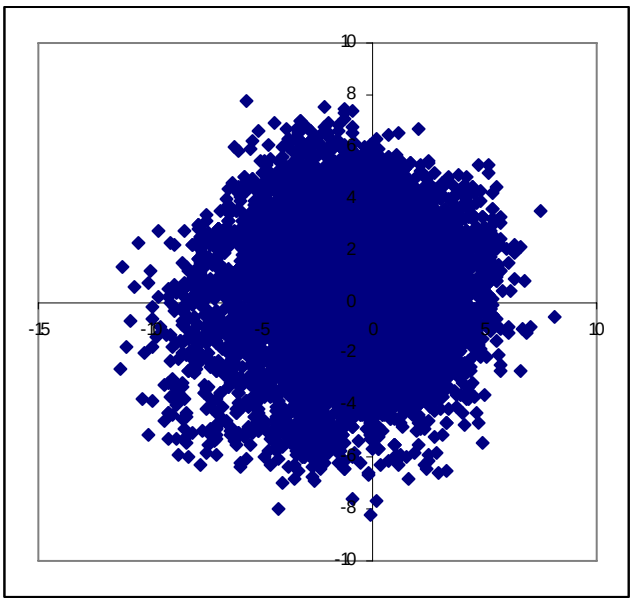


Figure A1.6 Near bottom current speed in 18 m water at low tide 130 m SE of 'F' walkway, 3/7/2003 to 27/8/2003, ,. max 20.4 av 3.1 km/h.

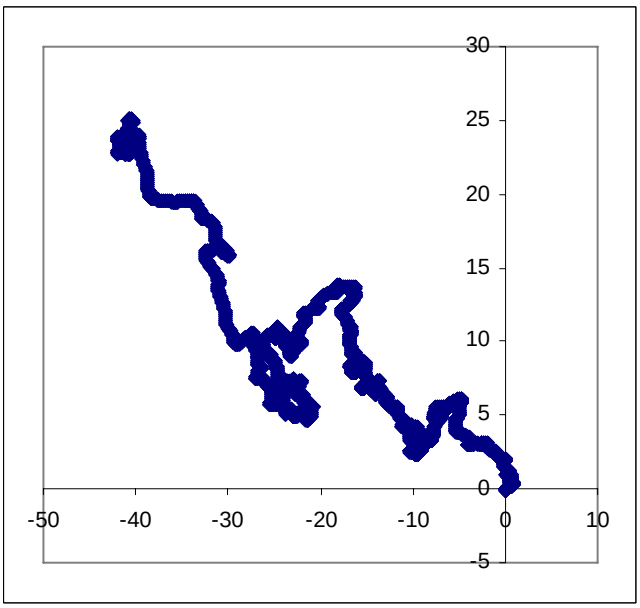


Figure A1.7 Near bottom cumulative distance. 3/7/2003 to 27/8/2003. Axis units are kilometres.

Badcall

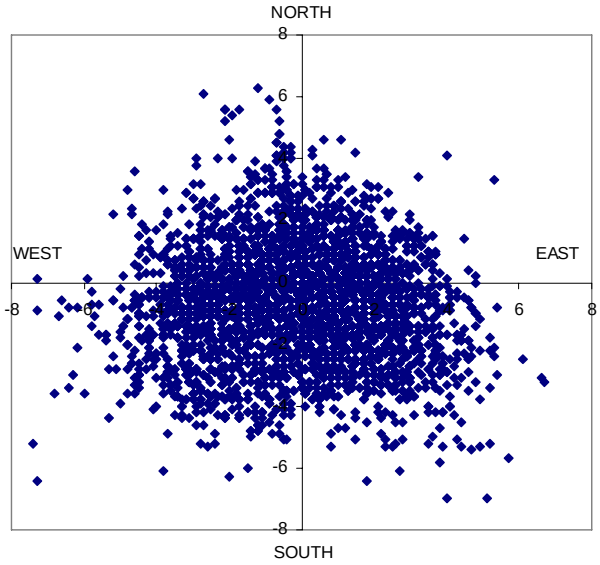


Figure A1.8 Current meter (715) approximately 5m below surface at low tide, Cabha Bay, D walkway. Speed, max 9.7, Av 2.19 km/h.

