Kelp culture in integrated multi-trophic aquaculture: expanding the temporal limitations

by

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B.Sc., Trinity Western University, 2001

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Abstract

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In integrated multi-trophic aquaculture (IMTA) production of cultured species may not align temporally. For instance, at an IMTA site in Kyuquot Sound, BC where the cultured species are *Anoplopoma fimbria* (sablefish), *Plactopentin yesoensis* (Japanese scallop) and *Saccharina latissima* (sugar kelp), sablefish are grown year round while the kelp culturing lasts from winter to summer. Kelp sporophytes become visible in early spring while harvest takes place in July. This indicates that at Surprise Island the time period of nutrient extraction by the kelp is limited to only a few months per year. Two potentials methods to lengthen the time in which the kelp component was on site were employed and evaluated: 1. the use of multiple kelp species with potentially differing seasonal growth strategies and; 2. outplanting kelp seed at four different times of the year. The first method involved outplanting seed of four kelp species, *Saccharina latissima*, *Costaria costata*, *Alaria marginata* and *Saccharina groenlandica* and monitoring growth parameters (blade length and yield). For the second method, a modified seed production method of Merrill and Gillingham (1991) with Luning and Dring (1973) successfully provided seed throughout the year. Seasonally out-planted seed was also monitored for growth parameters. Results were marginal for experiments and were confounded by the lack of growth rates due to infrastructure problems, grazing by

naturally setting marine snails and seemingly poor environmental conditions for kelp culturing at the farm site. However, data indicated that certain species in co-culture may slightly increase the time period, and strategically entered kelp seed may do the same. In particular the co-culture of *C. costaria* and *S. groenlandica* or biannual seed outplanting in fall and spring may increase the length of growth period of kelp provided certain limitations found during this experiment are overcome (i.e. pressures of grazing). Additional potential benefits with these kelp production strategies are the diversification of final kelp products, additional kelp harvests and increased production.

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Chapter 1- General Introduction

1.1 Global aquaculture

According to the Food and Agriculture Organization of the United Nations (FAO) the world's capture fisheries have reached their maximum sustainable harvest levels (2010). Meanwhile, the world's aquaculture production has been growing annually 8.3 percent since the mid 1980s. In the early 1950's the global annual aquaculture production was less than one million MT, and by 2002, production had increased to 59.4 million MT (FAO 2004). By 2006, production from aquaculture nearly reached that of capture fisheries (FAO, 2007). Predictions have stated that if aquaculture is to maintain its current level of per capita consumption that production will have to increase to 80 million MT by 2050 (FAO 2000). Intensification of production and modifications of current practices are growing trends in aquaculture (FAO 2007). However, many analysts have suggested that some modern aquaculture practices which contribute a large portion to global aquaculture production, are unsustainable (Folke & Kautsky, 1989; Naylor et al. 1998). In particular, intensive fed aquacultures (i.e. salmon farming) have been criticized over a variety of environmental issues (Naylor & Burke, 2005).

Despite prior predictions of the dramatic increases in the demand for aquaculture products, more recent figures indicate the aquaculture growth is starting to slow which is, in part, due to public concerns over aquaculture practices (FAO 2009). Certainly consumers have taken notice of aquaculture controversies and, if industry desires growth and profitability, increased production will depend on improved management with close attention paid to environmental issues and performance (FAO 2010).

1.2 Salmon farming in British Columbia

In British Columbia (B.C.), the greatest aquaculture production comes from salmon farming. Salmon farming was initiated in B.C. in the 1970's with many small family run operations in Sechelt Inlet and Alberni Inlet. Problems with farm sites, disease, and poor market conditions nearly destroyed the small industry. In the 1980's Norwegian salmon farming companies began to buy leases in BC and the industry grew. The number of operating farms increased and production rose swiftly in the latter half of the 1980`s and into the 1990`s. In 1984, ten farms produced 107 MT of salmon but by 1988 the number of farms grew to 118 which produced 6,600 MT (Bocking 2007). In 1991, a mere three years later, production rose to 24,000 MT.

In 1995 the B.C. provincial government imposed a moratorium on further expansion of the salmon farming industry by capping the number of farm sites at 121. Further growth of the industry would depend on the results of an environmental review of the industry by the B.C. Environmental Assessment Office (BCEAO). In 2002 the B.C. government retracted the moratorium after the implementation of a salmon aquaculture policy framework which addressed major environmental concerns from the BCEAO review. Since the lifting of the moratorium, salmon production has levelled off with little change in production or the number of active farms (BC MAL 2009). In 2007, B.C. was the fourth largest producer of salmon in the world culturing over 80,000 MT of salmon with a value in excess of CAN $400 million (BC MOE 2008). From surveys conducted in 2004 estimated that 2800 people were employed directly by farms and farm related practices (i.e. hatcheries and processing) (BC MAL 2004).

The salmon farming industry was considered by its proponents to be an economic saviour for coastal communities that had been hugely effected by downturns in many resource based industries (capture fisheries, forestry and mining). However, the industry still suffers from a poor public image (Cubitt et al. 2009).

Today, at a typical salmon farm in B.C., 400,000-500,000 salmon are grown out to marketable size which is approximately 5-7 kg. Nearly all the salmon farmed in BC are grown in flow through netcages suspended from grids of attached steel walkways or plastic circular floating cages. Grids are of various sizes but a grid formation of 2 x 6-12 is typical. Square pens, which were originally 30 x 30 ft or 50 x 50 ft, have been replaced with pens 100 x 100 ft and larger. Improved engineering and siting of farm installations now allows some growers to use circular pens as large as 130 ft in diameter. Fish are fed pellets from the time they are entered into the farm as smolts until harvest as adults. The growout period is variable taking 1.5-2.5 years, depending on a variety of environmental and oceanographic variables, and management practices (Bachman et al. 2009).

1.3 Waste release from fish farms

Despite the relative efficient use of feed in salmon production as compared to other farmed animals (Jackson, 2009), wastes from fish farming are the uneaten feed, dissolved inorganic and organic wastes, and feces (Beveridge et al, 1991). The uneaten feed and settable waste fall onto the seabed contributing to the total carbon flux on the local benthic environment. Furthermore, sediment free sulphide concentration increase and changes to oxygen demand can occur (Wildish et al. 1999; Hargrave et al. 1995). Sufficient deposition of the wastes can result in anoxic benthic conditions and changes to benthic community structure (Hargrave et al. 1997; Karakassis et al. 1999; Ritz et al.

1987). Increased water column turbidity and acidification have also been identified as other potential effects from benthic waste loading (Gowen & Bradbury 1987; Hargrave et al 1993).

Ackefors & Enell (1994) estimated that there was 2.5 MT of organic output for every MT of salmon produced on Norwegian farms. Weston (1986) estimated that for Washington State fish farms, 25-33 percent of the feed consumed by salmon was expelled as feces. In B.C., organic sedimentation rates under salmon farms were estimated at 42.7g TVS/m2 per day with a maximum of 94.5g TVS/m2 (Cross, 1990). Although the impacts to the ocean floor can be extensive (Johanneson et al. 1993; Pohle et al. 2001), impacts in B.C. are generally localized to within 50 m of farm operations and chemical and biological remediation has been well documented after fouling of farms (Brooks & Manken 2003).

In B.C. sediment waste accumulation under and near salmon farming installations has garnered attention from government agencies. In 2002, the B.C. Ministry of Water, Land and Air Protection instituted the finfish aquaculture waste control regulation (FAWCR; B.C. reg. 256/2002) which included benthic sediment monitoring component at all active salmon farms. In order for each individual farm to operate, the bottom sediments near the cage structures must not exceed particular chemical thresholds, effects of the farm must be reversible, and effects can not increase over time (i.e. from production cycle to production cycle). In other salmon farming jurisdictions around the world, environmental monitoring programs have been implemented though typically not as extensive as the FAWCR protocols (BC MAL 2005).

While most of the attention given to impact studies of marine fish farming wastes has focused on fecal deposition and benthic impacts, far less interest has been given to the potential environmental effects associated with the release of dissolved inorganic nutrients. Inorganic nitrogen and phosphorus from fish farms in both fresh and saltwater has been studied in Europe (reviews by Handy & Poxton 1993 and Gowen & Bradbury 1987), and to a lesser extent in the Northeast Pacific (Levings 1997; Rensel 1989; Korman 1989). Sources of inorganic nitrogen phosphorus are from ammonia released from gill epithelia and urine, and phosphate released from feces and urine, and resuspended ammonia from anaerobic benthic sediments. Estimates of the amount of inorganic nitrogen and phosphorus released from salmon farms equate to roughly 68 kg and of N and 14 kg of P per tonne of fish produced (Hall et al. 1992; Wu 1995). In B.C. in 1996, releases of nitrogen and phosphorus from salmon farms were estimated to be 844 MT and 188.6 MT, respectively (Levings 1997). In 2005, B.C. MOE estimated the loss of inorganic nitrogen from salmon farms to be between 4000 and 4500 MT (Cubitt, et al., 2009). Given that the nitrogen and phosphorous released from intensive aquacultures have the potential to cause eutrophication of surrounding water bodies (Troell et al. 1999; Folke et al. 1994), some authors are concerned with this form of waste (Chopin et al. 1999). Studies however, have found no environmental effects of inorganic nutrient release from farms even within poorly flushed areas (Gowen et al.1988; Weston, 1986). Typically nutrients associated with fish farms are diluted rapidly, and are not measurable beyond 50 m from farm installations (Brooks & Mahnken 2003; Gormican 1989; Rensel 1989). The common perception is that with modern feeding regimes/techniques equating to less feed waste, decreased protein fractions in modern

feed and proper citing of farms in higher current sites, potential effects from increased nutrients in the water column are unrealized (Brooks & Mahnken 2003; Davies 2000; Ackefors et al. 1994). Localized effects have been largely dismissed however, large-scale area wide concerns are still a concern (Folke et al 1994; Folke & Kautsky 1992; Chopin et al 2001). More contemporary research has indicated that there may be far field measureable nutrient levels in areas where intensive aquaculture operations exist (Nordvarg & Johansson, 2002; Pitta et al 2005). Nutrient residence times, which were once thought to be short-term in high current areas, can be much longer than expected (Page 2001; Sanderson et al. 2008).

The environmental effects of inorganic nitrogen levels cannot be entirely accounted for by local aquaculture operations as so many factors affect ocean nutrient loads (i.e. agricultural runoff, natural nitrogen fluxes, sewage release). No studies directly link intensive aquaculture with broad-scale ecosystem effects, though some authors suggest that the inorganic nutrient outputs of aquaculture are important given that the nitrification of coastal waters is a global phenomenon (Chopin et al. 1999).

1.4 Addressing the Nutrient Loading Issue

Two technologies that have been suggested for reducing the potential detrimental interactions between salmon farming and the environment are land based systems and closed containment systems (Naylor et al. 2003). In land based production systems the infrastructure consists of tanks and/or raceways on land where seawater is pumped in from a nearby source and effluent water can be treated. Closed containment systems are still considered open-water. However, the nets typically used by fish farms are replaced with bag-type or hard walled enclosures in which fish can be reared. Proponents of these

technologies emphasize the environmental benefits such as reduced waste outputs into the natural environment and reduced interaction between cultivated species and wild stocks. Producers emphasize the costs of such technologies by way of infrastructure, pumping of water and air, maintenance, added labour, and consider them largely unprofitable. The initial attempts at closed containment were considered by both industry and industry groups to be failures (B.C. Salmon Farmers Association 2007). To date technological advances in salmon farming (i.e. improved feeding, better management practices, etc.) have not satisfied opponents and likewise, proposed technologies like closed containment, are not considered viable options for farming companies.

One alternative that has been suggested is a balanced ecosystem approach to aquaculture that integrates existing culture types with the culture of species of lower trophic levels (extractive species, filter feeders, detritavores etc). It is seen as a means of overcoming many of the environmental problems associated with intensive aquaculture such as salmon farming (Folke and Kautsky 1992; Chopin et al. 2004; McVey et al. 2002; Costa-Piece 2002). Integrated multi-trophic aquaculture (IMTA) is the term used for such a system, and the concept has gained much attention throughout the world as a more sustainable form of production over intensive monocultures.

1.5 Integrated multi-trophic aquaculture (IMTA)

In nature nutrients are cycled between living organisms and their environment and the notion of waste is absent. As in nature, if wastes from existing aquaculture operations could be converted into biomass of other species, the aquaculture system would then become more balanced as the input energy could be used to enhance growth of multiple species (Chopin et al. 2004). Wastes from one culture become valued nutrients that are

important to the sustainability of the integrated production system. If wastes, which can potentially have negative environmental effects, are instead converted into biomass of another aquaculture, effects are could be mitigated and the environmental sustainability of the system would increase. As well, if the added species being cultured have value, the economic sustainability of the culture system could also increase (Ridler 2007).

IMTA systems require balance, in that the cultures must be complimentary in their trophic levels to properly utilize available nutrients. The integration of fed aquaculture, like salmon farming, and extractive cultures, like shellfish and seaweeds, could potentially create that balance (Chopin 2001). In IMTA systems, the culture species are connected by nutrients and energy transfer through the movement of water. Ideally, in an IMTA system, the chemical and biological processes would balance by selection and proportion of cultured species. Because of the inherent sustainability of IMTA systems, the practice is quickly becoming a new field of research in aquaculture and gaining much recognition (FAO 2009; Naylor et al. 2000).

IMTA is by no means a new concept as it has been in practice in China for centuries (Chopin 2001). The first known literary work on aquaculture, *Pisculture of Carp* by the Chinese politician turned aquaculturalist Fan Li, was written in the 5th century B.C., and instructs that ponds culturing carp should contain ample aquatic plants and turtles (Fan Li; FAO; Rabanal 1988). This type of aquaculture, termed polyculture or co-culture, characterizes the activities of early integrated culture settings where species were grown together for reasons to do more with water and land use, and nutrient utilization rather than environmental concerns and culture species were chosen to take advantage of every available niche (Li 1987). In China, the culture of cyprinids (carps) is

a classic example of polyculture where multiple species carps with different feeding requirements and differing ecological requirements are raised in the same pond. In these multi-species culture systems productivity can double over single species system with extra demands of input energy or space (Billard & Berni 2004).

1.6 IMTA Research

Integrated aquaculture work began in the western world with the research of Ryther et al. (1975). In a complex land based system, domestic sewage was treated using shellfish, microalgae and seaweeds. Though important in the development of integrated aquaculture there were doubts as to the method because of the value of organisms cultured on human waste. The idea was soon adapted to treat intensive aquaculture effluents (Huguenin 1976) but the interest in North America was not again revisited for several years.

A general definition of integrated farming was given by Edwards et al. (1988) as “an output from one subsystem in an integrated farming system, which otherwise may have been wasted, becomes an input to another subsystem resulting in a greater efficiency of output of desired products from the land/water area under a farmer’s control.” Folke & Kautsky (1992) applied the integrated approach to off-shore aquaculture suggesting that the culture of certain species could be brought into close proximity to intensive, fed cultures so as to benefit from increased nutrient availability. In the 1990’s various studies were carried out to develop and demonstrate the potential of integration to mitigate some of the possible environmental effects of intensive aquaculture (Haglund & Petersen 1993; Troell et al. 1997). Other studies showed the suitability of wastewater/wastes from intensive aquaculture being a suitable nutrient source for seaweeds (Neori et al. 1991;

Buschmann 1996; Krom et al. 1995; Chopin et al. 1999) and other culture species including shellfish and detritivores (Jones & Iwama 1991; Stirling & Okumus 1995; Ahlgren 1998).

IMTA is now mentioned by several aquaculture resources (Pillay 2005; Stickney & McVey 2002; Costa Pierce 2002; Bert 2007; Holmer et al. 2008). Extensive reviews of integrated aquaculture, with particular consideration to the importance of seaweeds in integrated aquaculture/IMTA, have been published (Buschmann et al. 2001; Chopin et al. 2001; Troell et al. 2003; Neori et al. 2004). In 2003, the European Aquaculture Association annual meeting was entitled “Beyond Monoculture”, and was dedicated to practices such as IMTA (as well as, polyculture, co-culture, integrated aquaculture etc.).

With growing public concern over aquaculture practices and product quality, particularly in developed countries, producers could gain a market advantage by adopting IMTA as their production system. The State of the World Fisheries and Aquaculture (FAO, 2008) noted that IMTA was an aquaculture method on the rise due to public concerns over current aquaculture practices and products. Indeed, IMTA is being recognized as a potentially more sustainable form of aquaculture by various groups. In Canada IMTA has become a national aquaculture research focus (Department of Fisheries and Oceans 2009). In Denmark, IMTA is legislated as a requirement for all new aquaculture farms (Chopin 2006).

1.7 IMTA Research

IMTA is now being studied around the world in different culture systems with a variety of organisms (Barrington et al. 2009). In the Northeastern United States, a demonstration-scale project was developed to grow Atlantic cod and two species of the

red seaweed *Porphyra* (nori) (Carmona et al. 2006). In Portugal, three potentially valuable red algal species, *Palmaria palmata*, *Gracilaria bursa pastoris* and *Chondrus crispus*, all showed effectiveness in removing nutrients from turbot culture effluents (Matos et al. 2006). Early experimentation with the effectiveness of Ulva lactuca in removing nutrients from intensive fish culture systems (Neori et al. 1991; Neori 1996) lead to the development of a commercial scale IMTA venture (SeaOr) incorporating cultures of fish, seaweed, abalone and sea urchins (Neori et al 2004). In South Africa a similar commercial scale IMTA farm is located where the kelp species Ecklonia maxima is grown in the effluent of fish and abalone to remove nutrients and then fed back to the abalone (Troell 2006). Gracilaria species have been shown to filter nutrient enriched water and are good candidates for IMTA systems in China (Zhou et al. 2006) and Chile (Troell et al. 1997; Buschmann 1996). In New Brunswick, Canada, a long term study has been underway examining aspects of an IMTA system comprised of Atlantic salmon *Salmo salar*, blue mussel *Mytilus edulis* and kelps *Saccharina latissima* and *Alaria esculenta* such as environmental sustainability, economic diversification, food safety and social acceptability (Chopin et al. 2004).

In BC in the early 1990's, some studies recognized the potential of integrated aquaculture. Jones & Iwama (1991) studied oyster and salmon polyculture noting that oyster growth benefitted from be cultured in close proximity to salmon farms. Subandar et al. (1993) suggested *Laminaria saccharina* as a macroalgal candidate for removing dissolved nitrogen from aquaculture effluents in land based systems. Petrell et al. (1993) modelled an integrated system of salmon and kelp in terms of nutrient uptake, respiration and economics. They concluded that a very large kelp farm would be needed to uptake all

dissolved N and P from a salmon farm. However, the constantly fertilized kelp would increase profitability over non integrated kelp farms. In 1995 kelp/salmon integrated systems were investigated at existing salmon farms and farm derived nutrients were found to have a fertilizing effect on kelp growth given ambient dissolved nitrogen was lacking (Ahn 1997). Integrated systems received little attention until a shellfish/salmon farm polyculture was studied on the basis of water quality interactions and food safety and quality (Cross 2004). Since that time, the first licensed IMTA farm in Canada was issued at a small-scale commercial site in Kyuquot Sound. The site, permitted to culture several species of invertebrates, seaweeds and sablefish, is a proof-of-concept operating aquaculture facility that includes IMTA as a design feature of its sustainable ecological aquaculture (SEAfood system) approach (Cross, in press).

1.8 Seaweed Mariculture

According to the FAO, global seaweed production has had an annual growth rate of 7.7 percent since the 1970s with a total of 15.8 million tonnes predominantly from aquaculture (2010). The earliest example of seaweed cultivation recorded was in China approximately 1000 years ago. Areas of intertidal shoreline were scrubbed to expose rocks and make more available substrate for what is now known as the species *Gliopeltis furcata* and later the same method was employed to enhance cultures of zicai (*Porphyra haitanensis*) (Tseng 1993). *Laminaria* and *Undaria* beds in Japan were augmented with the same method and, in more recent years, by blasting colonized rocky shorelines and by dropping stones and concrete blocks in the ocean to increase available substrate (Druehl 1988). Harvest of natural or semi-natural seaweed beds still takes place however the

mariculture of seaweeds has become dominant accounting for over 93 percent of utilized seaweed in the world (FAO 2010).

Seaweed mariculture was developed mainly by China and Japan in the 1950's. In China, before the development of modern cultivation techniques of the brown seaweed *Saccharina japonica*, kelp production peaked at 40.3 dried tons. Ten years later, after crucial developments in the culturing techniques, the production of the seaweed rose to 6253 dried tons, with the wild harvest accounting for only 15.8 percent of the production (Tseng 1984). Development of the culture raft, manipulation of the kelp life history and fertilization techniques were the key developments to kelp culture in China.

In Japan, the discovery of the forced cultivation method was the pivotal discovery in the development of the kelp mariculture industry (Druehl pers. comm. 2007). Before 1971, kelp culture was a lengthy operation. Seeded pieces of twine were placed in submerged grids of ropes in the ocean where they remained for two years (Kawashima 1984). This was the time it took kelp species to develop the characteristics desirable in Japanese cuisine (Druehl 1988). Hasegawa (1971) developed the forced cultivation technique whereby seed production was carried out in summer months and the seeded lines put out onto the farms months earlier than traditional methods. The kelp seed put out three months early would result in kelps behaving as if second year algae. Cultivation time was cut in half but with kelps having similar characteristics of two year plants (Druehl 1988).

In Porphyra mariculture, it was the discovery of the conchocelis phase in the seaweed's life history by Kathleen Drew (1949) that made mass cultivation of the seaweed possible. The subsequent growth of the industry made the annual value of Nori

in Japan, in 1986, worth 1 billion US retail making it the world's highest-valued near shore fishery (Mumford and Miura 1988).

