Ulva lactuca L. as an inorganic extractive component for Integrated Multi-Trophic Aquaculture in British Columbia: An analysis of potentialities and pitfalls

by

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B.Sc., University of Newcastle upon Tyne, 2003
M.Sc., University of Liverpool John Moores University, 2007

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Abstract

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Ulva as an aquaculture crop and IMTA component species has received mixed results globally; success has been achieved in South Africa and Israel, whilst in Europe the results have been poor. This project aims to determine if *Ulva lactuca* is a suitable candidate as an inorganic extractive species component within marine IMTA systems in British Columbia. The inorganic extractive feasibility of *U. lactuca* was determined with combination of real time growth and nutrient uptake experiments, alongside a SWOT analysis and literature review to reveal the possible potentialities and pitfalls.

U. lactuca was cultivated in 680 litre tanks in the effluent of Wolf Eels, *Anarrhichthys ocellatus* in a recirculation system at the Aquatics facility at the University of Victoria. Growth experiments of wild local *U. lactuca* strains attained summer growth of up to 17.43% specific daily growth rate, with winter growth of up to 4.26% specific daily growth rate. *U. lactuca* demonstrates a preference for Ammonia-N uptake over other forms of inorganic nitrogen and a reduced nutrient uptake capacity during dark periods. Nitrate uptake capacity up to 202µm N gDW^{-1} day^{-1} was exhibited. These figures display the excellent biological potential of local *Ulva lactuca* strains to act as an inorganic extractive. However currently, long term maintenance of the crop proved problematic with instability

with growth rates and nutrient uptake capacity. Cultivation issues in combination with poor economic outlook will restrict the feasibility of this species to specific types of IMTA system.

Beneficial steps towards the deployment of *U. lactuca* inorganic extractive components would include: (i) the identification of suitable sterile strains or employment of "germling" spore production, (ii) the use of a rotational, light weight, cage cultivation system, (iii) being farmed in combination with a dark period nutrient removal species, such as *Chondrus crispus*, (iv) being farmed in conjunction with in-situ algivorous species.

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Acknowledgments

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Chapter 1 - Introduction

1.1 Introduction to Aquaculture

Aquaculture is simply the cultivation of aquatic life. The purpose of which is to address an array of human concerns, which include conservation, energy production, feedstuff resource, human food production, pharmaceutical products, ornamental products, and recreation. The past five decades have seen a multiple disciplinary approach rapidly transform the industry of aquaculture from a millennia old art form to a modern multi-faceted science.

Humanity is fast approaching a major paradigm shift in aquatic product consumption from traditional wild harvests to a varied degree of artificial cultivation. The transition is analogous with the land-based hunter-gatherer society shift towards terrestrial agriculture that occurred several thousand years ago. The global demand for aquatic based products far exceeds the sustainable natural resource base. The upward trend in aquaculture production is clearly demonstrated with an increase from just over 44 million tonnes in 2001 to over 79 million tonnes in 2010 (FAO, 2012). The increase in population, reduction in natural resources, and land conflict issues will inevitably force the continuation of this trend, and the "*tipping point*" between proportion "*fished*" vs. "*farmed*" will be crossed. China for example has already crossed the threshold, in which it cultivates 64% of its aquatic produce (Ellis and Turner, 2009). True sustainability of many current aquaculture practices are under question however. The large scale industrialized monoculture establishments are not balanced and fail to address the three pillars of sustainability. Neori (2007) suggests a transition of funding with research and development from monoculture carnivorous aquaculture industry, to lower trophic order species and more ecologically balanced systems. He acknowledges economic benefits of intensive monoculture of carnivorous fish and shrimp. However, he argues that the industry is not socially or ecologically balanced, and thus is not sustainable. Current monoculture business models do not account for the environmental costs associated with such practices. Such as the economic value that bio-filters provide via bio-mitigation service. Currently, no monetary cost is associated with many existing aquaculture effluent discharges into natural systems (Neori *et al*. 2007). However, policy does impede the new aquaculture development in various locations dependent on effluent discharge reduction protocols (Tacon and Forster (2003).

Intensive fish/shrimp monoculture, even though it only makes up 9 percent of total mariculture production (as previously highlighted), has remained as the central focal point for the various shareholders of the aquaculture industry, in particular, the public, media, and policymakers (Neori *et al*. 2007). The social benefits of aquaculture include, but are not limited to employment, income, prevention of rural population degradation, species rehabilitation and conservation, pollution mitigation, and food security (F.A.O. 2006). Recent years have also shown an increase in consumer awareness and preference for sustainably sourced aquatic food (F.A.O. 2006). Future predictions for aquaculture products show a continued dependence on lower trophic level species, with carnivorous fish and shrimp continuing to count only for a small percentage of total production.

1.2 Integrated Multi-Trophic Aquaculture

Integrated multi-trophic aquaculture (IMTA) is a movement within the aquaculture industry that targets several key sustainability issues through the application and modernization of the ancient form of polyculture. The Integrated Multi-Trophic Aquaculture (IMTA) system is a solution advocated by the various aquaculture shareholders. An IMTA approach to aquaculture practices is far more ecologically balanced than current monoculture practices, and provides multiple benefits for multiple shareholders (Chopin *et al*. 2001). IMTA cultivates several products synergistically within one system, through the reduction of anthropogenic inputs and utilisation of natural energy and nutrient transfer cycles. IMTA adopts natural nutrient cycles to facilitate the culture of different trophic level species within one system. “Integrated” refers to intensive and synergistic cultivation, using nutrient and energy transfer. “Multi-trophic”, means that the various species cultivated occupy different levels within the food web (Chopin, 2006). The key goals of IMTA are: (1) Nutrient enrichment of natural water systems from aquaculture, through the utilisation of nutrients to generate growth of autotrophs and lower order heterotrophs (Harvin, 1978, Troell *et al*., 1999, Smith *et al*., 2002). (2) Diversify the production of a given aquaculture site, spread the economic risk and generate value added production species to supplement primary production income (Chopin, 2006). (3) Variation of product range provides alternative nutritional value products for the commercial market or locals in developing nation's community based aquaculture systems (Neori *et al*., 2007). (4) Movement away from monoculture practices to develop a more natural synergistic approach to farming, promoting and respecting natural ecosystems, and nutrient dynamics (Neori *et al*., 2007). (5) Provide in-house feed sources for other cultivated organisms on the site (Cruz-Suaruz *et al*., 2001, Neori *et al*., 1997, Robertson-Andersson, 2008).

The premise for IMTA systems is for each component to act as a series of ecologically engineered tools based on bio-filtration and self-generating feeding mechanisms. The theory that nutrient rich water should be viewed as a resource for the extractive species that benefit from this feed source led to the acknowledgement of intensive modern polyculture. Environmental mitigation using bio-filters began in the 1970's (Ryther *et al*. 1975, La Pointe *et al*. 1976, Harlin, 1978). Initially with the use of domestic sewage waste water as the primary feed source for a multi-trophic bio-filtration system. Nobre *et al*. (2010) highlight the many benefits of implementing an IMTA system over a traditional monoculture practice. Neori *et al*. (2007), also highlight that the nutrient consumption via the monocultivation of macro-algae and shellfish is substantially more than the nutrient levels given off by finfish farming. Both large scale nutrient removal and loading is detrimental to the balance of an ecological system. Integration of the various species within one system or at one site balances this disruption.

Within IMTA, the use of macro-algae and shellfish as bio filtration systems have received the greatest attention, (Ryther *et al*., 1976, La Pointe *et al*. 1976, Folke and Kautsky, 1989, Petrell *et al*. 1993, Neori, 1996, Chopin *et al*. 2001, Msuya and Neori, 2010). Justification for this research attention has evolved from one of aquaculture's greatest detrimental issues, nutrient enrichment of the surrounding water systems. Excess nutrient loading facilitates changes in the natural dynamics of an ecosystem and can lead to trophic disruption, through harmful algal blooms and eutrophication (Daalsgard, 2006). Macro-algal cultivation has been practiced successfully for generations in Asia, and as stand-alone crops support a substantial volume of cultivated aquatic species, in 2010 over 19 million tonnes of macro-algae was farmed globally (FAO, 2012).