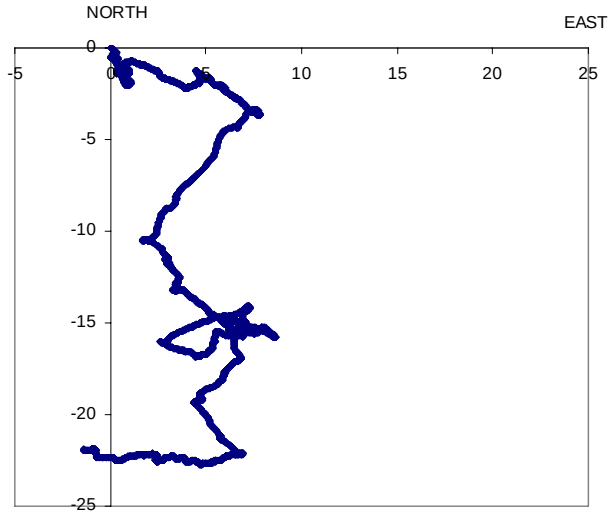


Figure A1.9 Current meter (715) approximately 5m below surface at low tide, Calbha Bay, D walkway, approximately 25 m SW of cages. 4/5/05 to 16/6/05. Cumulative distance axis units are kilometers.

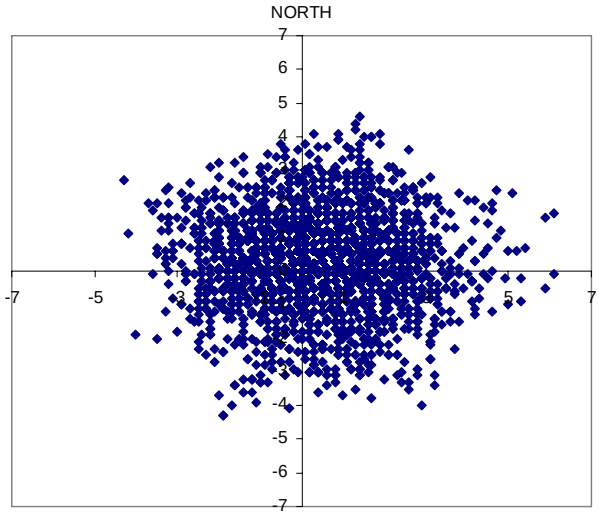


Figure A1.10 Current meter (716) approx 10m below surface at low tide, Cabha Bay, D walkway, 25 m SW of cages. Speed, max 6.3 Av 1.36 km/h

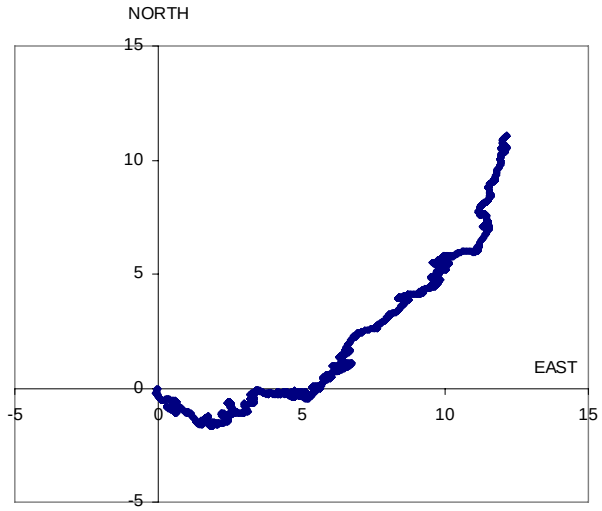


Figure A1.11 Current meter (716) approx 10m below surface at low tide, Cabha Bay, D walkway, 25 m SW of cages. 4/5/05 to 16/6/05. Cumulative distance, axis units are kilometres.