Many nations contribute to the global seaweed production but 99.8 percent of the production comes from Asia (FAO 2010). In 2008, nearly two thirds of Asian production was of the Japanese kelp, *Saccharina japonica* in China (Chopin & Sawhney 2008). In the Philippines, Indonesia and Malaysia intensive farm red algae mainly from the genera Euchema, are farmed as well as in Tanzania. In Japan, kelp and Porphyra cultivation dominate. For the production of agar, Chile and China and to a lesser extent lesser extent Brazil, Spain and Portugal, species of *Gracilaria* are cultivated. Of the 221 species of seaweed used worldwide, intensive cultures have been developed for ten species (Zemke-White & Ohno 1999).

The uses of cultivated seaweeds are mainly for food and food processing. Most species of seaweeds are dried and eaten directly, added to a variety of cuisines, or processed to extract cell wall components. Most notable are the phycocolloids; a group of cell wall polysaccharides from both brown and red seaweeds that have been utilized in food processing, pharmaceutical and other industries for their thickening, gelling, stabilizing and emulsifying properties. Alginates from brown algae, and carrageenans and agars from red algae make up the phycocolloids. In 1995, the annual production value of phycocolloids from both farmed and wild harvested seaweeds was worth approximately $2.5 billion USD (Zemke-White & Ohno 1999).

1.9 The Order Laminariales- the kelps

1.9.1 General biology of kelps

Kelps are brown seaweeds, or Phaeophytes, belonging to the order Laminariales. Within the Laminariales there are three families: Alariaceae, Lessionaceae and Laminariaceae. Recent genetic investigations, however, have called for a restructuring of kelps at the family level (Lane et al. 2006). Within the Laminariaceae family, from the Laminaria genus many species have been reordered into the new genus *Saccharina*. The largest genus was formerly *Laminaria*, which accounted for approximately 30 species of single bladed kelps (Guiry & Blunden 1991).

The kelp lifecycle consists of a dimorphic alternation of generations between a macroscopic sporophyte stage and a microscopic gametophyte stage. The kelp life cycle, like that of many other organisms follows a highly seasonal trend. In the Pacific northeast, kelps develop reproductive areas on their blades called sori in the late fall and early winter and release there zoospores in the winter. The flagellated zoospores are dispersed to find a favourable location for settlement and recruitment. After settlement, spores develop into male and female filamentous microscopic gametophytes which produce gametes. Male gametes are released and fertilize the female gametophytes to produce a diploid zygote. Zygotes develop into new sporophytes in the late winter, with elongation into large mature sporophytes happening in the late spring and summer. Reproduction ensues and the cycle is repeated.

1.9.2 Kelp farming

Kelp farming follows the same seasonality as the natural kelp lifecycle and has three components: a laboratory phase, grow-out phase, and harvest (Druehl 1988). The

lab phase consists of the induction of spores from kelp sori and the rearing of young sporophytes in isolated tanks. The growout phase consists of the growing and maturing of young sporophytes in the ocean. In Japanese kelp mariculture, Sanbonsuga (1984) describes the development of kelp blades as going through two separate phases: elongation and substantiation. Elongation involves the utilization of inorganic nitrogen for the increase of surface area and substantiation involves the products of photosynthesis increasing blade thickness.

In British Columbia, when blades of desirable characteristics for breeding reach maturity, in late fall/early winter, they are relocated to the lab from the farm to start kelp seed production. Spore release is induced and spore suspension retained from fertile kelp blades. Substrata (i.e. nylon twine) are inoculated with the spore suspension and spores are permitted to develop into juvenile sporophytes from 0.5-5mm. When sporophytes are of adequate size they are relocated to the farm. The substrata is put out onto the farm on longlines or rafts and submerged to a desired depth. Kelp farming is very similar in different areas where it is practiced, although there can be several subtle differences from region to region. Chinese methods of kelp culture also incorporate a green house phase which uses temperature controlled seawater to grow-out sporophytes to approximately 10 cm before they are put onto farms to avoid times of elevated, lethal ocean temperatures (Sahoo & Yarish 2005). Tseng (1987) reports of different infrastructure systems used in kelp cultivation such as longlines and rafts. Exact timing of farming operations can be slightly offset from region to region due slightly different timing of the kelp reproductivity or the use of different production strategies (i.e. the forced cultivation method). For example, an experimental kelp cultivation in New Brunswick

approximately two months earlier than in BC due to freezing of the ocean surface in winter and the availability of kelp sori in late summer and fall (Chopin 2004).

1.9.3 Kelps in IMTA Systems

Although many seaweed species have been recognized for their functionality in IMTA systems, most studies have focused on Rhodophytes (red alga; i.e. *Gracilaria sp)* and Chlorophytes (green alga; i.e. *Ulva sp.*) in closed systems with fewer studies on Phaeophytes (brown alga; i.e. kelps). This is despite the cultivation of kelps made up over 62 percent of all global seaweed mariculture in 2004 (Chopin & Sawhney 2009). This is not to say that kelps are unsuitable as inorganic nutrient extractors in IMTA systems, but rather that most studies have focussed on closed systems (Troell et al. 2003) which are not typically suitable for the production of kelp. However, in open water systems, where kelps are farmed already, the use of kelps as an inorganic nutrient extractor seems suitable. Despite the fact that kelps could be adapted to modern temperate open system culture systems to form IMTA systems, some authors suggest that the suitability of species within IMTA systems be based on other criteria.

According to Chopin & Sawhney (2009), extractive species would, not only be able to uptake nutrients efficiently and significantly, but also that the culture would have enhanced growth over its own monoculture. To date, studies which have used kelps in IMTA systems have had limited, but successful results. Chopin et al. (2004) found kelps growing adjacent to fish farm pens had growth rates 46 percent higher than kelps growing on reference rafts 2 km away. In a Scottish IMTA system, the sugar kelp *S.latissima*, also had enhanced growth near fish farm cages but isotope analysis also indicated kelps to uptake farm derived nitrogen up to 200 m away from cages (Kelly et al. 2007).

For kelps, the morphology and nutrient absorption characteristics of many species of kelps may be suitable as an extractive component in IMTA (Druehl pers. comm. 2006). Kelps are characterized as having complex morphologies that can have tremendous biomass which may represent high nutrient extractive capacity. The Giant Kelp *Macrocystis pyrifera* can grow up to 50 m long and the Bull Kelp *Nereocystis luetkeana* can grow up to 15 cm per day (Mondragon and Mondragon), while the single bladed kelp *Saccharina japonica*, which is the most farmed seaweed species in the world, can grow to 10 m in length (Kawashima 1984).

Biomass aside, seaweeds including kelps, can extract high levels of nutrients from the environment. In the case of inorganic nitrogen, seaweeds can absorb far more from their surrounding environment than actually required (Harrison & Hurd 2001). Nitrogen and Phosphorus can be concentrated approximately 100,000x in seaweeds over ambient seawater concentrations (Lobban & Harrison 1994). Kelps are no exception as Chapman & Craigie (1977) found one Atlantic kelp species to concentrate nitrogen 24000X over the highest ambient nitrogen concentration found in that year. Unlike marine microalgae or phytoplankton, which exhibit the Redfield ratio (intercellular nutrient concentrations of 106C:16N:1P), seaweeds require far greater amounts of nitrogen. Seaweeds demonstrate a general ratio of 30N:1P, with a range of 10:1 to 80:1 (Atkinson & Smith 1983). In kelps, the C:N ratio is seasonally dependant, as ambient nitrate concentrations diminish in summer months, with a wide range of recorded ratios. C:N ratios as low as 6 (Sjotum et al. 1996) and as high as 50 (Mizuta et al. 1997) have been reported.

Kelps absorb and can store excess nitrogen when it is available to be used for growth when ambient nitrogen becomes unavailable. In temperate waters, in the winter

months ambient nitrate concentration are high from coastal upwelling. In spring, when light conditions improve, kelps undergo a rapid growth period as nitrogen is still available. During summer, though light conditions are optimal, the ambient nitrate concentrations become depleted and availability of nitrogen becomes limited for marine algae (Chapman & Craigie 1977). Through the summer, internal nitrogen reserves are exhausted in kelps and internal carbohydrates increase as a result of increased photosynthetic activity (Chapman & Craigie 1978). Through the winter, carbohydrate reserves decrease (Chapman 1984), and appear to be used for early winter growth when ambient nitrate levels increase (Hatcher et al. 1997). New kelp plants will emerge in late winter after a period of adult fertility in the fall and early winter, and undergo the same patterns of growth and nutrient storage. Gagne et al. (1982) found exceptions to this general pattern. In the kelp *Laminaria longicuris*, in locations were ambient nitrogen did not deplete during summer growth was maintained through the summer and carbon reserves were not built up. A similar phenomenon was found when natural kelp beds were fertilized with nitrogen (Chapman & Craigie, 1977).

In an IMTA system, nitrogen could be available when fed cultures were in production which would include the summer months when ambient nitrogen concentrations are low. A kelp culture could take advantage of optimal light and enhanced nutrients during the summer months and potentially achieve enhanced growth over kelp cultures in a monoculture setting.

Where nitrate is the primary source of inorganic nitrogen for kelps in a monoculture setting, ammonia/ammonium is available to kelps in an IMTA system. Seaweeds will not only utilize nitrates and nitrites for nitrogen requirements but also

ammonia/ammonium and in many species ammonium is the preferred nitrogen form (Lobban & Harrison, 1994). In some seaweed species, absorption of ammonium can inhibit uptake of nitrates by up to 50 percent (de Boer 1981). Where nitrates require active transport into the algal cells, ammonium enters via passive transport and therefore its absorption is not energy dependant (Harrison et al. 1986). Therefore the effluents from intensive aquacultures may make inorganic nitrogen available year-round and in an ideal form for seaweed growth (Chopin 2004).

1.10 Study Rationale

The IMTA concept is still early in its development, and has not gathered much attention from large aquaculture producers despite its promotion by several authors (Troell 2009). In particular, is the endorsement of large-scale intensive monocultures with seaweed aquacultures (Chopin et al. 2001; Buschmann et al. 2004). Troell et al. (2004) offers a variety of directions for future seaweed culturing research which might answer questions to entice producers to adopt IMTA. Suggested research areas include nutrient efficiency, seaweed quality, design and scale of research projects and IMTA economics. Within those areas, there is discussion of the seasonality of seaweed aquaculture and lack of knowledge of seaweeds in open water IMTA systems. These two knowledge gaps are the broader motivation of this study.

As previously discussed, kelp farming follows the natural growth and reproduction cycle of kelps: 1) collection of sori in fall and subsequent seed production; 2) outplanting of seed in winter; 3) growth phase in spring and; 4) harvest in summer/fall. Depending on the initiation of the growth phase, the period where a visible and growing kelp crop exists on at a farm is from approximately March-April until harvest time. Based

on Druehl et al. (1987) which noted the seasonal elongation of the kelp species *Saccharina groenlandica*, in Barkley Sound, B.C., harvest might occur between July and August. Growth of blades continues however it is exceeded by blade erosion which occurs naturally and therefore continuing kelp culturing could lose kelp biomass rather than increase it. The period in which the kelp is of substantial biomass, actively elongating, and not losing excessive biomass to blade erosion would be the period in which the kelp will be absorbing the most nutrients from its environment (Barrington et al. 2009; Chopin pers. comm. 2008). In BC, this gives a period of approximately 4-5 months. When comparing the timing of kelp farming to fed aquacultures, the situation is very different. Initiation of a production cycle fed aquacultures is at the discretion of the producer and the growout period is variable depending on species, location, and other variables. In the case of salmon farming in BC, the entry of juvenile salmon into farms can occur year-round while growout to harvestable size takes 1.5-2.5 years. The greatest nutrient release on a salmon farm is at the peak of their biomass which is near the harvest time which could be at any time of year including months when kelp culture might not even be present. For an IMTA site, with kelp and salmon in the production model, the nutrient removal/fertilization benefits could only be realized for approximately 8-10 months over an 18-24 month growout cycle for salmon. This fact leads one to consider the potential for a year-round kelp culture, or at least expand the time at which kelp culture could be present.

To achieve a longer period of kelp benefiting an IMTA farm, the simplest solution would be to leave the kelp cultures on the farm for extended periods (i.e. greater than one year). Kelp seed could be input on the farm in the winter and instead of harvesting at the

end of summer/early fall, the kelp would be left on site. New kelp seed would be entered the ensuing winter and the first kelp crop be harvested after the newer kelp became of significant size and this process continually repeated. This technique would be similar to an older kelp farming technique used by the Japanese called the two-year method (Kawashima 1984). This has its drawbacks as the maintenance of a kelp culture for that long is expensive and difficult (Druehl 1988). Also, if left for longer periods of time blade erosion and fragmentation increase dramatically due to increased natural processes and blade fouling (Titlyanov and Titlyanov 2010), which could result in increased organic loading on the bottom and associated environmental effects (Phillips 1990).

Some authors have suggested the use of multiple seaweed species, including red and green algal species, to achieve longer periods of seaweed cultivation (Kang et al. 2008; Chopin 2004; Carton et al. 2010). Using red and/or green algal species to achieve this would require extensive background knowledge of their biology, knowledge of specific techniques for seed production and species-specific infrastructure. Though this may be plausible it is beyond the scope of this study. Considering other kelp research, the same effect may be achieved not by using other seaweed phyla but rather different multiple kelp species. For example, Luning (1979) found that kelp species grown in the same environmental conditions can exhibit different seasonal growth patterns. Studies such as this indicate that using different kelp species could grow at different times of the year and co-culturing kelp species may length the period of nutrient extraction. Seed production methods and grow-out infrastructure are the same for most kelp species eliminating extra effort associated with coculturing seaweeds of different phyla.

Taking advantage of differing growth strategies in one potential method to expand the time period of macrophyte growth but another potential method comes from the ability to manipulate all aspects of the kelp lifecycle. In the macroscopic stages sori formation can be stimulated by extended periods of short day length treatment (Luning 1988), or blocking the migration of sporulation inhibitor(s) by blade incision near the meristematic junction (Pang & Luning, 2004). In microscopic stages, egg release from female gametophytes can be prohibited by continuous white or blue light (Luning 1981), and gametogenesis inhibited by continuous red light (Luning & Dring 1972). These manipulations offer the opportunity to obtain kelp meispores at any time throughout the year. Subsequently, kelp seed could be outplanted into an IMTA farm setting at any time of the year potentially producing staggered kelp crops. This in turn, could lengthen the time of nutrient extraction of the macrophyte component of an IMTA system.

1.11 Thesis Goals

The primary goal of this thesis was to consider how existing kelp culture methods could be modified to enhance the effectiveness in an IMTA system. This of course is related to nutrient removal capacity and subsequent system efficiency which are topics often discussed in IMTA research and are considered but this thesis was also written as an aquaculture project. As an aquaculture project, a goal of this thesis was to consider the results of the experiments in relation to issues such as production, production strategies, product quality and markets. Few studies on kelp aquaculture exist in B.C. as it is not commonplace so little is known about these issues locally. This thesis is intended to contribute, not only to the broader scope of IMTA, but to IMTA and kelp farming in B.C.

1.12 Study Objectives

The overall objectives of the study was to investigate methods to expand the time a kelp component of an IMTA system would be in the culture setting. That is, expanding the time of kelp culture based on a simple kelp monoculture production model in Canada (Chopin 2004). This research comprised of two specific research projects: 1. multiple species trials and performance and; 2. timing of kelp seed entry and the resultant growth and performance. The approach, results and implications of the two areas of research are provided in chapters 2 and 3 of this thesis.

Chapter 2- Farming trials of five kelp species at an IMTA farm site: considerations of growth phase and productivity

2.1 Introduction and Rationale

In British Columbia there are 32 distinct local species of kelps, which inhabit all types of intertidal to subtidal areas. They are represented by three families historically, Laminariaceae, Lessoniaceae and Alariaceae, and the newly proposed family Costariaceae (Lane et al. 2006). For a kelp grower this offers a variety of potential culture species but for an IMTA producer, this may offer more.

Previous studies which have looked at the seasonality of kelp growth have found different growth patterns in different kelp species. Luning (1979) observed that species that occupied the same niche could have very different growth strategies. In that study, cultured juvenile sporophytes of the kelp species *Laminaria saccharina* (no known as *Saccharina latissima*), *Laminaria digitata* and *Laminaria hyperborea*, which all inhabited different sublittoral zones, displayed dissimilar growth patterns despite being grown in identical environmental conditions. Growth of *L.hyperborea* ceased in late June early July, but persisted till August in *S. latissima* and till October in *L. digitata*. Connolly & Drew (1985) found a similar growth pattern in the species *S. latissima* and *L. digitata* but along a eutrophication gradient indicating that the seasonal dependence of growth was innate and not nutrient dependant. Dunton (1985) found *S. latissima* and the arctic endemic species *Laminaria solidungula*, occupying the same area (including depth), to have very different periods of enhanced growth. *L. solidungula* growth period lasted from February to April and *S. latissima* lasted from April to July. Aside from scientific studies, the polyculture of the two commercially cultivated kelp species,

Laminaria japonica Areshoug and *Undaria pinnitifada* (Harvey) Suringar is suggested in the Chinese Kelp Culture Handbook (FAO, 1989), as a method of increasing overall output and market value as both species grow at different times of the year. Seed of both species is outplanted onto a farm at the same time in the fall, however *Undaria* grows rapidly being ready for harvest in the late winter and *Laminaria* grows slower and is ready for harvest in the summer. In this situation two species execute a period of elongation in different seasons which extends the nutrient extractive capabilities of the system over a greater period in the year.

An advantage of using multiple kelp species from the perspective of an IMTA producer is in both realized in the hatchery and production stages. Firstly, all species exhibit the same alternation of generation life history pattern which makes it possible to produce seed of each species without changing the method of seed production. If a producer had to alter hatchery methods to produce seed for different species, the extra time, effort and expense may not be worthwhile. During the growout phase, many kelp species could grow on identical infrastructure. Multiple species could be grown on subsurface grids of ropes whereas using species from different phyla may require the use of nets, raceways, different floatation and anchoring, and pumping of water. This could all require extensive effort and expense for the grower to set up, fit to existing infrastructure and maintain such a production strategy.

2.1.1 Objectives

The main objective of this study was to culture several species of kelp and monitor their growth. In doing so, the growth pattern of each species will be identified and compared. The results will indicate if using multiple kelp species can lengthen time

in which the kelp culture is in its growth period. Though the main motivation for this study is to lengthen the kelp growing season, this study also provides the opportunity to trial kelp species for their potential culture in the future. Performance of each kelp species during the growth trial will be evaluated with special consideration to yield, blade quality, growth rates.

2.2 Methods

2.2.1 Study sites

For this experiment kelp culturing was comprised of two components: 1. seed production and; 2. growout. The third stage, or harvest stage, mentioned previously, was not included in the scope of this project. Kelp seed for the study was produced at the SEAVision group algal seed production facility in Courtenay, BC. Growout of kelp occurred at the Kyuquot Seafood Ltd. IMTA farm at Surprise Island in Kyuquot Sound, BC (Fig. 1).

The Surprise Island farm site located in Kyuquot Sound, BC, is the first licensed IMTA farm in Canada and the first off-shore IMTA farm in Canada. The site consists of a relatively small 10 hectare lease in a sheltered embayment between Vancouver Island and Surprise Island (50° 02’ N, 127° 17’W). The farm installations consist of net cage system of several 15x15 m pens, work floats, and barge system with site accommodation. The relatively unidirectional current regime facilitates the flow of water and nutrients through the culture components of the IMTA system. The components of the Surprise Island IMTA system are: 1) Anaplopoma fimbria (Sablefish), the fed culture; 2)

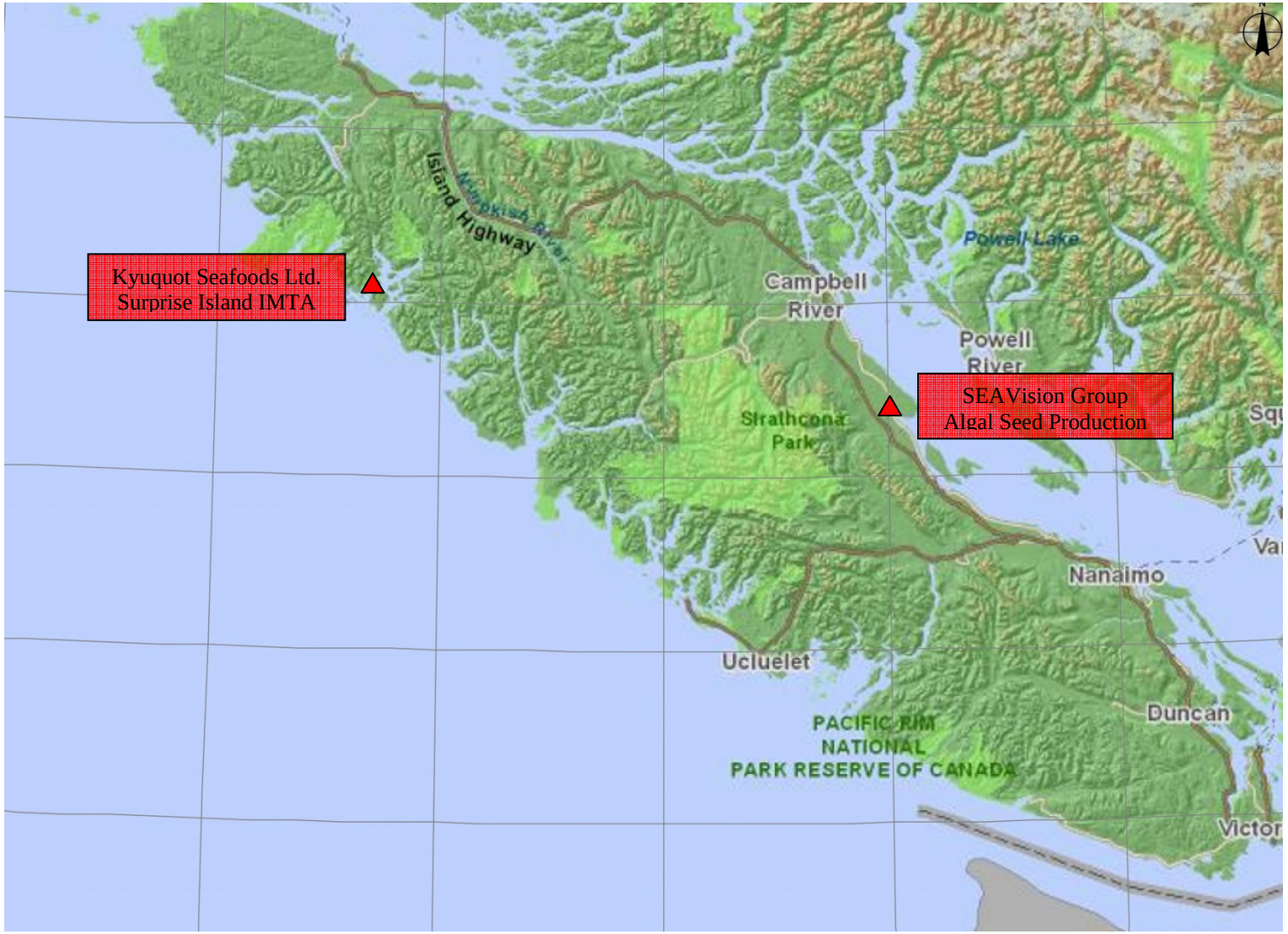

Figure 1- Study site and seed production locations

Platinopectin yessoensis (Japanese scallop), *Crassostrea gigas* (Pacific Oyster) and *Mytilus galloprovincialis* (Gallo mussel), the suspended organic waste extractors; 3) *Saccharina latissima* (Sugar kelp), the dissolved inorganic nutrient extractor and; 4) *Parastichopus californicus* (California sea cucumber), the detritivore (to feed on settable organic waste).