A vital component of an IMTA system is the co-cultivating of an inorganic extractive species, such as macro-algae. For example, 10-30% of total nitrogen added to marine fish cages as fish feed is harvested as product, while 10-40% is released as particulate matter, and the remainder is excreted in dissolved forms. Excess nutrient release necessitates the co-culturing of extractive components in the system to mitigate the environmental consequences of this nutrient influx. The precise proportions of excess nutrient release are dependent on environmental factors, species, culture system, feed consistency, and management practices (Hall *et al*. 1992). Numerous macro-algal species have been trialed and successfully used as biological filters for the extraction of the inorganic nutrient flux generated by the primary fed IMTA component (Ryther *et al*. 1975, Neori *et al*. 1991, Shpigel *et al*. 1993, Buschmann, 1996, Chopin *et al*. 2001, Robertson-Andersson 2008, Nobre *et al*. 2010, Abreu *et al*. 2011). The key to successful implementation of IMTA lies with the full understanding of the nutrient dynamics of the system. System design, efficiency, and the identification of correct synergistic species are determined by a multitude of factors, many of which still require substantial research.

1.3 Macro-algae cultivation: A global perspective

Although mariculture of seaweed is a global industry, the majority of seaweed is grown in Asia, of which China contributes 60% of the global algal production (Titlyanov and Titlyanov, 2010). Members of all three phylum are grown commercially, the main genera cultivated include: *Agardhiella, Caulerpa, Cladosiphon, Eucheuma, Gelidium, Gigartina, Gracilaria, Hydropuntia, Hypnea, Laminaria, Kappaphycus, Meristotheca, Monostroma, Porphyra, Saccharina, Ulva,* and *Undaria*. The estimated total value of aquatic plant production (both cultivated and wild harvest) was $7.8 billion in 2008, which equates to approximately to 15.8 million tonnes fresh weight of aquatic plants, of which 99.6% is dominated by seaweeds (FAO, 2010). The total of farmed algae in 2010 was estimated at 19 billion tonnes with a value of US$5.7 billion, in comparison to wild harvested algae which equated to approximately 800 thousand tonnes (FAO, 2012). These figures show the dramatic increase in the volume of product the industry generates, and the decrease in the proportion of wild harvest comparative to cultivated seaweed. The value of the seaweed is determined by its constitution and resultant use.

Pharmaceutical grade algal extracts command the highest price (FAO, 2010), whereas use as soil amendment and as fodder for marine and terrestrial herbivores have the lowest value. Cultivar selection and cultivation approach are determined by a multitude of factors as discussed in more detail later in the paper. The main selection criteria are a function of site determinants, economics, and environmental conditions.

The FAO`s (2012) State of World Fisheries and Aquaculture report outlines the disparate nature of the distribution of algal cultivation. Only 31 countries and territories actively cultivate algae.

“In 2010, and 99.6 percent of global cultivated algae production comes from just eight countries: China (58.4 percent, 11.1 million tonnes), Indonesia (20.6 percent, 3.9 million tonnes), the Philippines (9.5 percent, 1.8 million tonnes), the Republic of Korea (4.7 percent, 901 700 tonnes), Democratic People’s Republic of Korea (2.3 percent, 444 300 tonnes), Japan (2.3 percent, 432 800 tonnes), Malaysia (1.1 percent, 207 900 tonnes) and the United Republic of Tanzania (0.7 percent, 132 000 tonnes).”

The uneven distribution trend in algal production is dictated primarily by several factors. Firstly, the history of the industry; wild seaweed harvests have occurred globally over millennia. However, the cultivation of algae and its methods and approaches, have their historic roots in Asia. Secondly, in concordance with the history of algal production, the commercial market for seaweed base products (particularly human consumption) is significantly higher in many Asian countries. Thirdly, the low value of the product and high labour intensity of cultivation practices dictate maximum wage limits, which prohibit many nations from the seaweed cultivation industry. And finally, algal production requires a considerable amount of space and disrupts water usage; this has prohibited the development of seaweed cultivation in some regions.

1.4 Ulva: Background as an inorganic extractive species

1.4.1 Taxonomy

Hayden *et al*. (2003) describe in detail the problematic nature with the classification of Ulva. The relatively recent amalgamation of the genus *Ulva* and *Enteromorpha* under the genus *Ulva* only partially unravels the quagmire that presents itself when attempts are made to classify members of these taxa. The debate on classification stems up the tree, where ambiguity arises as to where to correctly situate *Ulva* within order and family. The classification of *Ulva* species is beyond the scope of this paper and author. This paper will use the following classification from Guiry and Guiry (2012) for all *Ulva* species.

Kingdom – Plantae

Phylum – Chlorophyta

Class – Ulvophyceae

Order – Ulvales

Family – Ulvaceae

Genus – *Ulva*

Defining the various species within the genus is even more difficult. Originally, identification of *Ulva* species was based on morphological, anatomical, and cytological characteristics. Thallus morphology, size, presence or absence of thallus dentation, thallus thickness, cell dimensions and cellular content were all thought to be viable differential traits. However, numerous studies (Wynne & Kraft, (1981), Womersley, (1984), Bold & Wynne, (1985), Joska, (1992), Hoek *et al. (*1995), Silva *et al. (*1996), Stegenga *et al. (*1997), Lee, (1999), Hayden *et al. (*2003), and Loughnane *et al*. (2008) have shown the scarcity of morphological conspecificity and the plasticity of these characteristics with external parameters. Such as, age, reproductive stage, hydrological disturbance, tidal factors, temperature, salinity, turbidity, light, and biological factors such as epiphytal growth, grazing, and diseases. Guiry and Guiry (2012) describe current identification methods rely on morphological and anatomical characteristics and genetic analysis of the material, there is however still discrepancy in the practical use of *Ulva*. Robertson-Andersson (2008) work describes the collection of a large sample of supposed *Ulva lactuca*, it digressed that there were in actual fact, five separate species within the sample, *U. lactuca, U. capensis, U. fasciata U. rhacodes and U. rigida*.

The algae database compiled by Guiry and Guiry (2012) taxonomically accepts 99 species and variations. The database however also lists a further 463 intraspecific names, which are either homotypic or heterotypic synonyms, or have not been verified by the database. *Ulva* distribution and the species variety is very location specific.

Numerous studies have been carried out to identify which *Ulva* species are present in a given region. The work done in Australia by Kraft *et al*. (2010) reported the presence of six species that conformed to classical anatomical and molecular identification. However, several cryptic species of *Ulva* did not conform to anatomical or molecular analysis.

In the Mediterranean and Adriatic Seas, the presence of two *Ulva* species is disputed. *U. laetevirens* is thought to be the only species present in lagoons (Sfriso, 2010). Sfriso (2010) uses anatomical and environmental analyses as evidence of the presence of *U. rigida* and *U. laetevirens*. The anatomical evidence is based on the morphology of cell structure in the cross section of rhizoidal and basal regions, however, the basal and rhizoidal cells do not exist in free-floating colonies. Thus, existence of two species is dependent on environmental preference. *U. rigida* prefers eutrophic (nutrient rich) environments, whereas *U. laetevirens* is more abundant nutrient poor waters. Molecular analysis of *Ulva* within the same region identifies six different species with morphological imbrication, *U. laetevirens* is considered conspecific with *U. rigida* in this study (Wolf *et al*., 2012). Also highlighted in Wolf *et al*. (2012) is the additional complexity of introduced alien species populations and their possible impact, in this case *U. californica* and *U. pertusa*.

The coast of British Columbia is supposed host to eleven different *Ulva* species (Druehl, 2001). Hayden and Waaland, (2004) identified twelve species of *Ulva* in the northeast Pacific: *Ulva californica, Ulva intestinalis, Ulva lactuca, Ulva linza, Ulva lobata, Ulva* *pertusa, Ulva prolifera, Ulva pseudocurvata, Ulva rigida, Ulva stenophylla, Ulva taeniata* and *Ulva tanneri. Ulva fenestrata* features in various research (Kalita *et al*, 2011) and is often described as a common species found in coastal waters of British Columbia. *U. fenestrata* is a heterotypic synonym of *U. lactuca* (Hayden and Waaland, 2004). A local *Ulva* sub-species, *U. scagelli*, researched and identified only in the coastal waters of Vancouver Island (Chihara, 1969), was classified by Hayden and Waaland (2004) as a heterotypic synonym for *U. californica*.