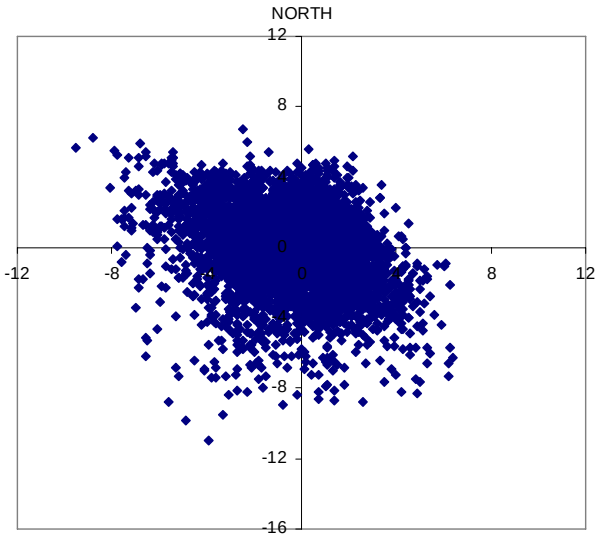


Figure A1.12 Near surface at low tide, Cabha Bay, D walkway, approx 50m north of cages. Speed, max 7.1 Av 2.15 km/h.

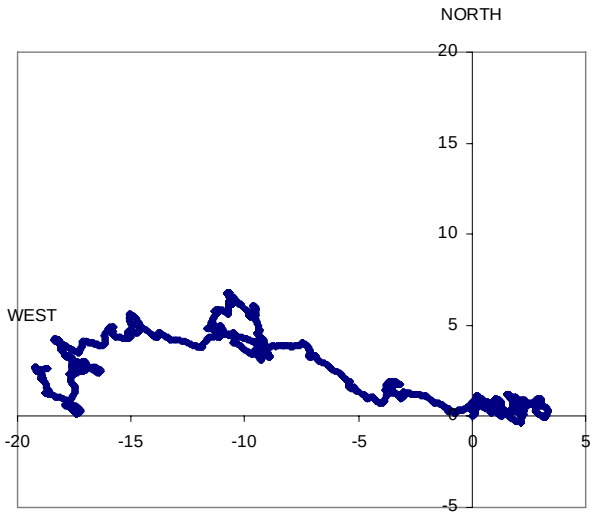


Figure A1.13 Near surface at low tide, Cabha Bay, D walkway approx 50m north of cages.. 4/5/05 to 16/6/05. Distance, axis units are kilometres.

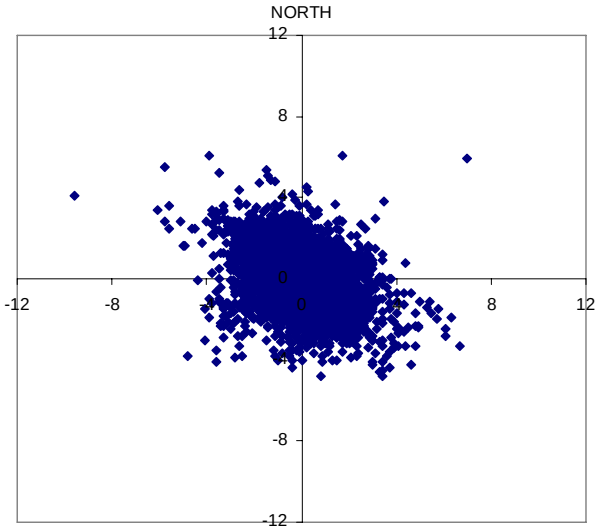


Figure A1.14 Cabha Bay, D walkway approx 50 m north of cages 10m from surface at low tide.Speed, Max 10.5, av. 1.46 km/h.

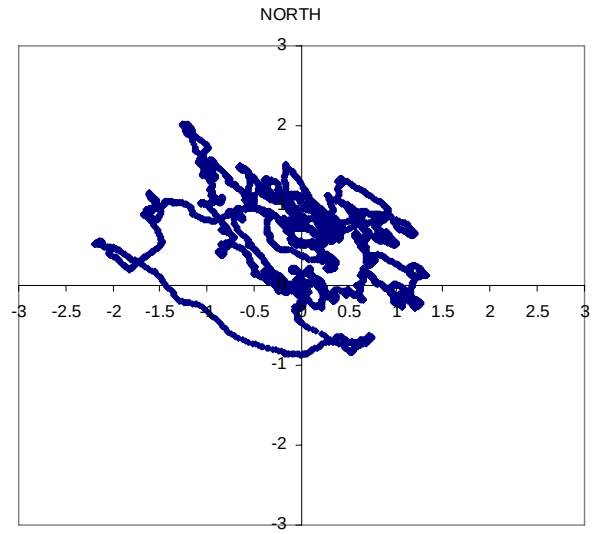


Figure A1.15 Cabha Bay, D walkway approx 50 m north of cage 10m from surface at low tide. Distance, axis units are kilometres.