2.2.2 Culture species

Criteria for choosing a kelp species was based on several factors including: 1) the availability of kelp sori at the time of searching for fertile alga; 2) the species had to be local (i.e. found in/around Kyuquot Sound); 3) morphology was to be a single bladed kelp species. The second criterion was included to avoid the potential introduction of species or species ecotype(s) that are non-native to the area. The third was included since kelps of the same morphology are more likely to be able to grown with the same methods and infrastructure (i.e. *Macrocystis integrifolia* and *Nereocystis luetkeana* require lower of anchoring structures as the kelp elongates).

Species selected for this study were *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, *Alaria marginata* Postels and Ruprecht, *Saccharina groenlandica* (Rosenvinge) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, *Costaria costata* (C. Agardh) De A. Saunders. The rationale for the selection of these species is summarized in Table 1. All seed production was performed by the author using the methods described below.

Table 1- Kelp species and rationale for their inclusion to the growth trial experiments

		Rationale for Trialing Species			
Species	Common Name	Farming status	Potential markets	Additional factor	Reference
Saccharina latissima	sugar kelp	experimentally farmed around world including BC	Kombu/Haidai; ethanol production	found in various environments including estuaries, subtidal, intertidal, outercoast	Druehl, 1998; Druehl, 1967
Alaria marginata	winged kelp	experimentally farmed in BC	Wakame		Chopin, 2004
Costaria costata	5 ribbed kelp	unknown	Thallasotherapy	found to grow on aquaculture infrastructure	Rensel and Forster, 2007
Saccharina groenlandica	sea tangle	experimentally farmed in BC	Kombu/Wakame; cosmetics		Druehl, 1980; Druehl, 1988

2.2.3 Seed production

Wild, sexually mature *S. latissima*, *A. marginata*, *S. groenlandica*, *C. costata* alga were obtained on October 17, 2008. *S. latissima* was acquired by dragging a small grapple-type implement, attached to several meters of rope, from a boat. The grapple was dragged from deep to shallow along the bottom and then retrieved at the surface. Areas to obtain plants were chosen based on gently sloping topography of adequate depth (4-10 m) and gravelly substrate (reflective of intertidal substrate). Several kelp thalli (blade,

stipe and holdfast) were captured by the grapple at three wild kelp stands all within 8 km of the Surprise Island farm (Fig.2). Plants were chosen based on their size and presence of sori. Larger alga with larger sori patches were taken preferentially.

Reproductive sporophytes of *Alaria marginata*, *Costaria costata* and *Saccharina groenlandica* were obtained from the outer coast at Kyuquot Sound on October 16, 2008 (Fig. 2). Thalli were removed by cutting the plants from rocks at low tide, placed in seawater-filled plastic totes and brought back to the farm. Kelp plants were put into scallop nets and hung for no longer than 24 hrs from the farm at a depth of 5 m. All thalli were put into large plastic coolers and taken back to the Pacific SEA-lab algal research facility the day after collection.

The following day, spore release from the kelp blades was induced using methods described by Merrill & Gillingham (1991) and Druehl (2007). Blades were scrubbed briskly with paper towels and cleaned of any fouling organisms and/or debris. The sori were excised from the kelp thalli using razor blades put into chilled (approximately 10ºC) heat-sterilized seawater. Seawater used for the entire project was obtained from a dock system in Brown's Bay, Vancouver Island. The site was chosen as it is located in a body of water with extreme tidal activity and little industrial activity. Seawater was collected from a depth of 4m, by a small Honda water pump, into 4 gallon plastic buckets and 20L plastic carboys. The water was heat sterilized by bringing the temperature up to 70-80 ºC for a several minutes. This technique has been adequate for sterilizing seawater for algal cultures (Chapman 1973) and at no time during the seed production was there noticeable contamination of kelp seed cultures. Sori were transferred to a 2L plastic container containing 10% Iodine spray in sterile seawater

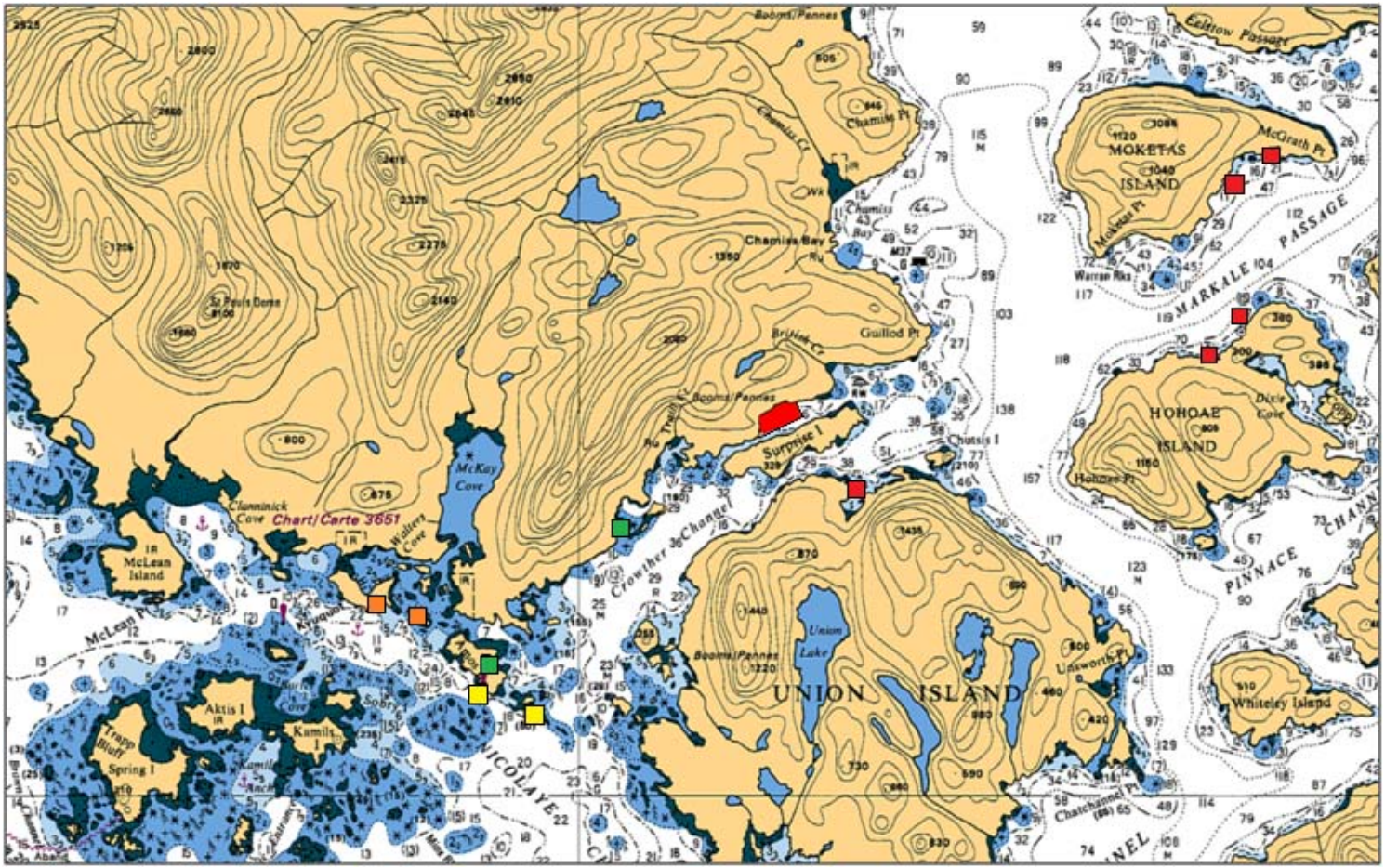

Figure 2- Locations of wild kelp beds near the Surprise Island farm (red polygon) from which sexually mature *S. latissima* (red markers), *S. groenlandica* (orange markers), *C. costata* (green markers) and *A. marginata* (yellow markers) were collected for seed production

and shaken vigorously for 30 sec. The solution was decanted off and the jar filled with sterilized seawater and shaken for 60 sec. This was repeated three times to ensure that the iodine solution was properly rinsed from the sori. The jar was filled again with sterile chilled seawater, lid secured and outside of jar cleaned with sterile water and fogged with a 10% ethanol in water. Sori were removed from the jar shortly afterwards, folded into paper towel, inserted plastic bags, and left overnight at approximately 5-8 ºC. The following day paper towel/sori were removed to examine if spore release was or had occurred. This was evidenced by brown staining of the paper towel. Once brown staining was observed the sori were removed and put into sterilized flasks full of sterile seawater. Spore release occurred 15 min to 2.5 hrs after insertion into the seawater. This was evidenced by the murkiness of the water and a fuzzy superficial layer on the sori. A few milliliters of seawater from each flask were viewed at 10X magnification to confirm spore release.

The spore solution was decanted off from the flasks into empty sterile flasks through several layers of cheesecloth to remove debris. The spore concentration was determined using a hemocytometer under 40X magnification. Final 20L spore solutions in sterilized 20L buckets were prepared at a concentration of 5000 spores/mL. The spore solutions were aerated for one hour using aquarium pumps and sterilized tubing and air stones to ensure adequate homogeneity of the spore solution. This procedure was carried out for each kelp species.

Culture tubes were prepared previously in advance by wrapping approximately 40m of no. 21 nylon twine around 30” lengths of 2” Schedule 40 PVC pipe. Culture tubes were soaked in a concentrated solution of $NaHCO_3$ for 24hrs to remove any dirt and/or

grease. The tubes were then rinsed several times with freshwater, scrupulously dried and stored until needed for seed production.

The tubes were put into the spore solution buckets and let stand. After 12hrs buckets/tubes were aerated for an additional 12 hrs. After the 24 hr inoculation, the tubes were put directly into 24 gallon sterilized aquaria containing sterile seawater enriched with F/2 Proline Algal Food. The aquaria were exposed to a long day light regime (16h day/8hr dark) provided by fluorescent shop lights containing 40W cool blue tubes approximately 30cm away, at temperatures between 8-12 ºC, and the culture media was changed every 9-14 days.

Seed was considered ready for outplanting when the young sporophytes growing on the culture tubes were approximately 0.5-2 mm. For each species of kelp, ten tubes of seed, each with the capacity for 25 m of kelp lines, were prepared.

2.2.4 Outplanting of Kelp Seed

When the tubes were ready for field deployment they were taken from the aquaria and placed in seawater-filled plastic buckets. The tubes were bound together in the bucket with elastic bands to prevent excessive movement during transportation to the farm. The same day the tubes were taken to the Surprise Island site and deployed onto a submerged grid of ropes underneath unused fish culture pens. Kelp lines were put through the kelp seed tubes and the seeded twine was fed through the tubes. As it was fed through the tube, the seeded twine wrapped around around the kelp line. After each of the tubes of twine was wrapped around the kelp lines, three kelp lines were attached end to end. The very ends of the new lines (i.e. three smaller kelp lines) were attached to sixty pound concrete anchors submerged below empty cages on the fish farm system. At two

locations along each of the long kelp lines 5 x 2 lb weights lines up and tied off on the netcage system, were attached. This was to provide access to kelp lines for measurements. After all the seed was deployed, there were three lines of each species approximately 65 m in length submerged at 5 m of depth.

2.2.5 Monitoring of growth and environmental parameters

Growth throughout the experiment was to be measured by growth rates. Growth rate measurements were attempted using the hole-punch method (Parke 1948). A small hole was punched in kelp blades approximately 10 cm from the meristematic junction. During subsequent monitoring events the distance of the hole from the junction is measured. The result is a linear blade elongation rate which can be calculated from the change in distance over time. During each monitoring trip to the farm 10 cm of sporophytes was cut from each kelp growout rope. The total weight of all the blades was measured as well as number of blades, blade length, and blade width. Estimations of the percentage of the blade surface covered with fouling organisms and species of fouling organisms was also recorded.

Triplicate water samples were taken from a depth of 5 m using a Niskan bottle at the edge of the kelp grid. Water samples were vacuum-filtered through a 0.45 µM filter into acid washed sample jars and transported in coolers with icepacks to North Island Labs, in Courtenay B.C., the same day. Samples were analyzed for nitrate (APHA 4500 Nitrate D method), nitrite and ammonium concentration (APHA 4500 Ammonia G method). Temperature, salinity and Secchi disk readings were recorded daily by farm staff as part of their site environmental monitoring program.

2.2.6 Statistical Analysis

Data were analyzed using SPSS version 17 statistical software package. Descriptive statistics were calculated and averages of blade length and yield for each species on each sampling day which were used for statistical analyses. Normality of data was tested using Shapiro-Wilk tests and Levene's test was used to test for equality of variance. One-way analysis of variance (ANOVA) was used to test for statistical differences in each parameter between species on each sampling date. Post-hoc tests were used to confirm which species were statistically different. For data with equal variances, Tukey's HSD post-hoc test was used. For data with unequal variances, Games-Howell post-hoc test was used.

2.3 Results

2.3.1 Environmental Parameters

The environmental data collected by farm staff are summarized in Table 2. Over the period of the experiment temperature ranged from approximately 9 to 15 ºC, salinity ranged from 23 to 32% and Secchi disks readings ranged from 1.5 to 8.5m of depth.

Table 2- Environmental parameters taken by farm staff at Surprise Island during the experiment

	Max	Min	Average
Temperature (ºC)	14.7	8.8	11.55
Salinity (%)	32	25	29.65
Secchi (m)	8.5	1.5	5.33

In winter, temperatures were lower than in other seasons. There was little pattern to Secchi disk and salinity readings throughout the winter and spring. In summer, there

were noticeable decreases in Secchi readings, and increases in temperature and salinity. Nitrate, nitrite and ammonium measurements are summarized in Table 3.

Table 3- Nitrogen concentrations from 5m of depth at the kelp grid during the experiment (n=3).

	9-April-2009		30-April-2009		13-May-2009		14-July-2009	
Nitrogen Type	Range (μM)	Mean (+- SD)	Range (μM)	Mean (+- SD)	Range (μM)	Mean (+-SD)	Range (μM)	Mean (+- SD)
Nitrate	4.19-7.42	5.54 ± 1.68	4.68-8.23	6.24 ± 1.81	2.79-24.84	14.75 ± 11.14	0.13-1.61	0.65 ± 0.84
Nitrite	0	0	0	0	0	0	0	0
Ammonium	0-0.29	0.10 ± 0.17	0	0	0.29- 0.82	0.51 ± 0.28	0.29-0.35	0.31 ± 0.03

2.3.2 Kelp Growth Trends

During the experiment, yield, blade length and blade width were obtained however, growth rates were not. Growth rates, which were to be used to identify the seasonal growth trend of each species, were not obtained due the brittleness of the kelp blades, and difficulties in repeated measures on the kelp grid. When attempting to hole-punch the blades, they would often break and crack. For blades which holes were successful punched, when returning for subsequent measurements, blades were often broken or completely missing. This occurrence lasted for the first three sampling periods Also, the lines attached to the farm system's walkways which held the long kelp lines in place (called taglines) were often caught up in debris floating into the farm. Branches, logs, and other woody refuse were frequently caught in taglines. The debris would then push adjacent kelp lines together tangling them, break taglines and, in one instance, break a kelp line. The result was difficulty in raising and lowering kelp lines, loss of kelp along part of the lines and an inability to perform repeated measurements at the exact same location along the kelp lines.

Since growth rates were unattainable, yield and blade length are used to infer the growth pattern of each species. Descriptive statistics of yield and blade length data that were tabulated for all species for the entire sampling period are given in Table 4. Of the four kelp species, *A. marginata* appeared to have the lowest blade length and yield over the entire experiment. The species grew to an approximate average length of under 30 cm and average yield less than 40 g/section. *S. groenlandica* achieved slightly better blade lengths, but yield was much higher reaching 300 g/section. *C.costata* and *S. latissima* were reaching average blade lengths of 42-43 cm, but *S. latissima* had average yields of nearly 100 g/section more than *C.costata*. Data for both parameters were graphed over the sampling period (Fig.3, Fig.4) and observable trends emerged for the different species. Blade length increased gradually and appeared to peak into early June aside from what was witnessed with *A. marginata* which continued to increase until the end of the sampling period. Yield peaked at the end of the sampling period for all species of kelp. However, the time at which the yield started to increase most dramatically was different for each species. *A. marginata* did not have an observable period when yield was increased more rapidly than at other times. *C. Costata* yield increased rapidly in late April to late May and plateaued after that time. *S. latissima* and *S. groenlandica* both had sharper increases in yield later than *C. Costata*. *S. latissima* initiated early than *S. groenlandica* and *S. latissima* yield also appeared to plateau however *S. groenlandica* yield did not.