1.4.2 Ulva Biogeography

This chapter will discuss the broad physical characteristics, physiology, life history, distribution, and ecology of the *Ulva* genera. *Ulva* is known as "*Sea Lettuce*" and "*Green Laver*" in many English speaking countries, *Tahalib* in Arabic, *Hai Tsai, Shih shun, Haisai Kun-po,* or *Kwanpo* in Chinese, *Glastan* in Irish, *Meerlattich* in German, *Alface-do-mar* in Portuguese, *Limu papahapapa* in Hawaiian, *Luchi* in Spanish, *Havssallat* in Swedish, *Laitue de mer* in French and *Aonori* in Japanese to name a few.

1.4.3 Spatial Distribution

Species of *Ulva* are globally distributed throughout coastal and estuarine habitats, with a few freshwater species. *U. lactuca* and the associated variants are together the most widely distributed of the *Ulva* species. The confirmed distribution of *U. lactuca* includes countries and regions within Arctic, Antarctica and Sub-Antarctic islands, Mid-Atlantic Islands, Oceania and the Pacific Islands, Asia, Europe, Africa, and the Americas (Guiry and Guiry, 2012). However, the defined ranges of *Ulva* species, particularly *U. lactuca* are subjected to controversy due to the difficulty in identification. The majority of distribution confirmations were solely based on morphological characteristics without molecular analytical support. Due to the plasticity and environmentally derived variance in *Ulva* morphology, many maybe deemed incorrect (Stegenga *et al*. 1997). Currently, there has been no evidence to prove that the various *U. lactuca* strains are genetically identical.

Ulva generally inhabits the upper to mid-intertidal (eulittoral, mid-eulittoral and supra-littoral zones), and occasionally the subtidal zone. The spatial distribution within the intertidal zone varies temporally. In the northern hemisphere, throughout the winter thalli tend to be smaller and more dispersed throughout the various intertidal zones. Whereas in the summer the fronds grow larger, and tend to be found within a smaller range within the intertidal (Lee, 1999, Druehl, 2001, Loughnane *et al*. 2008). Ulva are annual or pseudo-perennial in that the holdfast portions are perennial and grow new blades each spring (Lobban & Harrison, 1997).

Ulva have three habitat strategies, firstly, direct attachment to the base substrate via a discoid holdfast (epilithic), secondly, epiphytic attachment to various aquatic organisms and structures via a discoid holdfast. Finally, *Ulva* can grow in free floating form, particularly in sheltered lagoons; it often forms dense aggregations (Bold and Wynne, 1985). The formations of these large aggregations are often described as “Green Tides”, and are viewed as extremely detrimental to the ecosystem health and can be detrimental directly to human health.

1.4.4 Physiological Adaptations

Ulva is a robust species, in regards to its survival strategies. Ulva species tend to proliferate in nutrient rich waters, with low hydrodynamic forces. It displays both opportunistic and persistent traits (Vermaat and Sand-Jensen, 1987). Examples of specific traits that allow *Ulva* to proliferate in the intertidal include:

(i) The plasticity of the cell structure within the thallus can stretch an additional 35% prior to breakage (Svirski *et al*. 1993).

(ii) The flexibility of the frond allows the thallus to lie prone against the surface of the substrate to reduced water motion damage (Norton *et al*. 1980, 1982).

(iii) The extremely high surface area to volume ratio (SA:V), relatively large cell structure and uniformity of the cell activity throughout the thallus allows for increased exposure to photon flux and nutrient uptake (Littler, 1980, Littler and Littler, 1980, Sand-Jensen, 1988).

(iv) The capacity to mitigate the growth of *Gracilaria* through the production of allelopathic compounds (Svirski *et al*. 1993).

(v) Demonstrates the ability to survive periods of freezing, anoxia, sulphide exposure, and prolonged darkness (Vermaat and Sand-Jensen, 1987).

(vi) Adaptation to light level variation from zero/low light to high light intensity levels through the increase and decrease in chlorophyll levels which alters the photosynthetic capacity accordingly (Hansen and Jensen, 1993)

(vii) Total reproductive cell capacity is proportionally greater than many other algal species due to cell uniformity, which leads to higher reproductive rates, thus, rapid colonisation of newly cleared substrate (Littler, 1980).

However, as a result of the delicate nature of the thallus, there is increased risk of high desiccation rates, wave and substrate damage, susceptibility to later successional competitor species, outcompeted for light due to low profile nature of the thallus in comparison with the more rigid taller canopy macro-algal species, and increased damage from grazing impact. Ulva is viewed as a “pioneer”, opportunistic r-selected species (Littler, 1980), as opposed to a later successional K-selected macro-algal species such as *Laminaria*.

1.4.5 Morphology

The general morphological characteristic of Ulva is a distromatic green sheet-like thalli. The shapes of the Ulva thalli vary between lanceolate to broadly ovate, often ruffled along the margins. The average lengths vary in accordance to species and environmental parameters, local grown *U. lactuca vary between* 18-60 cm (Scagel, 1972). Thalli thickness ranges between 38 – 209 µm (Stegenga *et al*. 1997).

The cellular differentiation in *Ulva* species shows no specialization, every cell is capable of photosynthesis and reproduction. Cells are relatively large in comparison with other algal species. The cells are quadrate to slightly elongate and anticlinal (perpendicular to the surface). The cell walls are fibrillar, consist of cellulose, and store carbohydrates. Each cell is uninucleate, contains a cup-shaped parietal choloroplast and at least one if not several pyrenoids (Hoek *et al*. 1995). However, there are exceptions to the uniformity of cellular action within the thallus. Luning *et al*. (1992) identifies variation between the portions of the Ulva thalli, the upper portion grow more vigorously than the lower. Basal discs showed only vegetative growth after 8 days, whereas apical discs showed complete gametogenesis. Cell differentiation also occurs in the holdfast region of the thallus. Rhizoidal production is achieved by the proximal cell extension through the distromatic cell structure outward to form the holdfast. If the frond becomes detached a single rhizoid cell can generate a new thalli (Lobban & Harrison, 1997; Lee, 1999). Ulva generally displays parenchymatous uniformity, whereby cell division may occur anywhere on the thallus, this division occurs in a plane perpendicular to the thallus surface (Hoek *et al*. 1995). Fertile portions of the thallus change colour, from green to yellowish or brownish green as reproductive organs form (Chihara, 1968). The lack of plasmodesmata (microscopic channels that facilitate transportation and communication between plant cells) essentially means that *Ulva* are simply complex single celled colonies (Hoek *et al*. 1995). Semilunar (bi-weekly) gamete/sporophytes discharges for *U. lactuca* coincide with spring tides (Luning *et al*. (2008). Kalita and Tytlianov (2003) found that *U. fenestrata* displayed sporulation (gamete/sporo-genesis) approximately every ten days. The Kalita and Tytlianov (2003) study also highlights the ability to prohibit the maturation of the thalli through environmental controls, at 15°C, 71% tissue maturation occurs. At 10°C sporulation occurred for 6% of thallus surface area, at 5°C no sporulation occurred, only vegetative growth.

1.4.6 Life History

Ulva displays isomorphism and a diplohaplontic lifecycle with anisogamous gametes (Hoek *et al*. 1995) See figure 1.1.

Haploid gametophyte thalli (a,a') produce anisogamous biflagellate gametes (c,c') through mitosis (b,b'), the gametes display positive phototactic behaviour, thought to promote the mixing of the gametes and increase irradiance levels. Copulation (d) between the male (c') and female (c) biflagellate gametes creates a diploid zygote (e). The diploid zygote germinates into a filamentous and uniseriate germling (f), the germling develops into a pluriseriate filament, then into small hollow tube-like structures (g). The structures then "collapse" forming the characteristic distromatic thalli of the adult sporophyte (h). The sporophyte is morphologically identical to the gametophyte to the naked eye. The sporophyte (h) through meiosis in the sporangia (i) produces quadriflagellate haploid zoospores (meiospores) (j, j'). Morphologically, the development (k, l) (k', l') between zoospore (j, j') and the resulting gametophyte is similar to the diploid zygote development to a sporophyte (h). Half the quadriflagellate haploid zoospores develop into male gametophytes (a'), the other half into female gametophytes (a).