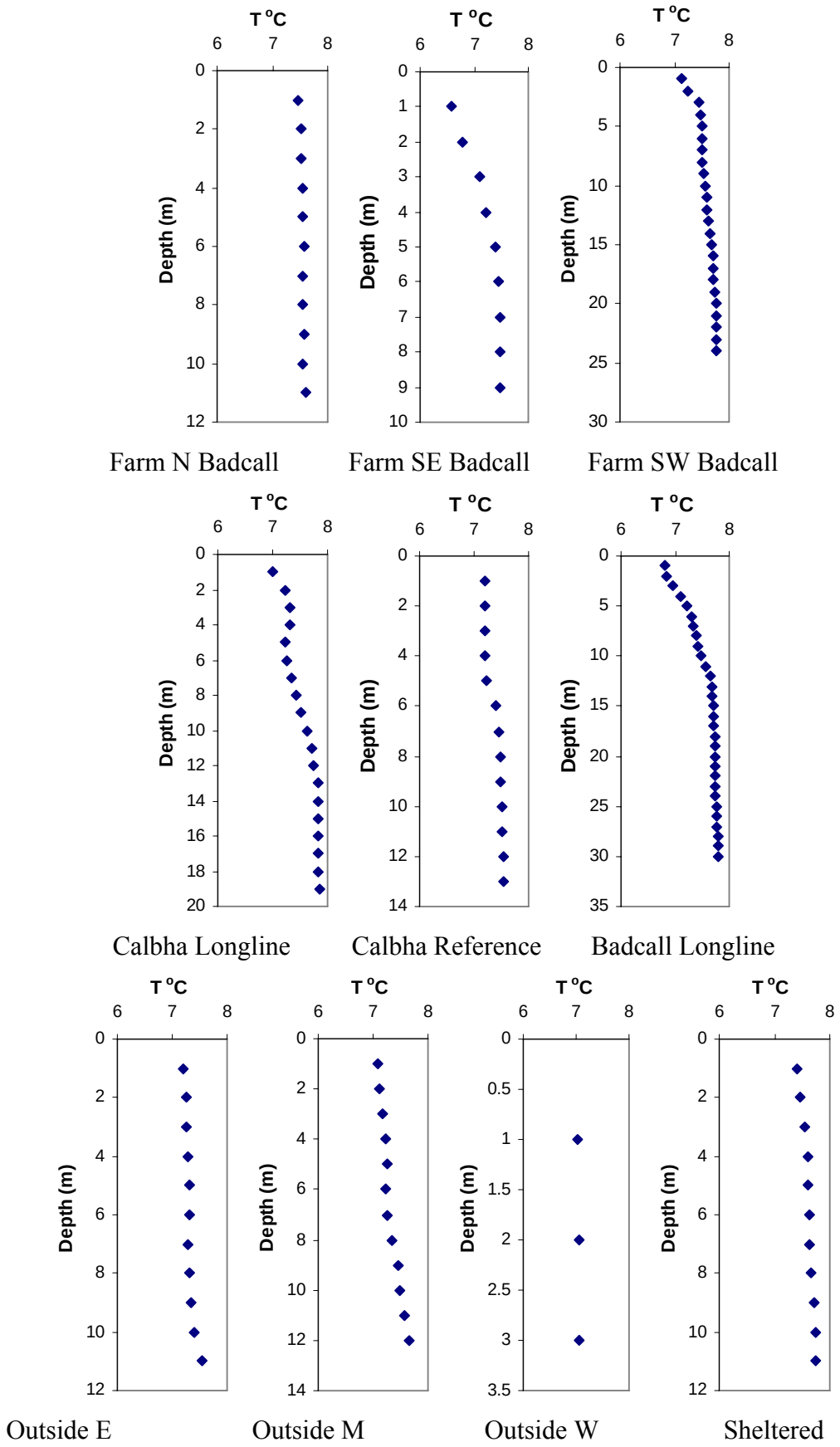


Figure A1.16 Temperature profiles for Badcall & Calbha sites, 25/2/05. See Figure 2.1 (Chapter 2) for site locations