Table 4- Descriptive statistics of blade length and yield for each kelp species for each of the sampling dates during the growth trials

			Blade Length (cm)			Yield (g/10cm)		
		N	Mean	Std. Deviation	Range	Mean	Std. Deviation	Range
April 9	*S. latissima*	6	11.232	4.207	6.7-18.9	22.500	14.843	9.0-50
	S. groenlandica	6	11.200	4.086	6.5-17.6	14.000	8.695	6.0-27
	A. marginata	6	8.733	4.422	4.3-15.3	16.667	7.967	8.0-30
	C. costata	6	16.967	4.394	12.3-23.2	34.167	13.273	15-49
April 30	*S. latissima*	6	23.690	7.797	13.9-33.1	99.500	41.225	50-167
	S. groenlandica	6	18.633	7.175	11.7-30.1	37.500	15.566	19-63
	A. marginata	6	13.600	2.782	9.8-17.4	21.167	9.304	10.0-36
	C. costata	6	21.826	5.453	16.1-30.5	132.333	37.623	87-190
May 13	*S. latissima*	6	26.605	5.027	22.2-36.1	157.167	59.456	84-224
	S. groenlandica	6	27.000	9.145	15.4-39.2	69.000	28.566	33-104
	A. marginata	6	18.203	4.106	13.6-23.5	32.500	8.019	21-42
	C. costata	6	33.750	8.268	23.3-43.6	321.000	86.475	222-447
May 21	*S. latissima*	6	34.145	7.070	23.8-41.0	280.167	91.966	164-417
	S. groenlandica	6	29.883	8.674	19.4-40.1	96.167	27.658	66-133
	A. marginata	6	21.517	5.014	15.7-28.6	42.667	13.866	29-65
	C. costata	6	38.817	3.895	32.6-43.2	374.500	65.723	290-472
June 1	*S. latissima*	6	42.538	8.963	31.0-56.2	486.000	57.061	427-589
	S. groenlandica	6	34.467	6.569	25.8-42.1	210.500	43.136	149-267
	A. marginata	6	22.833	3.677	19.3-27.4	50.667	13.574	36-67
	C. costata	6	43.483	5.416	35.1-50.2	417.167	107.013	324-612
June 14	*S. latissima*	6	42.142	5.946	35.8-50.9	513.667	68.751	437-625
	S. groenlandica	6	34.200	8.872	23.5-46.1	301.000	43.781	230-360
	A. marginata	6	31.817	5.682	23.6-38.4	64.833	22.781	39-102
	C. costata	6	42.417	5.470	36.2-50.1	436.000	62.766	376-526

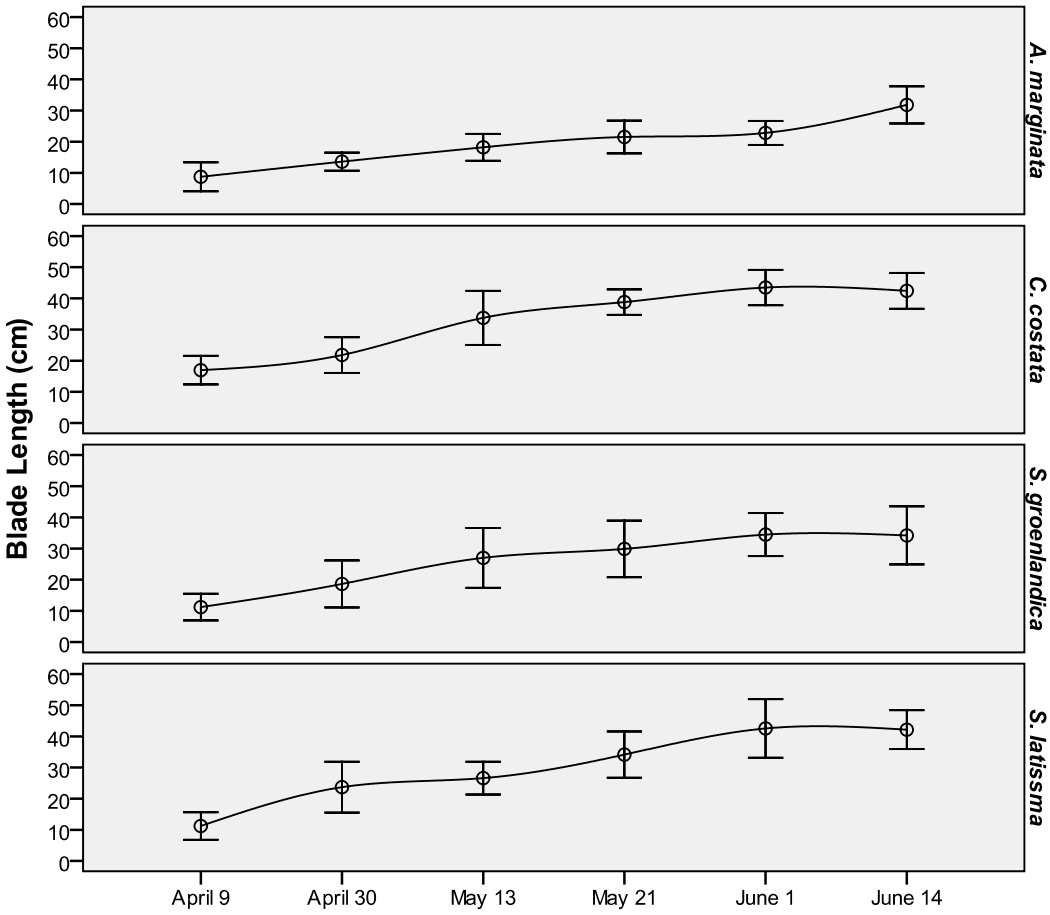

Figure 3- Average blade length for each species throughout the growth trial (error bars represent mean ± SD).

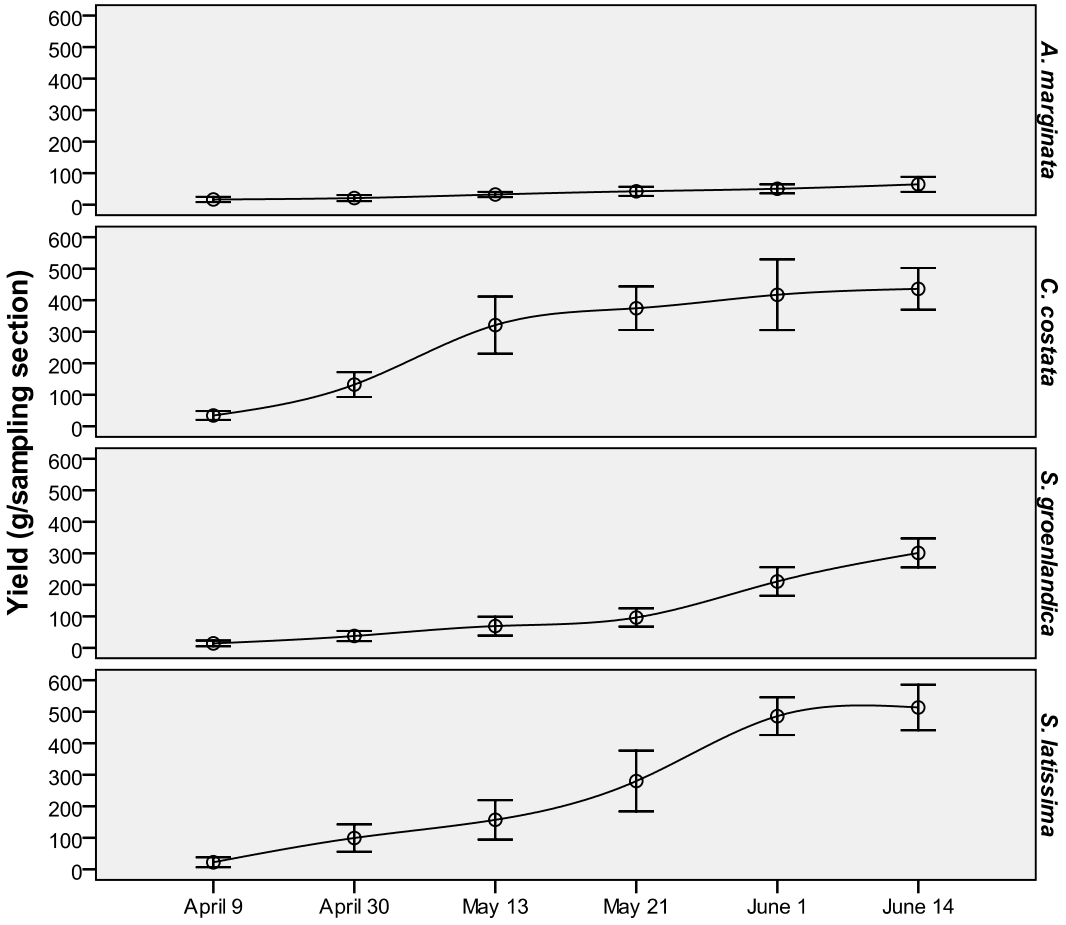

Figure 4- Average yield for each species throughout the growth trial (error bars represent ± SD).

2.3.3 Comparisons between species

Yield and blade length data for each species on each sampling date were normally distributed according to Shapiro-Wilk tests (df = 6, $p > 0.05$). Levenes' tests and ANOVAs, performed between each species for both average blade length and yield on each sampling day were summarized (Table 5). Equality of variance was witnessed in all cases ($p > 0.05$) aside from May 13th data for yield ($L = 13.61$, $p = 0.00$), and May 21st data for both blade length [$L(6) = 3.32$, $p = 0.041$), and yield [$L(6) = 0.420$, $p = 0.019$].

ANOVAs on each date, for each parameter, showed significant differences between species ($p < 0.05$).

Table 5- Levenes equal variance tests and one-way ANOVAs of average blade length and average yield between kelp species on each sampling date during the growth trials ($\alpha = 0.05$)

	Average Blade Length				Average Yield			
Sampling Date	Levene's Test for Equal Variance		One way ANOVA		Levene's Test for Equal Variance		One way ANOVA	
	Levene's Statistic	Sig.	F statistic	Sig.	Levene's Statistic	Sig.	F statistic	Sig.
Apr-09	.149	.929	3.993	.022	.952	.435	3.594	.032
Apr-30	2.678	.075	3.125	.049	2.967	.057	18.977	.000
May-13	2.351	.103	5.012	.009	13.606	.000	33.334	.000
May-21	3.319	.041	7.836	.001	4.198	.019	42.172	.000
Jun-01	1.301	.302	13.194	.000	2.738	.070	56.437	.000
Jun-14	.778	.520	4.031	.021	2.781	.068	83.674	.000

Post-hoc tests are summarized in Table 6, and indicate that over the sampling period there were an increasing number of statistical differences in blade length and yield between species. On April 9th, only two statistical differences were found, while on June 1st there were eight. Also, there were far more differences in yield between species than blade length between species (27 and 10, respectively). *C. costata* and *S. latissima* were most similar with one statistical difference in on May 13th ($p = 0.002$), whereas *C. costata* and *A. marginata* were the most dissimilar with nine statistical differences ($p < 0.05$). The

species with the most statistical differences with the others was *A. marginata* (19) followed by *C. costata* (16), *S. groenlandica* (14), then *S. latissima* (11).

Table 6- Post hoc tests between each species blade length (BL) and yield (Y) data on each sampling date during the growth trials. Numbers in bold represent statistically significant test results between two species. SL= *S. latissima*, SG= *S. groenlandica*, AM= *A. marginata*, CC= *C. costata* (α = 0.05).

		SL		SG		AM		CC	
		BL	Y	BL	Y	BL	Y	BL	Y
Apr-09	SL			1.000	0.590	0.745	0.819	0.127	0.327
	SG	1.000	0.590			0.752	0.978	0.124	**0.032**
	AM	0.745	0.819	0.752	0.978			**0.016**	0.072
	CC	0.127	0.327	0.124	**0.032**	**0.016**	0.072		
Apr-30	SL			0.495	**0.008**	**0.044**	**0.001**	0.951	0.244
	SG	0.495	**0.008**			0.499	0.771	0.803	**0.000**
	AM	**0.044**	**0.001**	0.499	0.771			0.125	**0.000**
	CC	0.951	0.244	0.803	**0.000**	0.125	**0.000**		
May-13	SL			1.000	0.051	0.191	**0.013**	0.313	**0.018**
	SG	1.000	0.051			0.161	0.088	0.361	**0.002**
	AM	0.191	**0.013**	0.161	0.088			**0.005**	**0.002**
	CC	0.313	**0.018**	0.361	**0.002**	**0.005**	**0.002**		
May-21	SL			0.789	**0.014**	**0.026**	**0.005**	0.524	0.241
	SG	0.789	**0.014**			0.249	**0.014**	0.187	**0.000**
	AM	**0.026**	**0.005**	0.249	**0.014**			**0.000**	**0.000**
	CC	0.524	0.241	0.187	**0.000**	**0.000**	**0.000**		
Jun-01	SL			0.166	**0.000**	**0.000**	**0.000**	0.994	0.284
	SG	0.166	**0.000**			**0.025**	**0.002**	0.105	**0.000**
	AM	**0.000**	**0.000**	**0.025**	**0.002**			**0.000**	**0.000**
	CC	0.994	0.284	0.105	**0.000**	**0.000**	**0.000**		
Jun-14	SL			0.196	**0.000**	0.062	**0.000**	1.000	0.082
	SG	0.196	**0.000**			0.924	**0.000**	0.174	**0.001**
	AM	0.062	**0.000**	0.924	**0.000**			0.054	**0.000**
	CC	1.000	0.082	0.174	**0.001**	0.054	**0.000**		

2.3.4 Final Kelp Yield Calculations

The measurement for yield in this study (grams/sampling section) is not typical but is used by other authors (i.e. Kain et al. 1990). The more common metrics for kelp aquaculture production are kg/m^2 (farm production) or kg/m (kelp rope/line production).

To estimate of the production capability at Surprise Island the yield data collected was converted to the latter yield metric.

Conversion from grams/sampling section to kg/m was calculated by the following equation:

Kelp yield (kg/m) = yield (g/10 cm section) × 10 (conversion to m) × 1/1000 (conversion to kg) × 1/2 (half sections had no growth)

The second term in the equation was the conversion of units and the third term was determined by the growth pattern along each kelp line. Along each rope short sections of growth and absence of growth occurred. This alternating pattern of growth coincided with the seeded twine being on top of the kelp line and being on the bottom of the kelp line and is possibly explained by the kelp line shading the kelp sporelings; enough to prevent their development early in the culture period. Several sections of growth and lack of growth were measured along the kelp lines and an average length of both sections was close to 10cm (Fig. 5). Traditionally, yield data is collected by randomly selecting locations along kelp lines which overlap sections of growth and non-growth and thus this phenomenon is accounted within the sampling. However this may require long lengths of continuous growth and numerous samples but, given the damage caused by debris floating into the kelp lines, this was not the case during this experiment. Using this method, it was determined that approximately half the kelp line exhibited growth. A final kelp production for each species was calculated based on when the greatest yield was witnessed. *S. latissima*, *S. groenlandica*, *A. marginata*, *C. costata* all hit their peak production on the final sampling period on June 14th at 2.57 ± 0.345 kg/m, 1.51 ± 0.220 kg/m, 0.324 ± 0.114 kg/m, 2.18 ± 0.314 kg/m, respectively.

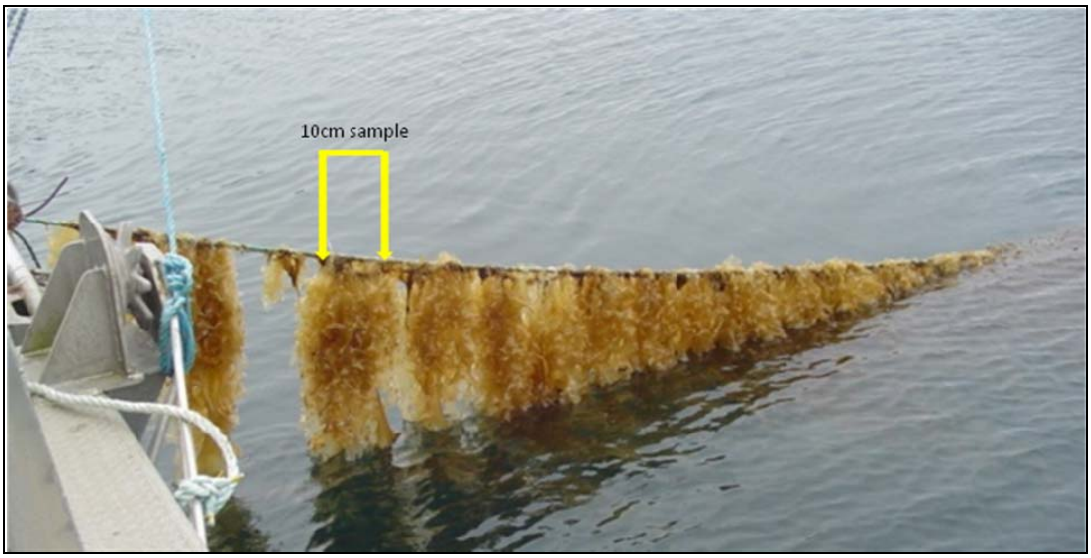

Figure 5- Example of sampling section of kelp used for blade length, width and yield measurements

2.4 Discussion

2.4.1 Environmental parameters

Environmental data showed salinity and temperature during the growth trial were adequate for kelp culture as they were consistent with ranges which supported *S. latissima* growth noted by Druehl (1967). Druehl also found the upper temperature tolerance of locations where natural populations of *S. groenlandica* were found was approximately 18 ºC, which was the upper level of the temperature range at Surprise Island.

Ambient nitrate concentrations at Surprise Island were similar to those found in areas around Barkley Sound by Druehl et al. (1988) with the exception of the measurements taken in July. The average nitrate concentration was 14.75 ± 11.14 µM which was unusually high with one of the three concentrations was 24.84 µM. The high

concentrations of nitrates may be explained by a large amount of rainfall and subsequent run off from the surrounding landscape before and during the water sampling. However, because of the wide variation in the three measurements on that day, it is likely that two of the three water samples were inadvertently contaminated. Ammonium measurements were all low which is typical of its ambient concentration; however it did increase slightly in the summer. This occurred during the summer when nitrate levels decrease and kelp are nitrogen limited. The increased ammonium concentrations may be contributed from the sablefish culture as fish were entered onto the farm during the spring of that year. However, sablefish were entered at only several grams in size and would not produce much waste and what was produced was likely diluted rapidly. In general nitrogen measurements indicated that at the Surprise Island site, despite being an IMTA system, the kelp cultures were still nitrogen limited (further discussion in section 2.4.3).

2.4.2 Growth Patterns of Kelp Species

Two occurrences limited the observation of a definite seasonal growth pattern of the kelp species. Firstly, the biomass and blade length parameters, rather than growth rates, were used to delineate the kelp's growth period. This is more accurately demonstrated with growth rates because of the natural erosion of the distal ends of kelp lamina. Blade erosion, which starts very early in the development of sporophytes, can turn over the entire blade area up to 5 times in a kelps growth cycle (Mann 1972). When a kelp lamina stops elongating based on observing blade length increases, growth has not necessarily stopped but it may be that the erosion rate has met or exceeded the elongation rate (Druehl 1987). The results and discussion would be greatly enhanced by having kelp growth rates because one could tell exactly when kelp growth slowed and/or stopped.

Because of unexpected infrastructure breakdowns and thalli brittleness, grow rates were not obtained. This is not to say that there is no value in the data collected but that the data obtained give an impression and not a definite period of growth. The value of the blade length data is particularly important to the culturist as blade length will affect the end product as is related to the yield.

The second incident that limited the observance of a seasonal pattern was the set of fouling herbivorous snails which grazed on the kelp blades. This seemingly annual snail set, which was noted the previous year during a grow trial of *S. latissima*, was assumed to be the cause of great biomass losses and deteriorating blade quality into the summer months. Production plans were made by farm staff that, if the snails were to set again, kelp would be harvested to prevent total loss of the product. Snails were witnessed on all kelp species and all kelp was harvested stopping the growth trial of the species in the middle of June. Snails may or may not have caused damage to kelp blades, but from the previous year's total disappearance of kelp crop by the end of July, it was decided by farm staff that kelp was to come out of the water. Therefore the opportunity to witness kelp growth of any of the species carrying on into the summer months, as witnessed by Luning (1979) and Druehl (1987) did not exist. If the kelp were left to grow, the likelihood would be that no growth would have been witnessed due to snail grazing. Further discussion is made on the impacts of snail grazing on kelp farming in later sections.

Despite lack of growth rate data and the set of snails, a seasonal pattern of kelp growth was observed albeit slight. Generally, the kelp species initiated in early spring and ceased elongation in early summer aside from *A. marginata* appeared to continue to

elongate to end of the trials without levelling off, as the other species. This is unlike what is witnessed with *Alaria esculenta* growth in New Brunswick which ceases elongation earlier in the growout season than *S. latissima* (Sawhey, pers. comm. 2010). However, because of its poor performance during the growth trial, the species will hardly be referred to further. A lag in the increase of yield behind blade length increase was witnessed. This was expected, as yield is largely affected by substantiation and related to light availability. Where little difference was witnessed in the intiation of blade length increase, differences were certainly noticed on the timing of the changes in yield. *C. costata* initiated its yield increases and plateaued earlier than the all the other kelp species. Visually this was noticeable as the alga plants grew rapidly and seemingly stopped while growth of the other continued. This plateauing of yield and blade elongation of *C. costata* may be representative of a species exhibiting an growth strategy earlier in the year the other species trialed in the experiment. This is supported by the observations of Druehl & Hsaio (1977), where intertidal *C. costata* did not persist through summer months but died off after becoming reproductive in April. They also found a similar pattern of growth of *S. latissima* where vegetative growth occurred through the summer and became reproductive in October. For *S. latissima* in other regions around this world, a similar growth period of cultured sporophytes was witnessed (Luning 1979; Kain 1990; Peteiro & Freire 2009). Again, *S. groenlandica* has been shown to have growth beyond blade erosion well into the summer, though not seen in this study. Existing literature and the witnessed growth pattern of *C. costaria* suggest strongly that a co-culture of this species with a summer grower, such as *S. groenlandica*, could

indeed lengthen the time in which a macrophyte component could actively absorb nutrients derived from the fed component of an IMTA system.

It was also interesting to note that the growth parameters of *C. costata and S. latissima* were significantly greater than the other two species throughout the growth trials. The two species have a wide distribution with populations in less exposed sites. This is unlike *A. marginata* and *S. groenlandica* which are located in more exposed locations. Given the low flow (i.e. slow current and little to no wave exposure) nature of the Surprise Island farm site, the location appears to support the growth of less-exposed kelp species. Druehl (1980), notes that locations of "vigorous water movement" or more suitable for culturing *S.groenlandica*. *A. marginata* trials brought poor results but motivation is high for the cultivation of *Alaria* species as they are considered a suitable for the Asian edible kelp product wakame (which is normally produced from *Undaria pinnatifida*, a non-native kelp in the eastern Pacific) with possibly high economic value (Chopin et al. 2004). An alternative species in the *Alaria* genus may be *Alaria taeniata* Kjellman, as it is considered a low exposure species.

2.4.3 Kelp Production of *S. latissima*

Kelp aquaculture, being a predominantly Asian practice, is not a widely published topic in North America or Europe as compared to China, Japan and Korea. *S. japonica* and *Undaria pinnitifada* are the species on which most of the research efforts have been focussed in Asia and therefore very few publications exist on other kelp species anywhere in the world. However, a few publications exist on the culture of *S.latissima* and production values from those publications are summarized in Table 7. Given the results from other studies, the production values at Surprise Island are quite low. The

discrepancy between production values at Surprise Island and published values could be due to a variety of abiotic parameters including light, nutrients, salinity, temperature and biotic parameters such as grazing.

Table 7- Published yield values of *S. latissima* in culture

Culture Location	Culture Duration (months)	Yield (kg/m)	Reference
Great Britian	6	4.2-28.4	Druehl, 1988
British Columbia, Canada	8	3.0-8.0	Druehl, 1988
New Brunswick, Canada	7.6-9.3	10.9-17.6	Chopin et al., 2004
Spain	4	6.2	Peteiro et al., 2006
Quebec, Canada	8	1.9-4.5	*Tamigneaux and Gendron, 2010

*note: species used in Quebec was *Saccharina longicruris*, a species very closely related to *S. latissima* and considered by some to be the same species.