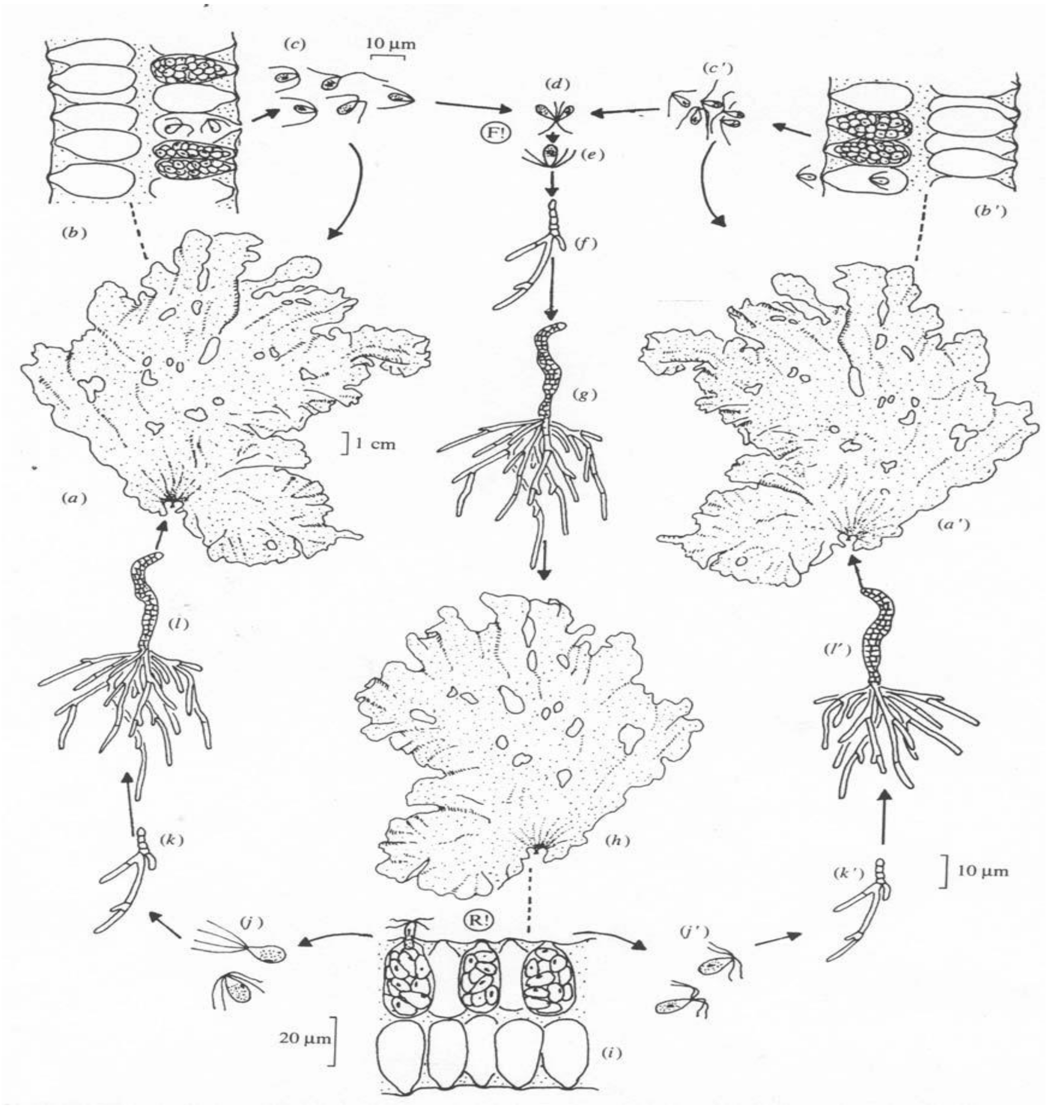

Figure 1.1. The life cycle of *Ulva lactuca L.* (Hoek *et al*. 1995).

1.5 Uses of *Ulva*

Ulva species have had an important role for many coastal communities, and are currently used for numerous purposes. The variety of applications is a result of the *Ulva's* physiology, accessibility, ease of processing, global distribution, and the high productivity rates. Various coastal communities have utilised this resource for millennia. Readily consumed worldwide as a raw ingredient, Ulva is primarily used in soups and salads. Ulva is relatively high in digestible protein and dietary fibre and comparable with other high protein level plants (Bodin-Donbidgeon *et al*., 1997, Ortiz *et al*. 2006). Ulva also contains relatively low lipid content, but proportionately high poly-unsaturated fatty acid (Omega-3) levels (Ortiz *et al*., 2006). Relatively high levels of vitamins and minerals are also exhibited, particularly tocopherols and tocotrienols, sodium and iodine (Ortiz *et al*., 2006). The average nutritional constitution of *U. lactuca* is: moisture 12.6%, ash 11%, protein 27%, lipid 0.3%, carbohydrate (incl. dietary fibre) 61.5% (Ortiz *et al*. 2006). The protein proportion varies up to 40% with increased nitrogen availability in unison with the variance in carbohydrate percentage (Msuyra and Neori, 2008).

In Asia, particularly China, the medicinal recognition of *Ulva* consumption has long been understood to be beneficial. Yu *et al*. (2003 a, b) showed that the consumption of *U. pertusa* polysaccharides significantly reduced the atherogenic index, due to a reduction in the plasma total cholesterol. In turn, *U. pertusa* shows potential for the prevention of ischemic cardiovascular and cerebrovascular diseases. The reduction in cholesterol is also supported in Jiang *et al*. (1994). Other beneficial medicinal traits of Ulva intake include anti-tumour and anti-aging properties (Wu *et al*., 2004).

Knowledge of the rich nutritional content of Ulva species has been apparent for generations. For example, Scottish farmers still graze their cattle and sheep on Ulva laden coasts to provide cost effective vital dietary additions. Poultry fed Ulva meal as part of their regular diet have shown increased growth and other beneficial cultivation traits (Ventura, 1994). Ulva as fodder for animals is the basis for relatively recent research into the benefits of using Ulva as a feed component for multiple modern aquaculture practices. Numerous dietary studies on the effect Ulva meal has on various cultivated fish species have resulted in mixed outcomes. Omnivorous and herbivorous species, such as common carp, *Cyprinus carpio* L. (Diler *et al*., 2007), Nile tilapia, *Orechromis niloticus* (Soyutu *et al*., 2009), black sea bream, *Acanthopagrus schlegeli B*. (Nakagawa *et al*., 1987), and striped mullet, *Mugil cephalus* L. (Wassef *et al*., 2001) all demonstrated beneficial cultivation traits when given a diet supplemented with *Ulva* meal. Diler *et al*. (2007) found that beneficial traits in common carp, *C. carpio* included, increased growth rate, higher quality carcass composition, increased feed utilization, and lipid metabolism. A 5% *Ulva* inclusion ratio resulted in the best performance. However, good performance for up to 15% inclusion was measured. An inclusion of *Ulva* meal in artificial feed allows for reduced dependency on fish meal and filler ingredients such as grain. Utilisation of Ulva species allows more appropriate use of previously mentioned resources.

The effect of dietary Ulva inclusion on carnivorous fish species shows fewer to no benefits. Low levels of Ulva meal inclusion in rainbow trout, *Oncorhynchus mykiss* (Walbaum) diets during starvation period, (i.e. before harvest or during periods of poor water quality) increases lipid metabolism to allow them to survive. However, regular inclusion of Ulva meal within the feed was detrimental to various cultivation traits (Guroy *et al*., 2011).