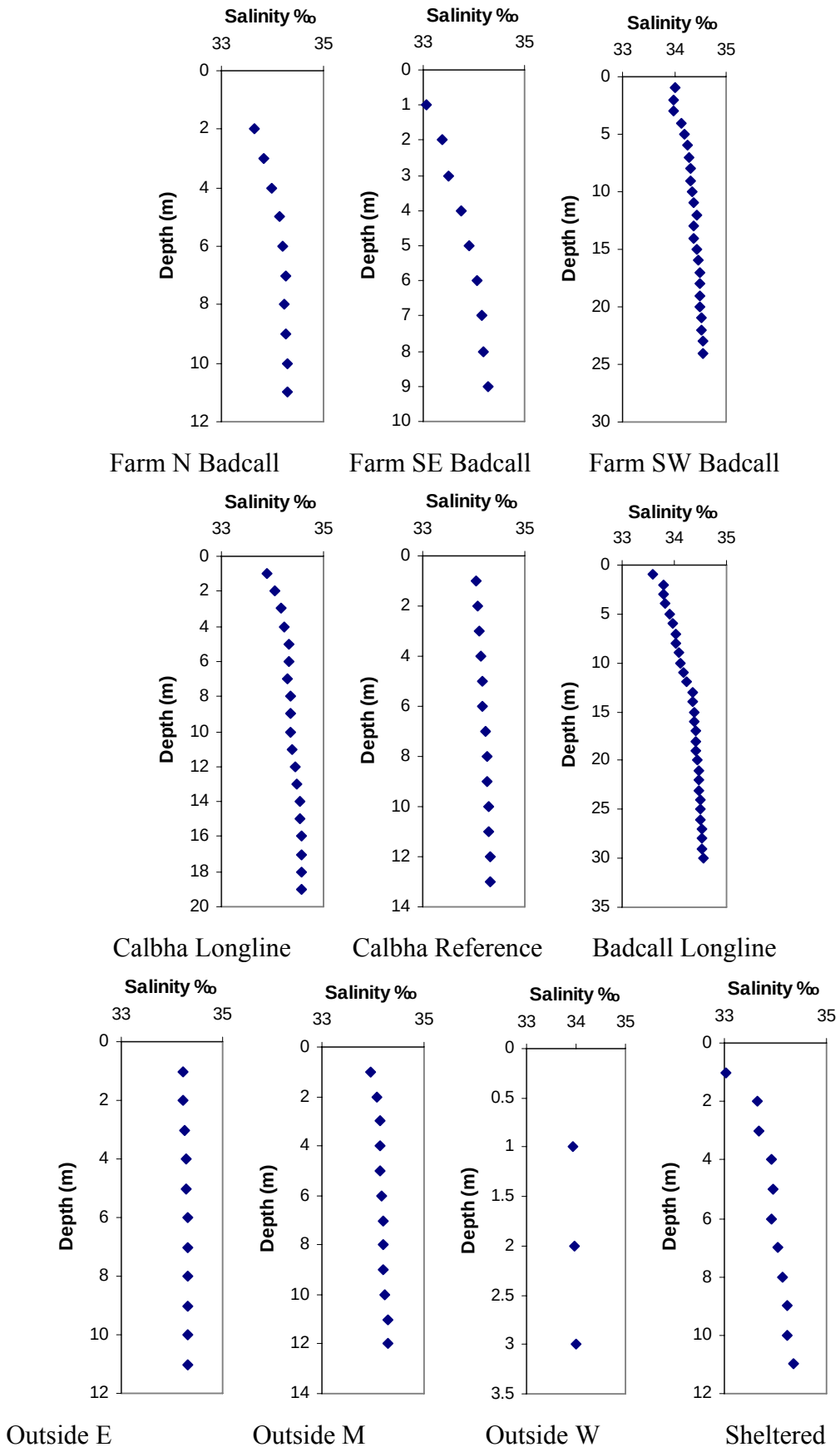


Figure A1.17 Salinity profiles for Badcall & Calbha sites, 25/2/05. See Figure 2.1 (Chapter 2) for site locations