Growth of kelps is largely thought to be limited in the winter due to reduced light, and in summer due to reduced ambient nitrogen (de Boer 1981). According to Gerard (1987), biomass production is maximized when algal growth is light limited rather than nutrient limited. Given that the measured values of nitrogen were constant low they could be considered nitrogen limiting (Chapman et al. 1978) and light-limited growth was not demonstrated. In China, efforts to curb nitrogen limitation in the late spring and summer months include spray fertilizers and/or in situ fertilizer filled porous containers. At less than 5 µM of dissolved nitrogen the ocean is considered infertile for kelp farming and fertilizers are applied (Tseng 1981).

A central IMTA premise is the availability of nutrients from one culture for transfer to another. Based on the ammonium measurements and low production

(compared to other published values), the transfer of nutrients from fish to kelps did not appear to occur. During the experiment the biomass of the fish culture was very low and thus the available ammonium may have been equally low not contributing to the available nitrogen for kelp. As well, the dilution of nutrients from an open water net pens is very rapid (Gormican 1989). Nutrients from fish farms with much greater production are virtually undetected short distances downstream from fish farms (Black & Carswell 1986). During the experiment, nutrient measurements were taken from water collected approximately 15 m downstream from the netcages at the edge of the kelp grid. At that distance, and the fact that production was low, the fed culture may have not provided very high levels of nitrogen to the kelp grid.

Low production may also be a factor of high rainfall, poor light conditions, and kelp blade grazing. In Kyuquot Sound, and certainly at Surprise Island, rainfall throughout the year is very high which relates to both the light availability and the sea surface salinity. Typical rainfall in a winter month is around 400 mm with 22-25 days per month having precipitation (CHS 1990). Freshwater from rainfall and runoff from the local landscape both contribute to freshwater intrusion into the bay. In particular, two streams of runoff empty large amounts of freshwater into the local marine environment around the farm contributing to a deep freshwater lens and creating estuarine-type conditions. As noted by divers at the kelp grid, the freshwater layer could be as deep as 4-5 m.

A freshwater layer can reduce salinity which is not necessarily a problem with natural populations of *S. latissima*, as it is considered a euryhaline species (Druehl 1967) with a wide optimal range of 23-31 percent (Bartsch et al. 2007). This broad salinity

tolerance range may account for the species wide distribution. However, Gerard (1988) found reduced growth rates of *S.latissima* grown in salinity of 21 % from 27 %. The culture lines are not subject to tidal cycles which can cause fluctuations in salinities for natural populations, but are at a fixed depth with the potential to experience extended periods of time in the low salinity conditions of the surficial freshwater lens at the farm. This was manifested in the physiological characteristics of culture kelp at Surprise Island. *S. japonica* blades experience "blisters" as a results of freshwater dilution (Tseng 1987) and hyposaline conditions can also result in brittle macroalgal thalli (Norton et al. 1981). Both conditions were found in Surprise Island kelp. Blisters were noted when blades became significantly large (approximately > 30 cm) and brittle kelp thalli were noted early in the monitoring period. Incidentally, one of the reasons growth rates were not calculated was due to kelp blade brittleness causing blade breakage. It is very likely that a freshwater layer which reached the depth of the kelp lines and had a negative effect on the growth and subsequent yield of the kelp.

Insufficient light was considered to be factor in low production values. In Kyuquot Sound the number of days with rainfall/precipitation per month in spring months is similar to winter months (CHS 1990). This suggests that during a kelp blade's period of rapid elongation in springtime, it experiences low light levels due to cloud cover. As well, rainfall transports particulates into the marine environment by runoff which would restrict the depth of light penetration from particle light absorption and light scattering. Often witnessed at the site after heavy rainfall are long periods of murky brownish water which can persist for extended periods of time. Though *S. latissima* is considered a shade loving species (Gerard 1990), reduced light can result in growth being

arrested (Burrows 1961). Luning (1979) noted higher growth rates of *S.latissima* at shallower depths in transplant experiments. Tseng (1981) remarks that in Chinese kelp farming, culture lines are raised when culture waters are murky with siltation from runoff to enhance photosynthesis and growth. At experimental IMTA farms in New Brunswick, culture lines are left at or just below the surface (Chopin 2004). At Surprise Island, previous growth trials indicated depths of 2-3 meters did not support growth (pers. obs.), which was likely due to persistant freshwater layers at the farm. Raising lines near the surface is not likely an option.

As much as light and salinity affect kelp production, environmental parameters are not mutually exclusive. Most parameters are intrinsically linked, so kelp production at Surprise Island may be due to several factors rather than one or two. Light can influence nutrient uptake by providing energy for active transport and conversely increased nitrogen availability can increase chlorophyll content (Chapman et al. 1978). Increased water motion will increase nutrient uptake by preventing boundary layer buildup (Wheeler 1980), and increase kelp production in culture (Sawhney pers. comm. 2010).

Marine snails, identified as *Lacuna vincta* and *Tegula funebralis*, were found on kelp blades from early May to the end of the experiment. Such species are known to graze kelps and are found in the local intertidal and on the outercoastal areas of Kyuquot Sound (pers. obs.). In Johnson & Mann (1986), *L. vincta* grazing, though it didn't affect kelp plant mortality, greatly reduced the kelp's canopy area. This was due to eaten areas, coalescing and weakening blades to the point of areas of blades breaking off. In seaweed farming, grazing is a common problem encountered around the world in a variety of culture settings (North 1987). In Russia, Ivin (1995) notes that nearly 85 percent of kelp

crop can be lost due to intense grazing by the marine snail *Epheria turrita*. He reports that thalli break off from a weakening of grazed blades. This appears to be the same occurrence at Surprise Island, and which may explain the sharp drop in yield after June. Holes in blades were noted on *S.latissima* blades with some having over 40 snails on their surface.

2.4.4 Project Considerations and Future Research

Although growth rates would have the extent of kelp growth, snail grazing would not have allowed for that measurement into the summer season. In the previous two years of experimentation with kelp culture at Surprise Island, sharp declines in kelp biomass were witnessed at approximately June-July. Only in the previous year was this phenomenon attributed to snail grazing. It was estimated in that in 2007, as much as 70 % of the kelp biomass was lost by July and 90 % by the middle of August. Therefore, it was a kelp management strategy of Surprise Island staff that if snails were witnessed and plentiful (i.e. > a few snails per blade) kelp would be harvested before snails could reduce the kelp biomass significantly. This was estimated to be the end of June as so kelp was harvested at that time. Measurements could not be made into the summer without the loss of much of the biomass of the farm. Even if growth measurements were obtained, the exact time at which the blades stopped elongating would not have been known. Previous studies do show growth of some species lasting into the summer (Luning 1979; Druehl et al. 1987) and that growth can be enhanced during that period by fertilization (i.e. nutrients provided by a fully stocked fish component at Surprise Island; Gagne et al. 1982). Though this study does not reflect this, further research with this method would need to include growth rate data and continued trialing of other species.

Although it is doubtful that snails could ever be eliminated from kelp without manually removal which would be far too labour intensive, reducing the numbers of snails on kelp thalli may be achievable through different measures. Firstly and most simply, Ivin (1995) found that reducing the density of kelp blades along culture lines reduced the number of snails per blade. Production could occur normally and before settling of snail larva on kelp lamina, plants could be thinned. Of course, this would take experimentation with timing. Martel & Chia (1991) noted peak recruitment of advanced larval stages on Macrocystis and Nereocystis in April-May.

Another possible mechanism for reducing snail herbivory is to culture species or phenotypes selected for with the greatest anti-herbivory chemical defences. Naturally kelps produce chemicals to defend themselves against herbivores called phlorotannins. Elevated phlorotannin levels have been shown to deter grazers, in particular, marine snails (Bartsch et al. 2007). They produce them both continuously and in greater amounts when grazed upon. Differences have been found in kelp phlorotannin concentration between species and between individuals of the same species (Connan et al. 2004), and in different areas of the thallus (Johnson & Mann 1986). If blades, which produce larger amounts of phlorotannins, are used for parental stock, grazing may be reduced. As kelp individuals were selected for their elevated iodine concentrations, which was determined to be an inheritable trait (Tseng 1981), so too may phlorotannin production. Increased phlorotannin production is not likely to prevent grazing since settling of gastropod larva will occur invariably and their presence only reduced the preference for feeding. If grazing is reduced the extension of the growing season into the summer may ensue which could lead to greater biomass and more nutrient absorption.

Unfortunately the quality of the farm environmental data was poor. Measurements became increasingly infrequent, so that by the end of the growth period data were collected twice a week at most with gaps of one week in between measurements. Therefore environmental data that was presented was only in ranges and in no specific time series data. It was also learned after the fact, that the data was collected by different individuals and in different locations. Although the environmental data were poor, the aim of the experiment was to look at the growth of species of kelps at Surprise Island. Several authors stress the importance of monitoring of environmental parameters in macrophyte cultivation for proper site selection (Sahoo & Yarish 2005; Kain et al. 1990), but in this case, the site was already selected. Despite any missing data, the best method for assessing a site for kelp culture and/or species for particular site is trial and error. Kain et al. (1990) agree stating "until it is tried, there is no certainty it is suitable" and Mumford (1987) echoes this sentiment with the cheapest way to assess a site is an "in situ bioassay". In the case of this experiment, poor environmental data were not a great concern but in future studies, accurate environmental data could only strengthen research.

2.5 Conclusion

Both abiotic and biotic factors at Surprise Island appear to limit kelp production and the list of kelp species for culture. By virtue of these factors, and the lack of growth rate data, this experiment was limited. Determining the value of using this production strategy to lengthen the period of nitrogen absorption at Surprise Island was difficult. This does not negate that this production strategy will not work as some of the results and existing published literature strongly suggest the contrary. As well, several kelp species exist in B.C. have not been trialed for kelp cultivation. Discovery of varied growth

strategies is a very real possibility which may produce better results than what was observed in this experiment. This may also include species that are not as susceptible to grazing which may promote better growth into the summer months. Species which naturally inhabit less-exposed areas appear a good criterion for selection for cultivation trials and within the lease of Surprise Island and there exists natural populations of *Eisenia arborea*, *Pterygophora californica* and *Macrocystis integrefolia*.

In BC, aquaculture operations exist in a variety of different oceanographic conditions which includes areas of high flow, vigorous water movement, less fresh-water intrusion, enhanced irradiance. At these sites, it is likely that kelp production could be enhanced and subsequently, differing growth patterns kelp species to kelp species could be expressed. For example, at a location less than two kilometres away from Surprise Island, but with very different oceanographic conditions, test lines of kelp seed produced yields as high as 12.5 kg/m (pers. obs.). As well, at other aquaculture facilities in BC, on subsurface infrastructure, various kelp species set and grow naturally. On one occasion, this author witnessed 5 kelp species growing side by side on a netcage at a salmon farm.

Literature shows that differing kelp growth strategies exist however, at the Surprise Island farm this was difficult to prove. At other farms however, it could be that with the proper data (i.e. growth rates), oceanographic conditions more suitable to kelp culturing and the selected species more suitable for that particular site, the multiple species method to expand the period of nutrient extraction at an IMTA facility is achieved. This author does note, however, that given the diversity of oceanographic conditions and the number of kelp species in coastal BC, that determining which species would grow best where, could be quite an undertaking.

Chapter 3- Multiple kelp seed entries at an IMTA farm to increase the time of inorganic nutrient extraction

3.1 Introduction and Rationale

Kelp farming very closely follows the seasonality of the natural kelp lifecycle. Again, this seasonality limits the time period of uptake and conversion of inorganic soluble wastes from fed aquacultures in IMTA systems where kelp is utilized as an extractive component. The maintenance of the kelp component of IMTA systems throughout the year, or at least throughout more of the year, may be facilitated by multiple kelp seed entries into the culture setting. Merrill & Gillingham (1991), noting peaks in sori production of the kelp species *Nereocystis luetkeana*, suggests this phenomenon provides the opportunity for two kelp seed entries and staggered crops of that species. This method of staggered production may be applicable for IMTA systems for the purpose of enhanced nutrient extraction. However, seed production from naturally occurring sori is not reported with other species (i.e. *S. latissima*).

Kelp lifecycle manipulations offer potential methods to maintain a kelp component on site year round, or at least extend the time period a kelp culture exists on a farm. Luning & Dring (1975), by withholding blue light from kelp gametophytes, arrested development and caused vegetative growth. When put back under blue or full spectrum light gametophytes regular development would resume and gametes would be produced. This technique enables the production of kelp seedstock year round (Lobban & Harrison 1994), as spores released from parent alga could be placed under red light conditions, brought back into white/blue light, and carry on with the laboratory stage of the kelp seeding process. Seed could then be entered onto a farm whenever desired. Under red light conditions, kelp gametophytes maintained for greater than thirty years are

still able to produce viable gametes and subsequent sporelings (Druehl et al. 2005; referred to as gametophyte banking). Sori formation can also be induced. In *S. latissima*, sori formation was discovered to be controlled by photoperiod (Luning 1988) and could be induced by extended periods in short day light conditions (8:16, light to dark). Sori formation was also induced in cut discs from kelp laminae (Buchholz & Luning 1999), and by making incisions across the blade (i.e. preventing the movement of an unidentified sporulation inhibiting factor) (Pang & Luning 2004). Though these techniques also offer the potential for seed production year round, an advantage of using red light to stall development is that the technique does not require a lot of additional equipment or space such as large tanks. Requirements of this technique above what is already needed for any kelp seed are only stable low temperatures throughout the year and potential long periods of culture maintenance (i.e. changing of media).

3.1.2 Objectives

The primary objectives of this study are to attempt to produce seed year round using the gametophyte banking technique adapted to seed production techniques of Merrill & Gillingham (1991), to outplant seed at each season, and to observe growth periods and growth parameters. Then with the results of growth trials of the seasonal entry of kelp seed consider both the implications of such a kelp production strategy in the context of IMTA and potential market of the kelp product(s). Also, the method of seed production will be evaluated.

3.2 Methods

3.2.1 Seasonal seed production

Tubes inoculated with kelp spores were produced with the same methods as described in section 2.2. After inoculation, they were placed into a 35 gallon aquarium with enriched seawater and continuously aerated as in the previous experiment but under 24 hr red light conditions (Luning & Dring 1972). Red light was provided by fluorescent shop lights with red Phillips 40W T12 tubes. The 35 gallon aquarium was jacketed by a larger glass aquarium filled with freshwater. The entire aquarium set-up was covered with a large black opaque tarp throughout the entire culture period to prevent natural light from reaching the tubes. Freshwater was circulated from the outer aquarium through approximately 25m of garden hose housed in a -4 °C deep freezer and back with a submersible 320gph pond pump. The cold-circulated water jacket was sufficient to keep the temp of the cultures below 13 ºC until late spring. A portable air conditioner was used continuously for the remainder of the seed culturing period. This kept the culture room sufficiently cool to aid the water bath in keeping temperatures in the culture aquarium below 15ºC. Water was changed every month and tubes rotated 180º or repositioned every two weeks in the dark. Approximately 50 days prior to the desired outplanting day, tubes were removed from red light conditions and placed in white conditions. In white conditions the methods described in section 2.2 were resumed to achieve kelp seed. The winter entry was deployed on January 4, 2008, the spring entry on March 20, 2008, the summer entry on August 27, 2008, and the fall entry on October 9, 2008.

3.2.2 Seed Deployment

All entries were deployed in random locations within the kelp grid and the deployment procedure for kelp seed remained the same for each entry. Each kelp line, 3/8” polysteel and approximately 45 m long, was secured on the fishcage system at one end at a depth of 15 m and on an anchored and floated backbone line at a depth of 3 m on the opposite end downstream from the fish and shellfish (Fig. 6). To deploy kelp lines, two individuals on the fish farm system fed the end of kelp grow out ropes through the PVC tubes and tied the ends of the seeded twine onto the end of the kelp growout ropes. A long line (longer than the length of the grid) was also attached to the end of the kelp growout rope. The long line was pulled on by an individual in a boat on the nearshore side of the grid. The two people on the fish farm system fed the kelp growout rope through the PVC tube and after the rope was pulled fully across the grid anchors were attached and the growout rope was submerged: 15 m at the fish farm system side and 4 meters on the far end. Ten lines were deployed for the winter entry and six lines during each of the spring, summer and fall.

3.2.3 Monitoring of Kelp Growth Parameters

Growth throughout the experiment was to be measured by growth rates. Growth rate measurements were attempted using the hole-punch method (Parke 1948). A small hole-was punched in kelp blades approximately 10 cm from the meristematic junction and during subsequent monitoring events the distance of the hole from the junction was to be measured. The result is a linear blade elongation rate which can be calculated by the change in distance over time. During each monitoring trip to the farm 10 cm of sporophytes was cut from each kelp growout rope. The total weight of all the blades was

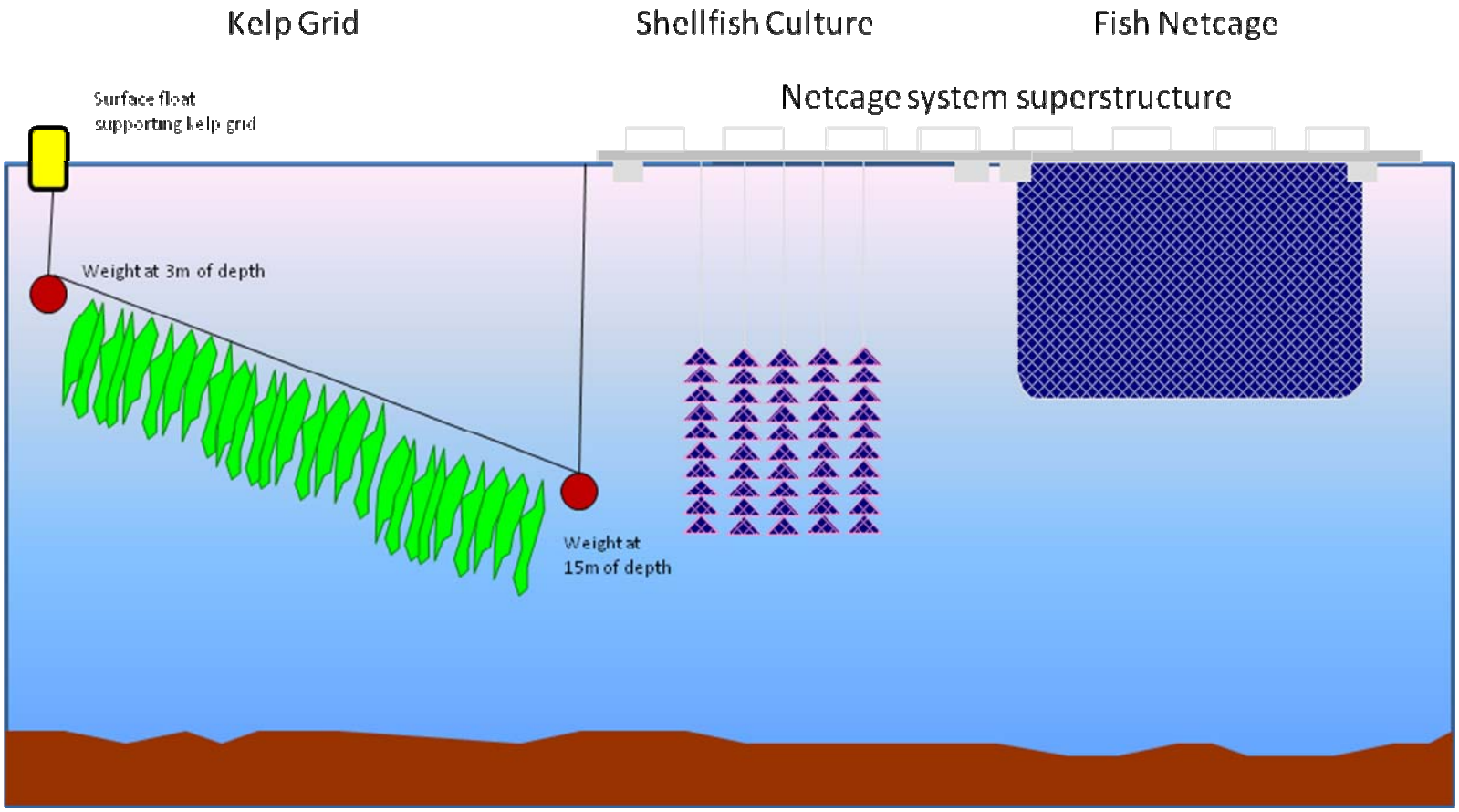

Figure 6- Cross-sectional infrastructure diagram of the Surprise Island IMTA site during the experiment. To the right of the diagram is the fish cage and scallop cages supported by the netcage system superstructure. To the left of the diagram is the angled kelp line setup (growth shown in diagram not representative of actual growth in experiment).

measured as well as number of blades, and blade length. Estimates of the percentage of the blade surface covered with fouling organisms and species of fouling organisms were also recorded. Growth across the entire depth was monitored and observed using a Seamor remotely operated vehicle (ROV). Periodically, the ROV was deployed at the deepest part of the randomly selected growout lines and manoeuvred along the entire length to observe growth, particularly at the deeper sections on of the lines.

An additional winter kelp crop for the subsequent year (after all the seasonal entries) was monitored for the same parameters as above. This was done to make proper comparisons with the data collected from the kelp produced from the fall seed entry.