Success with the use of cultivated Ulva meal as a feed source within aquaculture is demonstrated within the invertebrate cultivation sector. Cruz-Suarez *et al*., (2010) co-cultured *Ulva clathrata* with white-legged shrimp, *Litopenaeus vannamei* in the same system. This allowed *L. vannamei* to freely feed on *U. clathrata*. The *Ulva* acted as a multi-purpose bio-tool, and provided supplemental food, shade, oxygenated the water, reduced dissolved CO_2 levels, and removed potentially toxic inorganic dissolved nutrients, particularly ammonia. Co-cultivation resulted in increased growth rate of *L. vannamei* by 60% and increased production quality (carcass quality, docohexanoic acid levels). Robertson-Andersson *et al*., (2008) highlights the multiple beneficial effects of cultivated Ulva meal inclusion on the quality of abalone, *Haliotis midae* cultivation in South Africa.

Ulva, as with many algal species contains phycocolloids, these polysaccharides are found in the cell wall (Minghou, 1990). Algal polysaccharides exhibit excellent gelling, stabilising and emulsifying properties (Bixler, 1996). These properties make them commercially and economically significant, as additive products such as binder agents, thickeners, and gelling agents. Industrial, pharmaceutical, and food industries apply varying amounts of algal based phycocolloids to a surprising array of products. Phycocolloids are primarily sourced from red algae, (Rhodophytae)(Minghou, 1990). *Ulva* based phycocolloids have been shown to be of a lower quality and contain higher quantities of sulphur based polysaccharides, and thus require more processing to attain the high grade products required by modern industry (Lahaye *et al*., 1993, Siddhanta *et al*., 2001).

A further use of *Ulva* is as a soil amendment product. El-Naggar *et al*., (2005) found that the germination of the vegetable crop, broad bean (Vicia faba), was positively influenced by the addition of *Ulva* extract into the growth medium. *U. lactuca* extract promoted growth in chilli peppers, Capsicum annum, (Sridhar *et al*., 2012).

Humanity's interest in renewable energy sources has not overlooked macro-algae. Since the 1970's, research has investigated the utilisation of *Ulva* along with other algae as a source of biofuels. The potentiality of *Ulva* as an energy crop was investigated and found viable, however not economically viable (Rhyther *et al*., 1984). Increased concern with the planets insatiable appetite for energy has renewed interest in this potential marine energy source. Bruhn *et al*., (2011) analyses the potentiality of *Ulva* through the process of anaerobic digestion and methane generation. However, previous work by Morand and Briand (1999) demonstrated that whilst possible, anaerobic digestion as a pathway to methanisation simply was not economically sound. The work recommends instead, the methanisation of the liquefaction juices as a more plausible source of combustible biogas.

The primary use of Ulva species in the context of this paper is as an inorganic extractive component of IMTA or as a biological engineering tool for mitigating excess nutrients within marine and estuarine water bodies. Ryther et al., (1975) researched the concept of the utilisation of *Ulva* and other algae as extraction tools. Their work identified the ability of *U. lactuca* and *Chondrus crispus* as efficient inorganic nutrient removal components within an integrated waste recycling system that processes raw sewage effluent. The results showed *U. lactuca* to have the highest nutrient uptake efficiency within the sewage remediation system. However, the other species were more commercially viable. Hugenin (1976), Hughes-Games (1977) and Tenore (1976) were the first pioneers to establish the approach that seaweeds could be used to filter the excess nutrients from the effluent of cultivated fish. Gordin *et al*., (1981), Neori *et al*., (1991), Shpigel *et al*., (1993) continued the work, with the successful development and practical application of the first full IMTA systems with a variety of species. *Ulva lactuca* was one of the initial species identified for these systems due to the high productivity rate, ease of cultivation, availability, and high uptake efficiencies (Neori *et al*., 1991). These initial forays into IMTA have given rise to a new generation of seaweed integrated aquaculture. Multiple algal species including *U. lactuca* are currently cultivated in a variety of IMTA systems with the purpose of inorganic nutrient extraction.

1.6 Ulva Cultivation Methodology

The physiological traits attributed to *Ulva* species as previously described, make it, on paper, an ideal candidate for aquaculture, and particularly IMTA. The greatest detriment to the cultivation of *Ulva* on a commercial scale is the low economic value, and the high natural abundance. Thus, the challenge is to produce large volumes economically and reliably. Cultivation, as opposed to wild harvest of *Ulva* produces a crop that has the benefits of a higher quality and consistent product, an enhanced growth season, and dependent on the cultivation method, a positive impact on the environment. Numerous attempts at *Ulva* cultivation have been trialed. Cultivation methods can be divided up into Open water cultivation and Land-based cultivation, and then categorised under two life cycle categories (a) and (b). (a) Starting from microscopic spores, the whole lifecycle is controlled, e.g. *Laminaria, Porphyra*. (b) The cultivation from macroscopic algal fragments, e.g. *Gracilaria, Eucheuma* (Fei *et al*. 1998). Ulva species have been cultivated successfully with the use of both lifestyle methods. Hiraoka and Oka (2007), used their own ``Germling Technique`` for the production of *U. prolifera* spores. The production of spores and whole life cycle control enabled mass production and stock maintenance. The majority of growth experiments utilised fragments of wild harvested thalli to attain growth rates and conduct cultivation parameter experiments. Kalita and Titlyanov, (2011) obtained vegetative growth of *U. fenestrata* by cutting new material from grown vegetative parent material. No breakdown of the thalli was observed and the biomass increased 18-50 times (117days). Luning *et al*. (1992) identifies the difference between the portions of the *Ulva* thalli. The upper portion grows more vigorously than the lower. However, most literature states no differentiation between the cells. Later Luning *et al*. (2008), record basal discs showed only vegetative growth after eight days, whereas apical discs showed complete gametogenesis.

Cultivation method selection is dependent on a multitude of factors. The main factors include, but are not restricted to, the dimensionality of the proposed aquatic ecosystem, sediment type, the irradiance levels, temperature ranges, pH and salinity variability, nutrient intensity and fluctuation, site hydrodynamics, pollution levels, governance and economic issues, and other aquatic shareholder requirements (Titlyanova and Titlyanova, 2010). Each cultivation method has advantages and disadvantages.

Open Water Cultivation

Open water cultivation is the oldest form of aquaculture and the most widely practiced in terms of macro-algal cultivation. The main drawback to open water cultivation of algae is the infrastructure required to hold the algae in place to keep it within the cultivation area, for nutrient removal, light requirements, and ease of harvest. Several techniques are used to keep seaweeds in-situ. The most practiced technique is to attach the thalli, individually to a substrate or inoculate a substrate, directly with spores. These techniques are grouped into two categories. Firstly, bottom stocking or bottom culture, where the seaweed is anchored directly to the sediment. Secondly, suspended cultivation, where the seaweed is suspended within the water column to maintain the correct growth environment and prevent crop loss, and abrasion damage. Types of suspended seaweed farming methods include rope farming, the use of nets, floating rafts, and cages.

The main advantage to open water cultivation is the unnecessary requirement to transport water, or to provide the infrastructure or land to contain a body of water. Thus, the start-up and maintenance costs are relatively low. The main disadvantage to this methodology is the reduced level of control over the environmental conditions, resulting in variability in growth performance and the quality of the product. Open cultivation techniques are generally very low technology, but are labour intensive. This requirement restricts cultivation in developed nations where labour costs are prohibitive.

However, open water systems benefit from exposure to natural water conditions. Natural factors such as temperature, salinity and pH are relatively constant, although temporal changes occur. Cultivation of local species means these species are adapted to these temporal changes. The constant motion of the growth medium means continuous distribution of nutrients and the necessary removal of waste products. This has the drawback of having very little control over the environmental conditions if they are deleterious to growth requirements.

Bottom Culture

The main reason behind cultivation of seaweed in the natural sediment is to replicate nature, and the conditions the seaweed has evolved to thrive in. There are several methods of bottom cultivation.