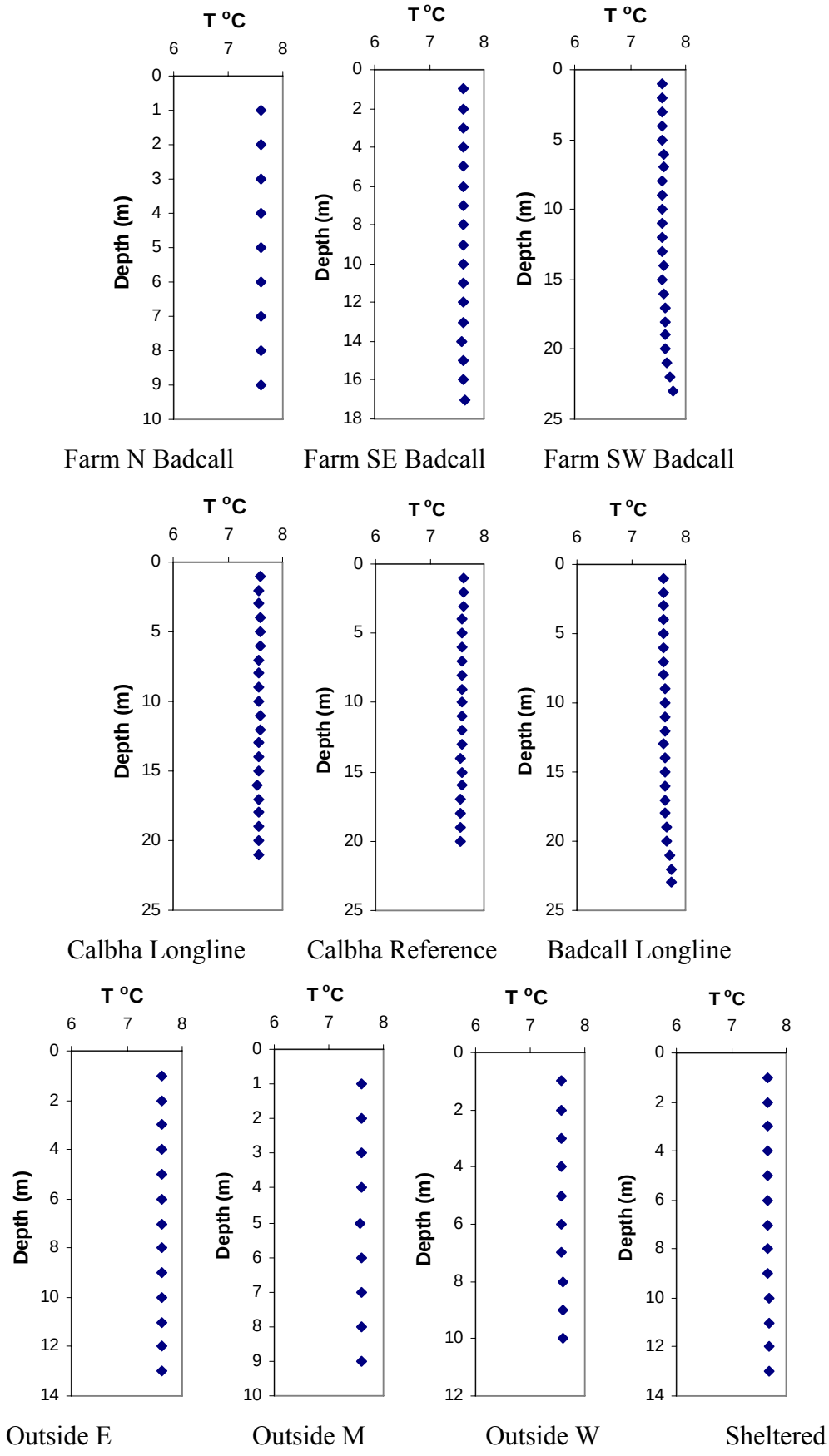


Figure A1.18 Temperature profiles for Badcall & Calbha sites, 29-30 March 2005. See Figure 2.1 (Chapter 2) for site locations