3.2.4 Estimation of fouling and correction of kelp yield

On the kelp from fall entered seed, Bryozoan fouling was estimated and a corrected yield was calcuated. From areas where there was 100 percent coverage of bryozoan colonies (distal ends of blades) and areas where no coverage occurred (closer to the meristematic junction), 6 cm diameter discs were punched out of areas of kelp blades. Discs were weighed and recorded each sampling event when considerable Bryozoan fouling occurred. Also, the total area per sample (i.e. blades from 10 cm along each kelp line) was estimated visually. From these measurements and approximations, and the measured yield, an actual kelp yield corrected for the presence of Bryozoans (i.e. unfouled kelp) was calculated.

3.3.5 Statistical Analysis

Data were analyzed using SPSS vs. 17 statistical software package. Descriptive statistics were calculated and averages of blade length, yield, and number of kelp blades for each seasonal entry on each sampling day were used for analyses. Shapiro-Wilk tests

were used to confirm normality of data and Levene's tests were used to confirm equal variances between data. For comparisons between seasonal entries data on each sampling date where homogeneity of variance between data was confirmed, independent t-tests were used. For comparisons between seasonal entries data where homogeneity of variance was not met, independent t-tests assuming unequal variance (Welsh's t-test) were used. Data from the winter of 2008 seed entry and spring 2008 entry were used for statistical comparisons and, the data from the fall of 2008 and the winter of 2009 used for statistical comparisons.

3.3 Results

3.3.1 Seedstock production

The time required for development of each seed deployment was different though this was confirmed by visual observation and not confirmed under magnification. The time needed for the development of each kelp seed is summarized in Table 8. Development of spring seed occurred much the same as the winter and it was entered on the farm on March 2. However, this was not the case for the summer and fall seed stock. For summer seed, tubes were removed from red light conditions and put under white light on April 26. Light banks remained the same however a cool water jacket around the culture aquarium was added and air conditioner to the culture room to keep temperatures below 15 ^{0}C. Development appeared to be very rapid as seeded twine turned from off-white to brown in color within 12 days. From this point of development, seen in the previous seed preparations an additional 21-28 days were necessary to grown out seed to 1-3 mm which was considered ready for deployment. If this were the case with the

Table 8- Actual, and intended, times of seasonal seed production and deployment.

Seed entry	Time in redlight conditions (days)	Date removed from red light conditions	Time to develop from gametophytes to juvenile sporophytes (<1mm) (days)	Time to develop from brown twine (>1mm) to furry twine (1-3mm) (days)	Time in white light to be ready for deployment (days)	Date of intended deployment	Date of actual deployment
Winter 2008	-	-	22	23	45	Early winter	Jan 08
Spring 2008	94	Jan 28	28	25	53	Mar 20-21	Mar 20
Summer 2008	186	Apr 27	12	*did not occur	Unknown	Jun 20-21	Aug 27
Fall 2008	278	Jul 26	17	55	69	Sept 20-21	Oct 09

summer seed would have taken a total of 33-40 days to become ready for deployment which would have been as much as twenty days quicker than previous seedstock. However, this was not the case. Though the twine became brown quickly, sporelings never grew to the 1-3 mm desired for before deployment. The summer entry, which was slated for deployment on June 21, was not deployed until Aug 27. At the time of deployment the seed had still not reached the desired 1-2 mm length though the seed was in white light conditions for approximately 4 months.

Fall seed was removed from red light conditions on August 13, but was not entered onto the farm until October 9. The development of seed after putting under white light was more similar to both the spring and summer seed. The seed twine turned from off-white to brown rapidly (as in the summer seed) and grew to approximately 1mm (like

the spring entry, albeit slower). The seed was ready for deployment approximately 72 days after being transferred from red to white light.

3.3.2 Kelp Growth Rates

Growth rates of kelp were not obtained due to rigidity of kelp lines. Infrastructure for this experiment, designed by farm staff, was made with such that kelp lines were to be kept extremely taut. This was in response to previous years' growth trials when kelp lines became entangled with adjacent lines. Lines were accessible at the shallow end of the kelp lines but were incredibly difficult to lift (often only achievable with boat winches). This made repeatable measurements, such as growth rate measurements, unattainable.

3.3.3 Depth

Kelp only grew at the shallow end of the kelp lines. ROV monitoring throughout growth trials at all times of the year showed growth from 7m of depth to the most shallow ends of the kelp lines. Though measurements were not taken, ROV footage showed clear transitioning into growth from no growth to little growth at 8-7 m of depth and a distinctive gradient of increased growth with decreased depth. For this reason, and the difficulties in accessing kelp lines, parameters were only monitored at the shallowest depth.

3.3.4 Winter seed entry

Kelp seed from the winter entry remained virtually unchanged from the time of deployment on January 4 until the end of February. At mid-March blades and stipes became evident in many algal plants. At that point, growth measurements were not performed as measured values would have been extremely low. Blade lengths were well

under 10 cm and yield was negligible. Monitoring of yield, blade length and number of kelp plants began on April 24 and ended August 29 (Table 9; Fig. 7). Average blade length increased rapidly from the start of monitoring until the end of May after which plateaued for approximately one month and then decreased rapidly. Kelp yield also increased rapidly at the beginning however did not plateau. Yield continued to increase until the end of June and then decreased rapidly to August. The number of kelp blades decreased consistently over the monitoring period.

Fouling organisms seen on the kelp blades were similar to those witnessed during a growout trial one year earlier. Filamentous algae, bryozoans (*Membranipora sp.*), glassy tunicates (*Corella sp.*), hooded nudibranchs (*Melibe leonina*), unidentified crescent shaped roe sacks, and marine gastropods *Lacuna vincta* and *Tegula sp*. and *Mytilus edulis* (blue mussels) were all witnessed on kelp ropes and blades. Filamentous algae was witnessed fouling lines in March to April and again in June-July but fouling was during the first colonization was controlled by “weeding”. The summer outbreak of filatmentous algae only occurred on the kelp lines and not the blades themselves therefore weeding was not employed. Bryozoans became noticeable on the ends of blades in the beginning of April. Colonies expanded, with some reaching as large as 14cm in diameter, however most colonies disappeared by the end of May. Tunicates and hooded nudibranchs were noticed by late May/early June with tunicates mainly colonizing on kelp lines and nudibranchs moving on the blade surface. The snails were first noticed at very end of May with a few very small individuals (1-2 mm). The number of snails continued to increase dramatically and by the end of June the number of

Table 9- Blade length and yield per sampling section of kelp from winter-entered seed (n = 10).

Sampling Date	24-Apr-08			11-May-08			28-May-08			06-Jun-08			23-Jun-08			23-Jul-08			11-Aug-08			29-Aug-08		
Statistic	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*
Blade Length (cm)	69.6	36.8-106.5	6.2	26.2	16.7-34.7	6.0	44.3	25.2-66.2	14.3	41.3	28.7-55.2	9.4	42.3	32.4-53.1	6.9	35.0	23.9-51	9.8	22.4	12.8-34.2	7.9	18.9	9.2-41	11.5
Yield (g/10cm)	18.6	12.4-29.6	23.9	279.0	142-361	77.7	436.9	304-604	112.0	516.5	316-714	128.3	761.8	377-1179	234.0	547.8	331-965	196.3	280.4	154-461	111.5	18.1	115-571	156.5
Number of Blades/ 10cm of Kelp line	38.9	26.0-51.0	8.4	36.5	14.0-46.0	9.5	32.4	21.0-46.0	7.9	30.9	18.0-42.0	7.9	27.8	19.0-38.0	6.6	23.4	12.0-31.0	6.2	18.7	13.0-27.0	4.7	233.4	9.0-34.0	6.8

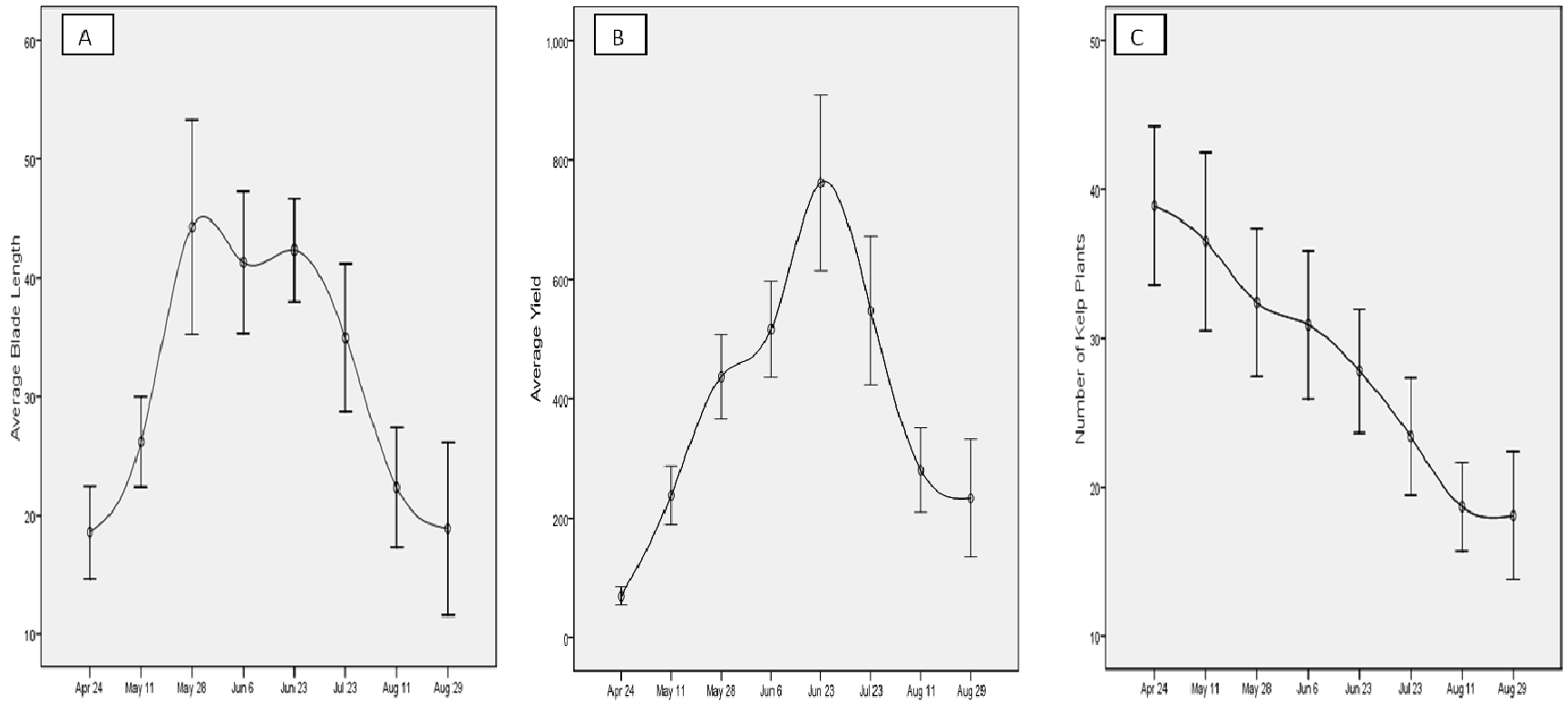

Figure 7- Kelp sampling parameters from winter-entered seed over the course of the sampling period. Graph A is the average blade length (in cm), graph B is the average kelp yield (in grams) and Graph C is the average number of blades; n= 10 and error bars represent standard deviation.

snails per kelp blade reached as much as 16. The size of snails also increased rapidly to that point as many of them reached 0.5 cm in shell diameter and by the middle of July many reached 1cm shell diameter.

3.3.5 Spring seed entry

Measurements of yield, blade length and number of kelp plants per sampling section were first made on May 28th, 2008 and continued until Aug 29th, 2008 (Table 10; Fig. 8). Initially, blade length rose rapidly but plateaued from June to July and decreased until the end of the monitoring period. Yield also increased rapidly, peaked near the end of June, and decreased after that point. The number of kelp plants had a decreasing trend over the monitoring period however the increased on two consecutive monitoring events after first but decreased steadily from that point on. Fouling organsims included those found on winter-entered kelp excluding the mussels. Bryozoan colonies (*Membranipora sp.*) were witnessed on the ends of very few kelp blades by the middle of June and unlike the winter kelp entry colonies never expanded to greater than 7cm in diameter. Nudibranchs and snails were first witnessed during the same monitoring event on June 6. Snails when first discovered were quite small (<0.3 cm shell diameter). However, by the end of July they became as large as 1cm shell diameter and as many as 9 snails per kelp blade were observed.

Table 10- Blade length and yield per sampling section of kelp from spring-entered seed (n = 10).

Sampling Date	28-May-08			06-Jun-08			23-Jun-08			23-Jul-08			11-Aug-08			29-Aug-08		
Statistic	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*
Blade Length (cm)	16.5	21.7-11.8	3.8	19.8	13-26.5	4.9	19.7	14.5-23.7	3.2	20.0	16.3-20.0	2.3	18.2	14.7-23.0	3.0	14.9	11.7-19.0	2.8
Yield (g/10cm)	66.2	22.7-102.6	30.5	87.1	34.5	37.5	158.8	84.1-248.7	55.1	141.1	71.0-202.7	43.7	137.3	94.3-167.7	43.7	134.1	76.2-185.6	44.6
Number of Blades/ 10cm of Kelp line	21.3	9.0-34.0	9.2	22.0	132.5	9.8	24.7	15.0-36.0	7.6	17.7	8.0-26.0	6.3	18.5	12.0-28.0	6.3	17.0	8.0-26.0	7.1

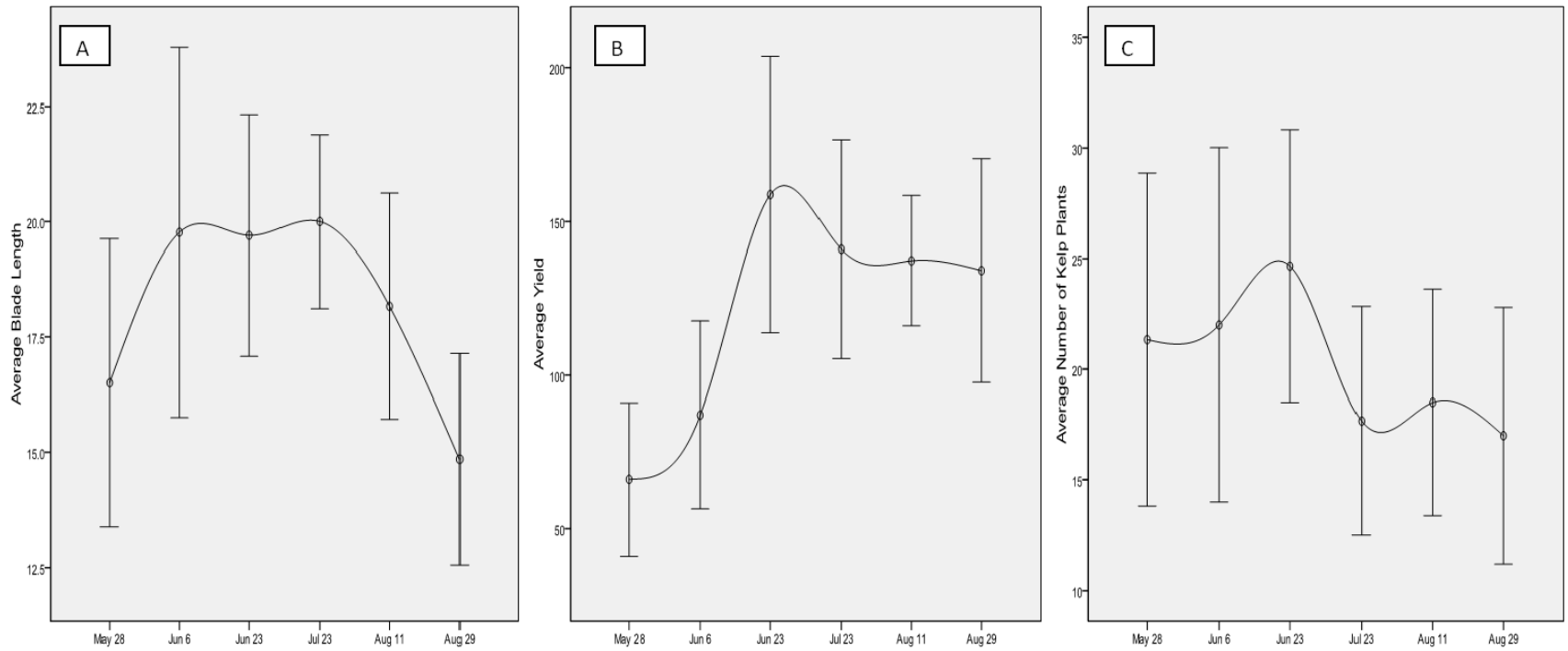

Figure 8- Kelp sampling parameters from spring entered kelp seed over the course of the sampling period. Graph A is the average blade length (in cm), graph B is the average kelp yield (in grams) and Graph C is the average number of blades; n= 10 and error bars represent standard deviation.

3.3.6 Summer seed entry

After deployment onto the farm, summer-entered kelp seed never developed into discernable kelp plants. No blades ever appeared despite monitoring lasting from the of 2008 until late spring of 2009. Lines were fouled by glassy tunicates, blue mussels, plumose anenomes and bryozoans which could not be removed by “weeding”.

3.3.7 Fall seed entry

Kelp blades and stipes became discernable in the beginning of March and the first measurements were taken on March 25 (Table 11; Fig. 9). Monitoring carried on to June 14th after which the entire kelp farm was harvested out. Average kelp blade length increased rapidly at the beginning of April until the beginning of May. Blade length plateaued until the end of June after which it decreased rapidly. Yield increased initially followed by a sharp decrease in the middle of May. Yield then increased dramatically until the beginning of June after which it declined rapidly. The number of blades per sampling clump showed a declining trend from March to June. Fouling of kelp lines and blades included filamentous algae, bryozoans, and gastropods. Filamentous algae was found on the lines in early spring and was removed by “weeding”. Gastropods were first observed on kelp blades at the end of May and persisted and grew in size however compared with other kelp entries abundance appeared to be lower. Of the fouling organisms, Bryozoans were the most abundant in terms of the surface area colonized. They colonized the kelp blades early in the year and were noted right at the beginning of the monitoring period on the longer kelp blades. Colonies grew to very large sizes (up to 17 cm in diameter) and abundance appeared to peak on or around May 13th and persisted until on or around June 1st with many kelp blades having approximately 50 percent of

Table 11- Blade length and yield per sampling section of kelp from fall-entered seed (n = 10).

Sampling Date	25-Mar-09			09-Apr-09			30-Apr-09			13-May-09			21-May-09			01-Jun-09			14-Jun-09		
Statistic	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*	*Mean*	*Range*	*St. Dev.*
Blade Length (cm)	13.6	9.8-17.8	2.7	18.9	13.5-26.1	5.3	63.7	47.9-86.3	15.5	74.3	58.7-87.4	11.5	60.7	42.4-98.7	21.2	69.2	50.0-98.7	19.1	51.7	40.0-64.8	8.6
Yield (g/10cm)	72.8	42.0-142.0	35.6	155.3	120.0-248.0	46.6	341.2	220.0-423.0	88.4	1431.0	1117-1952	356.2	797.3	376-1243	338.8	1649.8	987-2690	692.7	1015.0	602-1485	334.5
Number of Blades/ 10cm of Kelp line	17.5	11.0-23.0	3.9	12.2	7.0-19.0	4.4	10.3	4.0-15.0	4.4	12.0	7.0-17.0	3.9	9.3	5.0-15.0	3.7	10.3	7.0-16.0	3.6	9.3	6.0-13.0	2.9

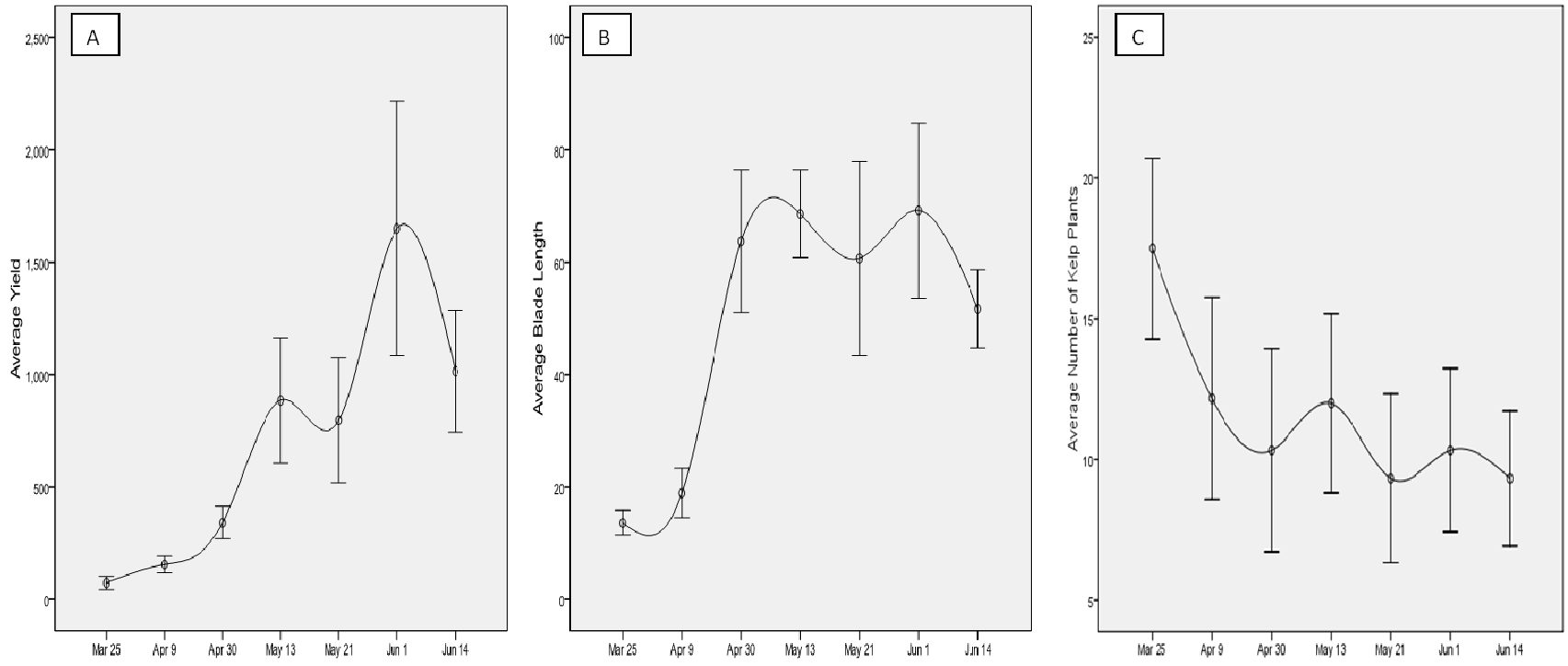

Figure 9- Kelp sampling parameters from fall-entered kelp seed over the course of the sampling period. Graph A is the average blade length (in cm), graph B is the average kelp yield (in grams) and Graph C is the average number of blades; n= 10 and error bars represent standard deviation.

their total surface area covered. During the final monitoring event on July 14 nearly all bryozoan colonies had disappeared. Only a few blades had fragments of colonies on there distal ends. At this point the kelp blades were nearly free of all fouling with only a few small snails present. The results of the yield corrected for fouling were graphed (Fig. 10) and summarized (Table 12). Of the total yield, estimations of 25-50% were accounted for by bryozoan fouling. The resultant yield curve from unfouled yield approximations shows a more gradual rise compared to the uncorrected yield curve.