A simple method is to transfer existing vegetative thalli, attached to a substrate, for example *Gracilaria* species attached to small stones, to areas where existing densities are low (Oliveira *et al*. 2000). Other methods include the direct planting of thalli into sub-tidal and intertidal sediments, or the attachment of vegetative thalli to poles as a substrate (Titlyanova and Titlyanova, 2010). The poles are then driven into the substrate (Oliveira *et al*. 2000). In Chile, *Gracilaria* plantlets are placed in plastic tubes and buried in the sediment (Buschmann *et al*., 1995). Another, more labour intensive method includes the inoculation of nylon mesh, which is stretched over rocks to keep thalli in place (Oliveira *et al*. 2000). General practice includes the partial collection of the crop, between 10 – 40% is left to allow future propagation and maintain natural ecosystem integrity (Titlyanova and Titlyanova, 2010). Another bottom culture method that could be described as a semi-closed farming practice, is the creation of shallow lagoon enclosures, whereby the thalli is not directly attached to sediment, but lies on the bottom, or is natural suspended close to it (Glenn and Doty, 1990).

In addition to the advantages already described, due to the shallow nature of the waters in which bottom culture is practiced, the final harvest is simple. However, the drawbacks of bottom culture, is that these practices are extremely labour intensive. They are only conducive to locations where the algae occurs naturally, thus, cultivation is simply to increase local density. The crop density with bottom culture is primarily a function of water clarity and water depth. The main problems with this type of culture are that tidal and wave movement; particularly storms can damage the crop. This depth fluctuation also alters the irradiance intensity and periodicity. Increased water movement due to the shallow nature of the location can increase turbidity, thus further reduce irradiance levels. The additional suspended sediment can them settle and suffocate the young thalli (Oliveira *et al*. 2000). Thalli transplants can be damaged or loosen from their substrate during transportation to the cultivation site. Finally, epiphytic growth and herbivorous presence can be particularly high in bottom culture, it can be difficult to mitigate and can dramatically reduce the quality of the crop (Oliveira *et al.*, 2000).

Suspended Cultivation

Suspended cultivation is attained by through the attachment of the seaweed to a ropes, lines, floating rafts, and nets or enclosed in cages. The substrate is then suspended at the appropriate height within the water column. The main aims are to maximise irradiance, and mitigate tidal influence, provide an increased circumferential water flow around the thallus, and restrict benthic grazers. The methods are generally more complicated and require more maintenance, and higher set-up costs. However, the systems are generally considered low technology. Suspended cultivation overcomes the issue of insubstantial or inappropriate substrate and local depth variations as seen found with bottom culture. However, there are disadvantages of suspended cultivation. Firstly, Santelices and Doty, (1989) noted that suspended *Gracilaria* crops tended to be more susceptible to epiphytic activity, increased grazing by fish species, and invertebrate species that cannot thrive in benthic conditions. Secondly, as with bottom culture, the systems tend to operate under intensive labour. This is not the case with some rope based systems, whereby, the lines are inoculated with spores, and strung out as required. Thirdly, there is an increased conflict with users of the water body (Critchley, 1993). Finally, suspended cultivation is more susceptible to strong wave action and currents.

Raft Culture

Multiple species are currently cultivated with the raft system, species such as *Undaria, Macrocystis, Laminaria, Gracilaria, Porphyra, Ulva* and *Enteromorpha* (Santelices, 1999). Two types of rafts are used: fixed rafts (those that “float” below the water surface) or floating rafts (those that float above the water surface) (Oliveira *et al*. 2000). The light attenuation coefficient is the primary factor to consider with raft culture. Too little irradiance is a limiting factor to the growth, whereas, too much sunlight can be detrimental to growth and thalli integrity (Critchley, 1993). The advantages of using floating rafts opposed to fixed rafts is that tidal level changes do not have an influence on light levels, and deeper water or higher tidal variances are less of an issue in terms of infrastructure.

Semi-Suspended Cultivation

With semi-suspended cultivation, ropes or netting is then suspended between the poles, which are attached to the substrate; this fixed position faces the net surface parallel to the surface. The primary gain of semi-suspended cultivation is that the nets are set at a particular height so the crop is exposed to air during low tides. This exposure mitigates epiphytic growth and can reduce grazing, the degree exposure is increased as the algae is suspended directly in air, as opposed to ling prone on the substrate, this extra exposure will further enhance the epiphytic and grazer pressure, with minimal damage to the algae. Santelices, (1999) recommends this system for the cultivation of *Ulva*. A variant of the semi-suspended system is to have a diver suspend a pre-inoculated line with macroscopic thalli, the line is stretched under tension between stakes. Thus, the line is suspended just above the sea floor (Oliveira *et al*. 2000).

Basket Culture

Basket cultivation is primarily found in South-East Asia. A variation of this technique is being tested for *Ulva lactuca* in South Africa (Robertson-Andersson, pers. comm). The algae thalli are placed free floating in baskets or net bags, these are grouped together to form floating rafts. With cage culture, fertilizer is placed in the basket, in a slow release system, in order to enrich the immediate vicinity of the alga with additional nutrients.

Long Line Cultivation

Long lines are constructed by suspending natural or synthetic lines between buoys. The lines are positioned perpendicular to prevailing currents. The lines can be pre-inoculated with spores or individual thalli prior to deployment, or have plantlets individually inserted into the ropes as the lines are being deployed. This cultivation method has the advantage over more rigid raft forms in locations with strong currents, heavy wave action or regular storm events.

Seaweed cultivation is presented as an environmentally beneficial industry with little impact. However, large scale monocultivation of algae causes detrimental changes within the surrounding ecosystem. The major changes are the decrease in irradiance levels, heavier sedimentation, reduced water motion and wave action, a dramatic reduction in artificial and natural nutrient levels, and the increase in ecological duress as the increase in human activity and operating mechanisms (Titlyanova and Titlyanova, 2010). The next set of systems negates these detrimental influences through the removal of their presence from the open water and into an artificial or enclosed water body.

Semi-Closed Systems or Land-Based Cultivation

Together with the reduced marine environmental impact, land-based systems also reduce many of the issues with open water seaweed cultivation. These issues include, epiphytism, grazer impact, poaching, weather conditions and marine hydrodynamics (Shpigel & Neori, 1996). The principle advantage of semi-closed and land-based systems is that overall control is possible through integration of all system components. In land-based systems the nutrition is artificially introduced to the seawater. This is via artificial fertilization and or as a result of the co-cultivation of marine heterotrophs. In addition, incoming water can be screened for pathogens, pollutants, and competing or detrimental species (Shpigel & Neori, 1996). Finally, the basis behind this investigation, utilisation of seaweed removes the excess nutrients from the effluents, whereby the treated water can then be recirculated or released back into the environment with little adverse impact and in accordance within local governance standards. The significance of this is that the excessive release of nutrients from many aquaculture facilities often prohibits the licensing of aquaculture productions (Shpigel & Neori, 1996). Land-based cultivation systems include pond and tank cultivation, raceways, and spray cultivation.

The primary drawback to these methods of cultivation is one of economics and energy consumption. Obstacles against the development of these systems include, but are not limited to, high initial infrastructure costs, the higher level of technology result in increased maintenance and increase skill level of the labour force, the utilisation of terrestrial property for marine based operations, the transportation of water, both to the facility and in-situ, pipe infrastructure, aeration, temperature fluctuations, and filtration of the seawater

Pond Cultivation

Pond cultivation can be divided into intensive and non-intensive practices. Non-intensive pond cultivation is one of the oldest forms of aquaculture. Non-intensive systems generally include simple holes, earthen banked ponds, or natural lagoons. These are uncovered with no artificial aeration system. The ponds are generally rectangular, but can be of any shape. Pond depth is dependent of the species chosen for cultivation. The main problems of non-intensive pond cultivation are the burial of seaweed in oxygen poor sediments. Wind can cause the thalli to bunch up, which can reduce growth rates due to competition. The shallow nature of the ponds and large surface area can generate large fluctuations in temperature and salinity. Excessive growth of epiphytes and grazers can be an issue for thalli damage and competition for space, light, and nutrients. The low level of water motion can lead to reduction in oxygen levels, low stock rotation and settlement, and amplify the temperature and salinity fluctuations and gradients (Boyd, 1990, 1998).