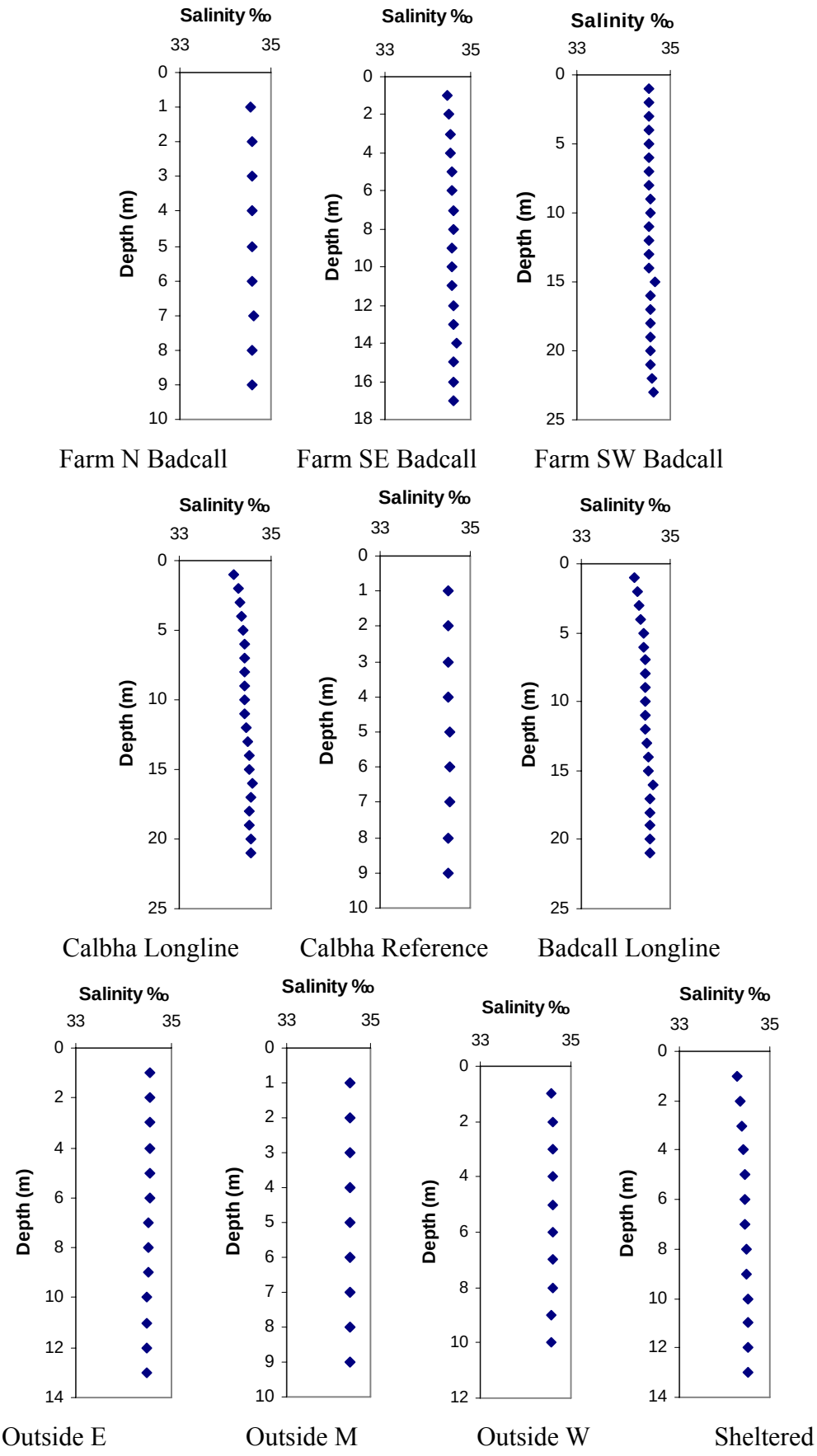


Figure A1.19 Salinity profiles for Badcall & Calbha sites, 29-30 March 2005. See Figure 2.1 (Chapter 2) for site locations

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