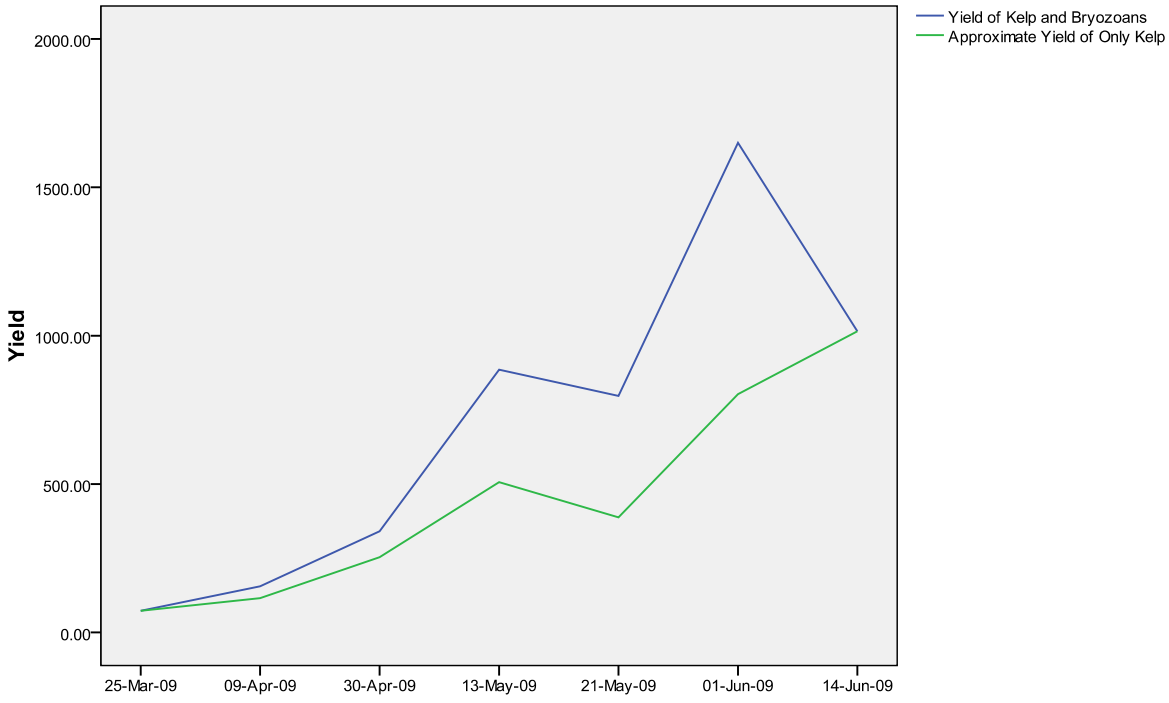

Figure 10-Average yield of kelp from fall-entered seed unadjusted and adjusted for Bryozoan colonization (n=6).

Table 12- Estimations of kelp yield from fall entered seed adjusting for the weight of Bryozoan colonies (n=6).

	25-Mar-09	09-Apr-09	30-Apr-09	13-May-09	21-May-09	01-Jun-09	14-Jun-09
Estimated % of total area of kelp blades fouled by Bryozoans (A)	10%	30%	30%	50%	60%	60%	0
Number of discs punched for weighing	Estimation of fouling weight not performed	8	10	10	10	10	Bryozoans abscent from kelp blades
Average weight of discs- 100% bryozoan coverage		5.0	6.4	6.9	8.1	9.0	
Average weight of discs- no coverage		0.7	1.1	1.2	1.4	1.4	
Approximate percentage of weight of bryozoans of covered areas (weight of Bryozoan covered disks - weight of kelp discs, no coverage/weight of bryozoan covered discs; B)		85.6	82.5	82.3	83.3	84.7	
Average yield of kelp and fouling (C)		155.3	341.2	885.5	797.3	1649.8	
Approximate weight of Bryozoans (C x A/100 x B/100) (D)		39.9	87.6	378.9	409.4	847.1	
Approximate percentage of Bryozoan fouling of the total yield (D/C x 100%)		25.7	25.7	42.8	51.3	51.3	
Approximate yield of only kelp (D - C)		115.5	253.6	506.6	388.0	802.8	

3.3.8 Comparison of parameters between seed entries

3.3.8.1 Winter seed and spring seed entries

From May 28th to Aug 29th of 2008, both spring seed kelp and winter seed kelp were sampled for average blade length, average number of blades and average yield per sampling section. Data was tested for normality using Shapiro-Wilk tests (Table 13). All data was normally distributed ($p > 0.05$), aside from the yield and blade length of the winter seed entry, on Aug 29th ($p < 0.05$).

Table 13- Shapiro-Wilk test results for winter and spring kelp data ($\alpha = 0.05$)

Sampling Date	Seed Entry	Average Blade Length			Average Yield			Average Number of Blades		
		Statistic	df	Sig.	Statistic	df	Sig.	Statistic	df	Sig.
May 28	Winter	.937	10	.515	.898	10	.206	.971	10	.899
	Spring	.946	6	.707	.926	6	.549	.983	6	.965
Jun 6	Winter	.927	10	.416	.958	10	.760	.965	10	.839
	Spring	.990	6	.989	.958	6	.801	.900	6	.375
Jun 23	Winter	.965	10	.843	.987	10	.993	.947	10	.628
	Spring	.973	6	.912	.969	6	.887	.985	6	.974
Jul 23	Winter	.891	10	.175	.875	10	.114	.950	10	.665
	Spring	.981	6	.956	.974	6	.921	.971	6	.897
Aug 11	Winter	.908	10	.266	.915	10	.320	.935	10	.501
	Spring	.963	6	.844	.952	6	.753	.930	6	.579
Aug 29	Winter	.796	10	.013	.782	10	.009	.873	10	.107
	Spring	.932	6	.594	.917	6	.484	.950	6	.739

Welsh t-tests between winter seed entry and spring seed entry kelp data were summarized in Table 14. The results of the t-tests indicated significant differences in average blade length, average yield from May 28 to July 28 ($p < 0.05$). However, significant differences in average blade count only occurred from the May 28 sampling event ($p > 0.05$).

Table 14- Independant samples t-test assuming unequal variances (Welsh t-test) results for winter and spring kelp (α = 0.05)

	Results of Independant Samples T tests assuming unequal variance (Welsh t-test) comparing average blade length					Results of Independant Samples T tests assuming unequal variance (Welsh t-test) comparing average yield					Results of Independant Samples T tests assuming unequal variance (Welsh t-test) comparing average blade count				
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
May-28	5.816	11.006	.000	27.790	4.779	9.875	11.059	.000	370.750	37.544	2.451	9.340	.036	11.067	4.515
Jun-06	5.990	13.875	.000	21.533	3.595	9.903	11.332	.000	429.400	43.361	1.887	8.863	.092	8.900	4.717
Jun-23	8.890	13.509	.000	22.630	2.545	7.797	10.575	.000	603.017	77.340	.842	9.494	.420	3.133	3.720
Jul-23	4.613	10.577	.001	14.960	3.243	6.299	10.420	.000	406.750	64.577	1.764	10.467	.107	5.733	3.250
Aug-11	1.497	12.522	.159	4.183	2.794	3.888	10.547	.003	143.083	36.806	.067	8.382	.948	.200	2.964
Aug-29	1.056	10.681	.314	4.030	3.818	1.883	11.229	.086	99.267	52.731	.305	10.254	.766	1.100	3.604

3.3.8.2 Fall seed and winter seed entries

From April 9th to June 14th of 2009, both fall seed kelp and winter seed kelp were sampled for average blade length, average number of blades and average yield per sampling section. Shapiro-Wilk test results are summarized in Table 15. Aside from the fall kelp on April 9 ($p < 0.05$), the test results data were normally distributed ($p > 0.05$). Results for all t-tests are summarized in Table 16. T-test results of average blade length between entries showed statistical differences on every sampling day ($p < 0.05$) aside from on the last day of June 14th [$t(10) = 1.78$, $p = 0.105$]. Significant t-test results were witnessed on every sampling day for number of blades ($p < 0.05$). Yield data for each kelp seed entry were statistically different every sampling event ($p < 0.05$) and adjusting yield for Bryozoan fouling changed results on two sampling days. On May 21st [$t(6.4) = 0.188$, $p = 0.857$] and on June 1st [$t(6.6) = 1.605$, $p = 0.155$], yield results were not significantly different.

Table 15- Shapiro-Wilk test results for fall and winter kelp data ($\alpha = 0.05$)

Sampling Date	Seasonal Entry	Average Blade Length			Average Yield			Average Number of Blades		
		Statistic	df	Sig.	Statistic	df	Sig.	Statistic	Df	Sig.
Apr 9	Fall	.883	6	.285	.716	6	.009	.965	6	.854
	Winter	.926	6	.552	.912	6	.453	.962	6	.832
Apr 30	Fall	.921	6	.510	.818	6	.085	.909	6	.430
	Winter	.864	6	.204	.869	6	.223	.930	6	.577
May 13	Fall	.881	6	.274	.867	6	.215	.943	6	.686
	Winter	.975	6	.921	.813	6	.077	.995	6	.998
May 21	Fall	.856	6	.175	.939	6	.647	.928	6	.566
	Winter	.835	6	.118	.963	6	.844	.964	6	.847
Jun 1	Fall	.851	6	.160	.885	6	.291	.902	6	.387
	Winter	.903	6	.393	.971	6	.897	.920	6	.506
Jun 14	Fall	.986	6	.976	.960	6	.823	.908	6	.425
	Winter	.982	6	.963	.815	6	.079	.906	6	.409

Table 16- Results of t-tests for comparisons between fall seed-entered and winter seed-entered kelp crops (α = 0.05)

	Levene's Test for Equality of Variances		Test Used as determined by Levene's test (1= independant t-test; 2= Welsh's t-test)	Results of Independant t-tests or Welsh t-test comparing **average blade length**				
	F	Sig.		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apr 9	3.816	0.079	1	4.535	10	0.001	11.755	2.592
Apr 30	7.346	0.022	2	7.186	6.262	0.000	48.271	6.717
May 13	0.929	0.358	1	9.032	10	0.000	44.973	4.979
May 21	1.917	0.196	1	2.583	10	0.027	24.549	9.506
Jun 1	0.623	0.448	1	2.778	10	0.020	27.614	9.939
Jun 14	0.353	0.566	1	1.784	10	0.105	10.281	5.762

	Levene's Test for Equality of Variances		Test Used as determined by Levene's test (1= independant t-test; 2= Welsh's t-test)	Results of Independant t-tests or Welsh t-test comparing **average number of blades**				
	F	Sig.		T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apr 9	0.453	0.516	1	-7.010	10	0.000	-19.500	2.782
Apr 30	4.542	0.059	1	-4.053	10	0.002	-19.167	4.729
May 13	1.488	0.251	1	-4.684	10	0.001	-15.833	3.380
May 21	0.988	0.344	1	-4.179	10	0.002	-11.333	2.712
Jun 1	0.027	0.873	1	-2.490	10	0.032	-5.667	2.275
Jun 14	2.576	0.140	1	-2.337	10	0.042	-6.167	2.638

	Levene's Test for Equality of Variances		Test Used as determined by Levene's test (1= independant t-test; 2= Welsh's t-test)	Results of Independant t-tests or Welsh t-test comparing **average yield**				
	F	Sig.		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apr 9	1.429	0.260	1	6.064	10	0.000	124.833	20.585
Apr 30	4.769	0.054	1	6.549	10	0.000	268.167	40.947
May 13	9.205	0.013	2	4.326	5.897	0.005	632.167	146.141
May 21	14.223	0.004	2	3.007	5.337	0.028	422.833	140.636
Jun 1	11.589	0.007	2	3.765	5.397	0.011	1085.667	288.352
Jun 14	3.368	0.096	1	2.831	10	0.018	426.500	150.661

	Levene's Test for Equality of Variances		Test Used as determined by Levene's test (1= independant t-test; 2= Welsh's t-test)	Results of Independant t-tests or Welsh t-test comparing **average yield adjusted for bryozoan weight**				
	F	Sig.		T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apr 9	0.649	0.439	1	5.257	10	0.000	85.025	16.174
Apr 30	1.476	0.252	1	5.461	10	0.000	180.575	33.065
May 13	3.611	0.087	1	2.800	10	0.019	253.267	90.451
May 21	7.479	0.021	2	0.188	6.398	0.857	13.516	71.952
Jun 1	5.599	0.040	2	1.605	6.635	0.155	238.650	148.719
Jun 14	3.368	0.096	1	2.831	10	0.018	426.500	150.661

3.4 Discussion

3.4.1 Use of the redlight/spool method in seed production

Seed was successfully produced at all times of the year redlight/spool method without difficulty. Aside from a temperature mishap do to a late spring warm spell and inadequate cooling, no known problems with this technique were encountered. Practically speaking, this technique required little space above what was required for regular seed production but did require the space requirements for a longer period of time. In the case of this experiment, there was a lot of time and effort dedicated to acquiring sterile seawater however, if one had a source of sterile or filtered seawater this technique would be much simpler and less expensive than other means of acquiring seed year round.

As previously discussed the production of kelp seed at any time is not limited to only to this red light technique. Spores may be obtained from kelp which produce sori year round (Bartsch et al. 2007), or at least other times of the year. The advantages of this technique over the use of natural occurring sori, is that an individual does not need to find sori which can be difficult, time consuming, and may require SCUBA diving. Though a variety of authors report sori on various kelp species much of the year including *S. latissima*. For this author sori from *S. latissima,* were only available in late fall/early winter. Only in the kelp *Nereocystis luetkeana* was sori witnessed throughout the year (pers. obs.). After being dislodged from its holdfast, this kelp was often seen floating by the farm and frequently became entangled in farm infrastructure. The blades commonly had sori however experimentation with spore release from this species seldom produced sufficient spores (if any) for seed production (pers. obs.).

Environmental and physiological manipulations which can enduce sporogenesis include dark day regimes (8L:16D) immediately after a period of long day exposure (Luning 1988), disc excision from the blade (Buchholz & Luning 1999) or making an incision across the blade length just below the meristematic junction to block sporogenesis inhibitor(s) (Pang & Luning 2004). These techniques do require tanks/flow through systems which in turn require space and potentially expensive flow through systems, whereas the red light/spool technique did not require a lot of space or expensive equipment. For this experiment, a very simple recirculating chilled water bath system and infrequent water changes were employed.

A short coming of this technique is pronounced in its application and not equipment. It was determined that entering kelp in the fall, produced a better kelp crop over other seasonal entries (discussed further). However, this was the last of the entries and required the longest time in red light conditions and thus, required the most maintenance. Though water changes and cleaning of tanks did not require a lot of effort each time this occurred, the cultures were in that setting for 7-8 months.

3.4.2 Kelp seed production times

Reported culture periods of 30-60 days for juvenile sporophytes up to a few millimetres from spores are typical (Merrill & Gillingham 1991; Chopin et al. 2003). The time period of winter and spring seed development was in within that range but the time period for the summer and fall seed production was much longer. This is likely caused by the unexpected hot period in the May of 2008 while those seeds were still under red light. Cooling was not being supplied to the culture room where seed was being cultivated since ambient temperatures were still adequately low enough to maintain gametophyte

cultures. Temperatures of 27 ^{0}C were noted in the culture aquarium despite the use of the circulating cool water jacket. Immediately after that temperature was recorded additional cooling was provided via a portable air conditioner. Afterwards the recorded temperatures of the culture aquarium never exceeded 15 ºC however it is likely that the kelp seed experienced the very high temperatures on the afternoons of three consecutive days before the problem was corrected. In experiments studying temperature tolerance of kelp gametophytes, there is agreement with different studies that *S. latissima* gametophytes from all around the northern hemisphere have an upper temperature limit of 23-24 ºC (Bolton and Luning, 1982; tom Dieck 1993). In these experiments however, incubations times were long in comparison to what gametophytes underwent in this situation (i.e. two weeks compared to three days). However, observations from both studies may be indicative of events that may have occurred in the summer and fall seed.

In Bolton & Luning (1982), at 23 ºC gametophytes were damaged but were able to regenerate from only a few cells. In the tom Dieck study, survival within gametophyte masses was greater than outer gametophytes when undergoing the temperature stress. The same kinds of occurrences may have happened with the high temperature bursts in the aquarium. It is likely, that some gametophytes died but not all as evidenced by the seed production for the fall entry. Gametophytes on seed twine could be layered, especially given the amount of time they would have had for vegetative growth under the red light. Aside for mortality, gametophytes may have been stunted in their growth and development by the elevated temperatures. Previously stated, it was thought the seed progressed rapidly from gametophyte to sporophyte stage however this was not proven. The seed may have continued on as gametophytes never developing because of the stress

of the heat. Gerard (1997) applied various stresses onto young *S. latissima* and found that temperature had the most profound effect on growth and produced the longest recovery period after different stress treatments. That study was on a different life stage, but the elevated temperature may have stunted growth and development, which could be why the seed never progressed to the 1-2 mm stage. As well, the remaining kelp seed reserved for the fall entry, while in red light conditions could have been given enough time to recover from the temperature stress.

3.4.3 Seasonal Kelp

The winter entry resulted in a peak average yield of 761.8 ± 234 g per 10 cm of line. When converted to actual yield per meter of line taking into account the twine effect (as in Part II) the peak yield was 3.81 ± 1.17 kg/m which was lower than other published values (Part II, Sect 2.4.2). The spring entry of 2008 resulted in a mean yield of 158.8 ± 55.1 g/10cm of kelp line. This results in a converted actual yield of 0.79 ± 0.28 kg/m. Kain et al. (1990) are the only other published values of yield of kelp cultivated from spring entered seed. They reported an average of approximately 40 g per clump dry weight of *S. latissima*. The occurrence of sectional growth along the kelp lines was also seen by those authors and where they refer to it as clumps, this author found each clump to be approximately 10 cm. Per 10 cm section, as stated above, the yield was approximately 160 g. It was found that the approximate water content of those kelp blades was 90 percent; this result seems consistent with other studies on kelp farming (Sanbonsuga 1984; Druehl 1980). This leads to a dry weight of approximately 18 g per 10 cm which is notably lower than the results of Kain et al. (1990). Those authors mention a persistence of nitrates in there cultivation area which might have led to greater

biomass than in this culture area. In theory, available ammonium from the adjacent fish culture should have been available for the kelps as well. However, at that time the fish were very small and likely not contributing nitrogen to the culture environment. Other authors (Lee & Brinkhuis, 1988) who successfully grew algal plants from spring-entered kelp seed reported an average length of *L. saccharina* sporophytes of 17.4 ± 10.4 mm from seeded lines entered into the field near the middle of March and measured near the middle of June. This was a culture period of 92 days. At Surprise Island, after a 95 day culture period, sporophytes averaged 197 ± 32 mm. Again there is a large discrepancy between results however those researchers were working in Long Island Sound; not only were they in a different ocean but at the southern limit of distribution in the Atlantic.

Statistically, yield and blade length were significantly greater in winter kelp than spring kelp from May 28 sampling through to July 23rd sampling. After this point blade length and yield decreased dramatically which largely coincided with the observance of fouling marine snails. Snails were not only seen on winter kelp but on spring kelp as well and the decreases in blade length and yield of both crops coincided with snail fouling. As a result, it is likely that snail herbivory also prevented spring kelp from entering into the summer and potentially large increases in yield due to inadequate substantiation.

Production from the fall seed entry was analogous to the forced cultivation method in Japan. Japanese farmers select and remove mature blades from farms in late summer before harvest, and have seed ready in October (Kawashima 1993). The forced cultivation method has in Japan revolutionized kelp farming by yielding kelp blades of the same characteristics of second year kelp and for this reason the expectation of the fall kelp crop would be that blade characteristics, such as length, and yield would be greatly

enhanced over kelp crops from seed entered later in the season. Therefore it was expected that this seed entry would produce larger kelp thalli with greater yield than the other seed entries. The final yield of fall and winter kelp, after conversion using the formula previously described, were 5.08 ± 1.67 kg/m^2 and 2.94 ± 0.78 kg/m^2, respectively. The blade lengths between the two kelp crops differed little on the final sampling day.