However, with intensive cultivation, the ponds generally consist of a concrete structure with a water agitation system (Friedlander & Levy, 1995). The intensive cultivation of free-floating seaweeds has developed over the last three decades, and started with the cultivation of *Ulva* in the U.S.A (Hanisak and Ryther, 1984). The advantages of the intensive system is the high yield potential, the additional control over environmental factors, the mechanization of operation systems to reduce labour intensity, and the ability to use seaweed ponds as an inorganic extractive component for effluent discharge. The disadvantages are the initial infrastructure costs, the additional energy costs, and the maintenance involved. Excessive epiphyte growth has been identified as a major problem with intensive pond culture (Friedlander & Levy, 1995). Integration within the ponds of select herbivorous species can mitigate epiphytic growth (Oliveira *et al*. 2000).

Tank Cultivation

Tanks can be constructed from a variety of materials, fiberglass, treated wood, rubberised (manufactured density board), concrete, or PVC plastic. Size ranges from tens of litres to thousands of cubic meters (Oliveira *et al.* 2000). The design of the tank is important to maximise efficiency. The main considerations for tank design are size, water movement facilitation, ease of construction (economic and material constraints), and in-built infrastructure requirements. Ideally, the tank design should allow for a V or U-Shaped bottom with an aeration system, this facilitates more efficient water movement, and reduces the volume of “dead zones” within the tank (Vandemeulen, 1989).

All environmental conditions can be controlled with tank cultivation; this allows for the production of high yield crops regardless of temporal fluctuations. The efficiency of these systems is dependent on the design, the complexity of the infrastructure including the maintenance, and the input of energy. As a result of the control attained with these systems, these systems are the most expensive. The economic viability of intensive tank cultivation is the main obstacle with land-based aquaculture, the value of the product has to justify the initial outlay and continued maintenance costs. The low values of the majority of algal species cannot currently warrant commercial scale cultivation (Hanisak & Ryther, 1984; Critchley, 1993; Oliveira *et al.* 2000). The main exception, and justification for the high costs associated with tank cultivation, is the value added benefit of IMTA. The monetary value of nutrient mitigation via algal uptake has only recently been acknowledged and quantified. For example in Denmark, the value of nitrogen mitigation is $44 per kg (nitrate) (Holdt *et al.*, 2006).

Raceway Cultivation

Raceways are another form of intensive land-based cultivation. The structures are basically elongated tanks or ponds. Generally shallow as with ponds, they allow excellent irradiance capabilities and rapid water movement. The flow rate of the system is determined by the species, stocking density, water temperature, and fertilisation strategy. The primary mechanism to regulate water flow is through paddle wheels, and efficiency of inflow and discharge systems. An issue for raceways is the disparate concentration of nutrients within the system, with decreases in nutrient concentration as a function of increased distance from nutrient influx. Another problem can arise with excessive flow rates, which can lead to excessive "clumping" of algae (Shpigel *et al.* 1997). The additional length of raceways allows higher total removal efficiency, as opposed to tanks or ponds. The reduction in crop yield is outweighed by the increased nutrient removal capacity which is the principle aim of IMTA. Raceways have been successfully integrated in IMTA systems (Lapointe *et al.* 1976; Ryther *et al.* 1978; Shpigel *et al.* 1997). A variation on the traditional raceways is the “double-ended D” raceway. These systems have increased water retention, which results in more effective nutrient removal.

Spray Cultivation

The concept behind spray cultivation is to maximise light absorption, gaseous exchange, eliminate epiphytes and grazers, and reduce water requirements. The thalli are spread onto nets, which are then suspended over a container that collects the sprayed with nutrient rich water before it is returned to a reservoir. However, the insufficient diffusion medium can result in reduced gaseous exchange, and reduced nutrient uptake (Lignell *et al*. 1987). Another problem is the force of the spray can cause the thalli to clump on the nets, which results in shadowing (Robertson-Andersson, *pers. com*.).

1.7 Wolf Eels, *Anarrhichthys ocellatus*: Introduction

Wolf-Eels, *Anarrhichthys ocellatus*, are a Northern Pacific species of Wolf-Fish. Although well-known very little research has been carried out on this species. Adult *A. ocellatus* inhabit rocky reef areas in shallow water to depths of 250 metres (Eschmeyer and Herald, 1983). *A. ocellatus* is a territorial species that inhabits crevices within the reef, the dens are defended and inhabited for indefinite periods until either outgrown, or forced out by competitors (Armstrong, 1996). Juveniles are pelagic for approximately the first two years (Love, 1996). The wild diet of *A. ocellatus* consists of crustaceans, molluscs, fish and echinoderms (Eschmeyer and Herald, 1996). Males can reach sizes of 2.4 metres in length, and attain weights of 18.6kg (Love, 1996). In British Columbia, there is no legal fishery for *A. ocellatus*. However, due to their sedentary and predatory nature, they are easy targets for poachers and as by-catch. *A. ocellatus* are considered a threatened species (Love, 1996). Behaviour, territorialism, and interactions with recreational SCUBA divers have increased the profile of *A. ocellatus* as a key reef species in the diving industry. There is recent ongoing research at Vancouver Aquarium and other research facilities into the development of *A. ocellatus* as a potential aquaculture species. Cultivation of this species will ensure the protection of wild stocks, and hopes to conserve conventional eel stocks through the provision of similar cuts of very palatable meat.

1.8 In-Situ Optical Nitrate Analysis

Current literature in regards to uptake nutrient capacity of *Ulva* has previously attained data by analysis of tissue sample composition, and laboratory testing of water samples. These studies have fully documented the nutrient uptake capacity of *Ulva*. This study aims to utilise a relatively new form of technology in order to determine the effectiveness of the technology for future studies of this nature. Johnson and Coletti, (2002) describe the sensor and the reasons for the development of the device.

> *"Satlantic's ISUS V3 nitrate sensor as a real time, chemical free sensor designed to overcome the traditional challenges associated with reagent-based nitrate analysis*

> *in aquatic environments. The ISUS technology uses advanced UV absorption technology to provide accurate nitrate concentration measurements in real-time.”*

1.9 Research Approach and Questions

Based on previous successful attempts and failures with *Ulva* cultivation around the world, a multifaceted research approach aims to explore the feasibility of cultivation of *Ulva* within Canadian West coast IMTA systems.

1. Cultivation experiments of wild local *Ulva lactuca* in a recirculating aquaculture system. Wolf Eel, *Anarrhichthys ocellatus* effluent will be the principle fed component and provide the inorganic nutrient load.
2. Real time optical nitrate uptake analysis of *Ulva lactuca* to analysis nitrate uptake capacity.
3. SWOT analysis of the potential for the use of *Ulva* as an inorganic extractive component within a West coast IMTA system.

Chapter 2 - Growth and Nutrient Uptake Experiments

Introduction

2.1 Ulva Cultivation Parameters

The accumulation of investigations over the last few decades on the cultivation of *Ulva* and other macro-algal species (Duke *et al*. 1986, Sand-Jensen *et al*. 1988, Duke *et al*. 1989, Friedlander *et al*. 1990, Lüning, 1990 & Critchley, 1993, Taylor *et al*. 2001, Kalita and Tytlianov, 2003, Malta *et al*. 2003, Msuya and Neori, 2008, and Mantri *et al*. 2010, Vaibhav *et al*. 2010, Mvungi *et al*. 2012), has identified the key biological and environmental parameters conducive to the efficient tank cultivation of *Ulva*. These include: irradiance levels and medium attenuation coefficient, system aeration capacity, temperature range, water volume exchange rate, pH variation, stocking density, the presence of epiphytes and marine herbivores, and finally, nutrient concentration and constitution (Robertson-Andersson, Pers. comm.) Correct identification and manipulation of these parameters determine the maximum growth rate, crop yield, and quality of the *Ulva* produced, as with the creation of artificial ecosystems, the full understanding of the complexity of the interactions between all variables may never be achieved. However, the aim is to generate conditions whereby the maximum growth rate, nutrient uptake, yield, and crop quality can be achieved. For example, the simple decrease in CO_2 and O_2 capacity of water as a function of temperature increase. Successful *Ulva* cultivation requires a minimal gaseous exchange, whereas a higher temperature within the algae`s biological range is required for maximum physiological activities, and growth rate.