Sanbonsuga (1984) reported that from forced kelp (seed entered in October), average blade lengths are doubled and blade areas and fresh weights increase three-fold at peak times over kelp from seed entered in December. Also, kelp elongation and yield start to increase 1-2 months earlier. At first glance this was witnessed Surprise Island kelp entered in the fall exhibited as similar increases of fall kelp over winter kelp. Fall kelp blades became distinguishable nearly two months before winter kelp blades and blade length and yield from the fall crop was significantly greater than that of the winter crop. The difference in blade length between the two crops became increasingly less significant which was not unexpected. Throughout all growth trials of kelps, blade length became more and more uniform which is likely the consequence of the slowing of blade elongation into summer and blade erosion of distal tips. Visually, this trend can be likened to side-by-side kelp parabolas along the length of each kelp line becoming a hanging kelp sheet when the lines are hoisted out of water. This was the case in both fall and winter entered kelp. The difference in kelp yield was significant throughout the growing season which was not unexpected given that the fall kelp had a longer growing season. However, when the kelp yield was adjusted for bryozoan fouling (i.e. the estimated contribution of weight bryozoans to the yield was negated), the results became very different. On the sampling days of May 21st and June 1st, when bryozoan fouling

appeared at its greatest (i.e. colonies were largest and covered the most blade surface area), the adjusted yield values of fall kelp were not significantly greater than the winter kelp. On the last sampling day, June 14th, all bryozoan colonies disappeared from the fall kelp crop and the difference between yields was significant again. In calculating the percentage of yield due to bryozoans, non-fouled disks showed an increasing average weight over the experiment. This is likely explained by increased substantiation of kelp as the photosynthetic capacity of the blades as they approached the summer months.

In regards to the number of blades, the fall entry had significantly less blades on every sampling day and this result could be a sign of a problem with the kelp seed, and subsequently why fall yield, adjusted for bryozoan weight, was not statistically greater than winter yield.

When summer and fall seedstock were in red light conditions previous to deployment, the culture room was not sufficiently cooled at one point and culture temperatures may have briefly exceeded the lethal temperature of *S.latissima* gametophytes. Though fall entered kelp seed resulted in a kelp crop indicating that gametophytes were not killed, the fact that the number of blades was comparatively low to the winter kelp crop may indicate that the seed quality was negatively affected by the period(s) of high temperature.

One very interesting occurrence during the fall kelp culture period was the amount of bio-fouling found on the kelp blades. The bio-fouling of Bryozoan colonies was first witnessed on kelp blades in late March and their size increased very dramatically from April to May. The fouling was so severe and it was estimated that 30 percent of all blade area of the fall kelp crop was fouled. By weighing disks cut from

fouled and unfouled blade parts it was also estimated that half the kelp yield could be attributed to bryozoans. However, in May, the kelp yield dropped dramatically (near half) and almost all of the bryozoan colonies disappeared. It is likely that the bryozoan colonies became so heavy that they either tore off or were a victim of natural blade erosion. The former is more likely as the blades would have had to erode nearly half their length in one month. After the bryozoans disappeared, yield increased dramatically and blade length also rose but more importantly bio-fouling of blades became almost non-existent aside from the presence of snails. This is potentially important for future considerations as clean blades are much more attractive in terms of their end-product. This is discussed in more detail further-on.

When summer kelp seed was deployed it was not at a desired length. But because the seed seemingly stopped development during the lab stage and over month had past after the intended outplanting date, the seed was entered onto the farm. At the time of entry the developmental stage was not confirmed. The seed string appeared light brown but it did not reach the “fuzzy” stage which is indicative of seed reaching the sporophyte stage of adequate blade length to be outplanted. It was speculated previous to this experiment that the seed would simply “oversummer” and growout in much the same manner as the fall seed entry, acting as kelp seed in the forced cultivation method. This of course was not the case and if the summer seed were still viable at the time of deployment, there are a variety of possible explanations as to why it did not grow. Aside from heat stress, temperature, fouling, grazing and/or, natural circannual rhythmns could be responsible for the lack of sporophyte growth.

In British Columbia, Hsiao & Druehl (1972) witnessed year-round sporophyte fertility with two marked peaks in sori production in May-June and October-December. They also witnessed *in situ* gametogenesis and subsequent microscopic sporophytes from cultured gametophytes year-round however not macroscopic sporophytes. Yarish et al. (1990) reported a similar occurrence in Long Island Sound, USA. Despite the ability for kelp to successfully complete virtually half of their lifecycle at any time, the development from microscopic sporophytes to macroscopic sporophytes appears to only occur in winter/spring. While it was previously suggested that kelp gametophytes might "over-summer" because of their high temperature tolerance (Lee & Brinkhuis, 1986), Lee & Brinkhuis (1988) reported this did not occur and fall-winter sporulation results in the only sporophytes surviving through the following summer. Though they could culture juvenile sporophytes throughout the year, finding the growth was largely dependent on temperature (range of 7-14 ºC) and there field deployments of gametophytes and juvenile microscopic sporophytes never resulted in macroscopic sporophytes beyond the month March. Those authors and Hsaio & Druehl (1972) both suggest that biological factors such as grazing and/or microbial activity may limit the growth of microscopic sporophytes from further development. Vadas et al. (1992) state that following secure attachment, foraging activities of herbivores appears to constitute the greatest source of mortality to early post settlement stages of many benthic algae. At Surprise Island fouling species start to appear early in the year however most fouling occurred in the summer months. Given the amount of fouling on kelp lines in the summer and the presence of *Lacuna vincta* in large numbers at that time, this may be one reason for the lack of success of the summer entered sporelings.

Chenelot & Konar (2007) found higher densities of *L.vincta* on juvenile *Nereocystis leutkeana* plants than older plants. Recruitment of *L. vincta* on newly deployed lines of the summer entry could have occurred as Martel & Chia (1991) reported. Their study showed two spikes in *L. vincta* larval recruitment in April-May and late summer, early fall and also year-round presence of pelagic veligers. On kelp blades from the winter entry, *L. vincta* become evident in later May as stated previous and their egg masses (small doughnut shaped or crescent shaped, roe white to yellow in color) became noticeable in later June. Also, ROV surveys (pers. obs.) note large numbers of snails on kelps in the local subtidal. So, not only was *L. vincta* in witnessed in the local intertidal and on the farmed kelp blades, and their larval stages could have the recruited on lines of newly deployed summer kelp seed, but the species also shows preferential feeding of younger kelp tissue. It is very likely that grazing pressures had negative effects on summer seed growth.

Other fouling organisms, in particular hydroids, may have had smothering effects on juvenile kelps. As seen on experimental lines previously outplanted with the kelp *Agarum fimbriatum*, they became fouled with the hydroid *Obelia dichotoma*. Unlike the filamentous algae(s) which fouled summer lines and was removed by “weeding”, the hydroid was removed only by picking it off by hand. *O. dichotoma* typically grows to 12 cm in 2-3 months however *Obelia sp* on the east coast of the U.S. can elongate 20mm/day given optimal temperature and nutrients (Morris et al. 1980). Rapid growth and colonization of the hydroid was witnessed on the summer lines with hydroids reaching up to 10 cm one month after deployment. The seaweed-like organism very

likely would have shaded kelp sporelings out-competing them for space and smothered them.

The lack of growth by summer kelp entry may also be linked to more than external factors. In a few kelp species, circannual rhythms have been described which govern their seasonal growth cycle (Luning 1991; Schaffelke & Luning 1994). The rhythm, which is cued by daylength, is responsible for the reduction in growth into the summer and fall months and increase in growth in the winter and spring. If the same circannual growth rhythm occurs in *S.latissima*, which is thought to be present (Luning & Kadel 1993), the newly entered kelp seed would have been entered into a season of declining day length and become entrained to a period of growth cessation.

The summer seed entry failure to grow could have been due to a host of factors, including but not limited to poor seed quality, potential grazing pressures, competition, potentially poor culture conditions, internal rhythms failed to grow. However, the outplanting of seed of juvenile sporophytes by researchers has not generated mature sporophytes in summer and reasons that have been previously suggested reasons were all observed at Surprise Island. It is unlikely that summer outplantings would ever produce a growing kelp crop in the summer and/or fall months to effectively absorb nutrients during that period.

3.4.4 Seasonal kelp in IMTA systems

From the results of this study, the potential of seasonally entered kelp seed producing kelp crops to lengthen the time which the macrophyte component of the Surprise Island IMTA system could not be determined. Growth from fall entered kelp started earlier than winter entered kelp which represented an extension of the growing

season earlier in the year but, because of grazing, an extension of the growing season into the summer could not be verified. The spring kelp looked promising for a crop with growth into the summer however the crop began disappearing with the increased presence of snails. Had the spring kelp been able to grow undisturbed it would have likely produced a significantly larger crop than what was subject to grazing. Druehl et al. (1987) found increases of both yield and blade elongation until the latter half of July in *S. groenlandica.* After that point blade erosion exceeded elongation however growth still continued into September.

What appears to be a potential IMTA kelp production strategy, where grazing is not an issue, would be to enter kelp biannually. A first entry could be made in September to produce a crop ready for harvest in late spring and second entry could be made in late winter or early spring to be harvested in mid to late summer. In this case, two kelp crops of different characteristics would be produced with a combined growth period approximately 2-3 months greater than a single winter seed entered kelp production strategy. The staggering of seed entries and harvesting of crops in such a manner would help maximize production, while at the same time allowing for kelp to grow without density problems. The first crop would be harvested before the second became of significant biomass avoiding competition for resources between crops. To confirm the biannual strategy, growth rates and timing of seed entry in the spring would have to be determined. Kain et al. (1990), report greater growth rates in summer months from April entered kelp seed of *S. latissima* over seed entered in February but Lee & Brinkhuis (1988) report no growth from juvenile sporophytes entered after the beginning of March.

3.4.5 Potential markets for Surprise Island seasonal kelp crops

Considering markets for the kelp produced at Surprise Island, there are several options. Kelp edibles such as Kombu or sea vegetables, invertebrate fodder, fertilizer type uses, nutraceuticals, and spa applications have all been purported as uses for kelp in Canada and B.C. (Chopin et al. 2003; Druehl 1988). However, the kelp produced at Surprise Island is likely to only satisfy a few those markets.

Kombu, which has been widely suggested as a potential use for kelp from Canadian IMTA farms may be a high valued product. The popularity of Kombu, as evidenced by the number of increasing local suppliers, is increasing in North America which does not traditionally utilize kelp for Kombu (Druehl 1998). Based on this fact the use of kelp for Kombu looks very promising given its increasing awareness in North America. However, the properties of the kelp produced at Surprise Island may not be advantageous for Kombu production.

Kombu in Japan is produced from their native kelp species *Saccharina japonica* Areschoug. The species is fast-growing with certain chemical and physical characteristics which the Japanese prize in their Kombu. Physical characteristics such as blade length, width and weight are used to calculate a measure of quality of kelp for Kombu called substantiality value (SV; Kawashima 1984). The formula is as follows:

SV (mg/cm) = Blade Wet Weight (mg) / Blade Length (cm) x Blade width (cm)

References for SV values are scarce however Mairh et al. (1991) report values of cultivated S. japonica of 60 mg/cm^2, and Asaike & Tsuda (2005) report a SV of approximately 140 mg/cm^2. In both cases SV values are greater than what was observed at Surprise Island. Though not part of the results of this exercise, SV values were

calculated on several blades from winter and fall entered kelp seed and SV was not greater that 40mg/cm^2 for any individual kelp blade tested. From the formula SV is largely dependent on blade thickness which is enhanced mainly in the later spring and summer. This period is substantiation and on which Kombu quality is dependant (Sanbonsuga 1984). In the culture of *S. japonica*, harvest is typically in the latter half of summer so blades have a longer period of substantiation than what is the case at Surprise Island.

Techniques for farming in Japan and China are very different than what was employed in these growth trials. Their seed string it cut into short sections and inserted into culture ropes in either horizontal or vertical positions. When blades elongate, the density of the blades is reduced to approximately five individuals per seed string and holdfasts are tied onto the culture ropes if needed. Thinning of excessive fronds is considered an important technological feature for stimulating growth and making high quality Kombu (Kawashima 1993). No thinning ever occurred at Surprise Island which is very likely to have had an effect on the SV values. Had there been less competition for light and less shading, blades may have had increased photosynthetic activity which in turn may have increased substantiation.

Of the kelp crops the fall entry showed the most promise for the use of Kombu. The blades exhibited the highest yield while having similar blade lengths to winter entries (indicating greater level of substantiation) and had very little fouling present. Tseng (1987) describes the practise of “tip cutting” the blade ends as another practice to increase blade quality and the sloughing of bryozoan colonies from the distal ends of the kelp blades mimicked that practice. Unfortunately kelp was harvested in June of that year

for fear of snails destroying the kelp crops. It would have been interesting to monitor the blade length and yield after the loss of bryozoan colonies.

If the intent of kelp farmed at Surprise Island is Kombu production, the farming techniques will likely have to be changed to those employed by Asian countries to increase the level of substantiation. This is not to say that Kombu from Surprise Island kelp could not be destined for North American markets where potentially less discriminating pallets may welcome it however blade quality would still be an issue. The phenomena of Bryozoans colonizing on kelp blades causing blades to rip and removing most of the fouling organisms, is not likely to happen every year, unfortunately. The producer still has to consider blade fouling and how it relates to blade quality as a very important issue to work through to produce Kombu at Surprise Island. After those issues are worked through the another question to ask is will North American consumers be interested in Kombu grown adjacent, and potentially fertilized by, fish farms?

A use for IMTA kelps which is less promoted than Kombu, is the use for the kelp for intertebrate fodder. Sea urchins and abalone are two aquaculture species which have been considered for IMTA production and can both be fed kelp diets (Pierce et al. 2004; Troell et al. 2006). What is appealing about this application for kelp is that the condition of the final kelp product is not important. For instance, when Kombu requires a particular SV and clean blades free of fouling, kelp for fodder requires no optimal SV, particular quality or blade cleanliness. At Surprise Island, the level of fouling was very high at time and kelp blade cleaning could be cumbersome requiring many man hours. If fed to invertebrates at the farm kelp would simply be removed from production lines and added to the invertebrate production unit/area. Staggered kelp seed entries could also fit into this

model. Kelp would be available earlier in the year with seed entry in the fall and potentially later in the year with seed entered in the spring. As well, using products of IMTA to feed back into the production system only promotes greater sustainability as more cultures are supported by the energy provided by the initial feed inputs and a greater recycling of resources is achieved.

Another potential use of the kelp cultured at Surprise Island, that has a more traditional application in Europe and North America is as a fertilizer. Kelp is applied to cultivation areas as nutrient enhancers and/or conditioners in soil, in several different forms. The most common form of kelp fertilizers are the concentrated kelp purees and extracts. They are produced by various processing techniques (from grinding to lyophilisation) and are manufactured mainly for garden enthusiasts rather than for industrial scale agriculture. The benefits of such a product to Surprise Island is similar to the use of kelp as invertebrate fodder in that blade quality is not of great concern. Processing of kelp into fertilizers, is performed by combinations of washings, millings, dryings, chemical treatments and other processes (Verkleij 1992) which is likely to rid the kelp of fouling organisms. The staggered kelp seed entries could add to the kelp biomass available and potentially offer more steady supply of kelp for the fertilizer product. A fall entered kelp seed could yield a biomass ready for harvest in April/May, a winter entered kelp seed a could yield a biomass ready for harvest in June/July and a spring entered kelp seed could yield a biomass ready for harvest further into summer provided issue of grazing is overcome.

The high level of bryozoans fouling on fall kelp may offer and interesting opportunity for kelp fertilizer products. The exoskeleton of bryozoans, which is

composed of calcium carbonate, could be used for conditioning of acidic soils in much the same way maerl is used in Europe. Maerl, which is the common name for coralline red algae dredged from the ocean bottom, dried and sold to organic farmers, is utilized in France and Ireland and is considered to be harvested unsustainably (Wilson et al. 2004). If the kelp farm and bryozoans fouling were sufficiently large, blade areas with such fouling could be snipped, dried and milled to produce a fertilizer product with benefits of both kelp fertilizer and maerl while leaving a “tip cut” kelp crop to continue growing until harvest. This method of farming would utilize the fouled blade areas for a useful valuable product instead of letting those blade sections go to waste, while at the same time potentially yielding two different kelp products.

3.5 Conclusion

Seasonal seed deployment gave mixed results as to the value for a kelp producer and in an IMTA system. However, mixed results are likely due to biotic factors rather than the seed deployment itself. The fall entry was shown to give greater yield, less fouling and enhanced blade characteristics than the winter entry which would appeal to any kelp producer. Unfortunately the cultivation of the fall, winter and spring seed entry failed to show growth into the summer months due to snail grazing. From previous kelp research, it is likely to have seen growth and yield increases for an additional month for fall and winter kelp. Since this would have been at a time of greater day length and potentially elevated irradiances at the kelp blade surface, substantiation of kelp was never realized which limits the potential of the kelp being used for Kombu, which has been largely reported as a final product for Canadian IMTA kelp (Chopin et al. 2004). For spring entered kelp, the grazing pressure is also limited this study since this represents a

secondary crop that did not gain much biomass when some literature suggests such an entry could continue growth and yield increases into the summer (Kain et al. 1990).

For the benefit of an IMTA system the cultivation the growing season lengthened by entering seed in the early fall/late winter and this was realized by expansion earlier into the year rather than later. This seed entry produced kelp of enhanced characteristics over the winter seed entry.

To the Surprise Island IMTA system, entry of kelp in the fall meant an elongated growing season earlier in the year by one month which was also witnessed by Sanbonsuga (1984). It cannot be fully be determined how much longer this is over a winter entered kelp because there is no data into summer months. However, if the season is from April to July for winter kelp for instance, this is an increase of time of 25 percent. The question to follow then would be is entering seed in fall worthwhile? The benefits of the longer growout season were greater production and better kelp blade quality (i.e. less fouling). Of course this comes with a greater effort in producing seed, which for the sake of this experiment was not extensive, however for a full production at one or possibly several farms, this could be substantial (i.e. maintenance of cultures for approximately 9 months as opposed to under 2 months).

Chapter 4- Final Conclusion

Abiotic and biotic parameters, in particular freshwater intrusion, limited light and grazing, were all likely to affect production which was considerably lower than other published kelp yields. The discrepancy between Surprise Island kelp production published values of IMTA kelp production from New Brunswick (Chopin et al. 2004) of the same species was particularly surprising. Though much closer to published kelp yield in B.C. (Druehl 1988), Surprise Island yield was quite low. Low yield values, albeit not as low as what were witnessed, were not unexpected given the early stages of development of the kelp culture at the location. Yield may be increased through a variety of measures such as blade thinning, clipping, density experimentation, and further experimentation with outplanting times of seed, different species and multiple species. It is probable that the greatest kelp biomass increases will be realized through a concerted breeding program of selecting for certain blade characteristic which increase yields (Wu, 1999) and by overcoming grazing issues, if possible.

Of the kelp species attempted sheltered species preformed better than exposed species; *S. latissima* and *C. costata* performed better than *A. marginata* and *S.groenlandica* in terms of yield and blade length. Future experimentation other species may increase production at the site. Located within the Surprise Island bay is the perennial species *Eisenia arborea* and based on its natural presence this could be a "natural fit" for kelp culturing.

The two methods employed to increase macrophyte growth period both showed promise. Production and blade length curves showed differences between species but low production and lack of growth rate data made it difficult to define species growth period.

Therefore, the enhanced time period provided by the two different could not be properly calculated. However using the data collected, the two methods showed modest time increases over a conventional single species (*S. latissima*) kelp entry in winter. These methods employed at sites where conditions were more favourable for kelp culture (i.e. less freshwater, greater water movement and light) could show more dramatic results.

The results of these experiments showed that the increased time of kelp on site was made in the earlier part of the year but the summer months were limited by grazing. At Surprise Island, if grazing of kelp isn't overcome, an actively growing macrophyte component through the summer months may only be achieved by the addition of seaweed cultures from different phyla (i.e. greens and reds). This has been proposed by others however, as previously mentioned, but additional time and expense would likely be associated with developing those aquacultures.

Wheeler North in the book chapter entitled "Oceanic Farming of Macrocystis, the Problems and non Problems" summarizes years of experimentation of farming *Macrocystis pyrifera* in California. His experiences are not unlike this author's where problems existing from interactions between kelp and farm structures, such as tangling of kelp with lines, were a common occurrence. Early experimentation with kelp farming at Surprise Island noted tangling of parallel lines which took off all kelp plant from those lines. In response kelp lines were spaced further apart with the taglines to superstructures at the surface. Those taglines became targets of floating debris which caused numerous problems. This problem lead to the heavy anchoring of individual kelp lines on the designated kelp grid (used for the seasonal entries). Lines were anchored so taught that access to them was incredibly difficult. These problems are characteristic of open water

systems where so many factors can result in ruinous effects (in particular on thesis experiments). As well, grazing was a major issue for their farming operation. Grazing, which was probably the major factor limiting the results of this thesis, is not limited to North's or this author's experience but referenced with seaweed farming operations around the world (Ivin 1995; Doty 1987; Santelices & Doty 1989). These are only two of many issues associated with kelp farming at Surprise Island and make it very clear as to why most IMTA research projects are carried out in tank/land-based systems. However, in temperate regions around the globe, intensive monocultures are located predominantly in open water systems. If IMTA is to become an adopted production strategy in those regions, experimentation in open water systems and overcoming associated hurdles must be realized.

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