2.1.1 Irradiance Levels and Medium Attenuation Coefficient

The photobiological responses of marine algae demonstrate a broader physiological diversity because of the variation in photosynthetic pigments in comparison with higher plants. This is presumed to have evolved due to the greater variation in natural light conditions (Lopez-Figueroa and Neil, 1989). Generally, Chlorophyceae and Rhodophyceae have broader tolerances to temperature and light variation, than do Phaeophyceae (Mathieson and Dawes, 1986). Many algal species have the ability to adapt to low irradiance conditions by increasing light absorption to the maximum attainable (Markager, 1993). *Ulva* shows a remarkable ability to cope with highly variable irradiance levels, such as that which occurs in the intertidal zone of a temperate coast (Sand-Jensen, 1988). *Ulva* also has the ability to photo acclimatise within days to lower light levels and can to some extent maintain growth rates (dependent on the total decrease in irradiance. This physiological trait is particularly useful when high densities can promote self-shading, (Sand-Jensen, 1988, Vandermeulen and Gordin, 1990, Malta *et al*. 2003). The physiological responses of *Ulva* to temporal variations in light include, a variation in light harvesting pigment concentrations and their ratios, and the change in the activities of Calvin Cycle enzymes, particularly ribulose biphosphate carboxylase-oxygenase (RuBPCase) (Hoek, 1995). The light harvesting pigment concentrations and accessory pigment typically increase as light levels decrease. Conversely, the RuBPCase levels increase with light (Duke *et al*. 1986).

As a result of the effluent attenuation co-efficient and the maintenance of a minimal light supply, a key factor to remember when considering the irradiance levels within the tank system is depth. Shallow tanks have high light intensity; however, if combined with high temperatures can cause the tank water to exceed the safe cultivation range. Conversely, in winter shallow tanks maybe prone to lower temperature as a greater portion of the tank water is exposed to the air. Furthermore, in colder latitudes, there may also be the possibility of freezing. However, cooler climate *Ulva* varieties are adapted to cold temperatures, and whilst the crop will not be lost. The productivity will be dramatically reduced or cease altogether (Sand-Jensen, 1988). In tanks that are too deep, light may become a limiting factor, the depth is a function of the light attenuation coefficient of the growth medium. The majority of tank cultivation depths are designed within a range of 50-100cm (Robertson-Andersson, Pers. Comm., Ryther *et al*. 1975, Shpigel *et al.* 1993, Neori *et al*. 1998, Van Khoi *et al.* 2011, Abreu *et al*. 2011.

Light becomes a limitation factor for *U. lactuca* below 2.5 µmol m-^{2}s^{-1}, as *U. lactuca* loses absorption efficiency and cannot function fully at a cellular level (Sand-Jensen, 1988). Sand-Jensen (1988) study also suggests that light saturation for the growth of *U. lactuca* occurs at 55 µmol m-^{2}s^{-1}. There is some species specific variation in optimum irradiance absorption levels. For example, *U. curvata* and *U. rigida* display optimum growth at 18µmol m^{-2} s^{-1} and 54µmol m^{-2} s^{-1} respectively (Taylor *et al*., 2001). Previous investigations have used artificial cool white light sources to cultivate Ulva with a 16:8 light:dark cycle (Ale *et al*. (2010), Khoi and Fotedar, 2011). In the cultivation of *U. pertusa* under various light conditions, broadband isoquantic red light (RR) (600-700nm) was found to be insufficient to support all metabolic functions, broadband isoquantic blue light (BR) (400-500nm) supported growth and all metabolic functions (Muthuvelan *et al*., 1998). The use of white light (WR) culture compared with broadband isoquantic red and blue light cultures showed increased growth (specific growth rates at WR, BR, and RR were 8.6, 2.15, and 1.2 %, respectively) and nitrogen uptake (WR, BR, and RR were 42.1, 27.0, and 16.7 %, respectively). White light culture gave the optimal cell growth, integrity, and function Muthuvelan *et al*., (2001). Light intensity and periodicity in regards to algal cultivation is generally uncontrollable. Artificial lighting systems can be incorporated to provide additional lighting during low light conditions if a consistent year round crop is necessary (Sand-Jensen, 1988, Kosaka *et al*. 2008, Kalita and Titlyanov. 2011). Lignell *et al*. (1987) found that the cultivation of *Graciliaria secundata* in plexiglass cylinders with submerged and intensive top lighting gave significantly higher growth rates and yields in comparison with natural growth rates and other tank cultivation techniques. However, as with most energy consumptive technology and seaweed cultivation, the product value cannot justify the financial expenditure.

Another issue related to light is the stocking density. For maximum system efficiency, all the biomass in the system must be exposed to light. If the density is too great, no amount of aeration will provide sufficient crop rotation for even light distribution.

Regular harvests must occur, whereby optimal stocking densities are maintained in order to maintain illumination efficiency (McLachlan, 1991).

2.1.2 System Aeration Capacity

Unlike heterotrophic aquaculture (piscivores, invertebrates), the primary purpose of aeration in *Ulva* cultivation tanks is not necessarily to provide gaseous exchange (although it does). The goal is to provide water motion to keep the crop within the water column. Hanisak & Ryther (1984) observed in free-floating algal cultivation, water motion plays a key role in the prevention of thalli settlement. Later DeBusk *et al*. (1986), found that in non-aerated tanks, the algae floated to the surface as a result of O_2 bubbles formed during photosynthesis. The additional temperature and high light levels caused desiccation of the thalli. Aeration systems within tank cultivation maintain the necessary water motion to keep the thalli in circulation within the water column, and prevent floating and sinking of the thalli. DeBusk *et al*., (1986) study on *Ulva* cultivation found continuous aeration resulted in the highest yield. The lowest yields were recorded in tanks with no aeration. However, DeBusk *et al*. (1986) experiment also showed that growth rates could still be maintained with only twelve hours aeration. The reduction in aeration time results in a substantial reduction in energy and thus cost. Doty (1971) and Parker (1981) both observed that the provision of artificial water movement enhanced the algal growth rates. Parker (1981) went on to note that along with an increase in growth rate, ammonium uptake was increased, and nitrogen and carbon composition of the thalli was also increased. The reasons aeration stimulates growth is due to the breakdown of the diffusive boundary layers at the surface of the thalli, this increases the physiological uptake processes (Hanisak & Ryther, 1984). The aeration also results in crop rotation, which increases the exposure of individual thalli to light, and gives a more balanced distribution of irradiance levels to the entire crop (DeBusk *et al*. 1986).

Aeration has another benefit of providing mass movement within the tank, which causes abrasion between the algae itself, and the tank walls. This abrasion serves as a mechanism for epiphyte control (Hanisak & Ryther, 1984).

Ulva cultivation tank aeration systems do have drawbacks however. The installation and maintenance of aeration systems is an expensive initial and ongoing outlay. The increase in stocking density requires increased aeration to maintain the optimal stock rotation. Regulation of the aeration level has to be adjusted to suite the thalli size and stocking density to optimize energy consumption. Excessive aeration can damage the delicate thalli, however economic restraints generally prevent the use of excessive aeration due to energy consumption. Aeration can also increase dissolved O_2 concentrations, this results in an increase in the oxidative metabolism (Hoek, 1995). This can be neutralized with the use of CO_2 gas as the aerator medium.

2.1.3 Temperature Range

The broad distribution and habitat of the genera dictates that Ulva is adaptable to wide temperature fluctuations. The extent of the temperature fluctuations depends on the latitude and habitat. Physiological cell activity of the thalli is determined by temperature. Temperate regions experience the highest temperature fluctuations. Kalita and Titlyanov (2003) determined that in temperate waters, the vegetative growth rate of *U. fenestrata* reached optimal levels at 10°C. A further increase in temperature led to increased maturation of the thalli. At 5°C the growth rate was significantly reduced. Ulva can survive periods of extreme cold, below freezing for extended periods, (Sand-Jensen, 1988). The growth rate is negligible at low temperatures. Hence, the seasonal difference in *Ulva* production rates. Hiroake *et al* (2007) noted that an increase to temperatures exceeding 20°C led to almost instantaneous sporification. However, with the use of deep seawater continually pumped through a system at a temperature of between 11-14°C, maturation of the thalli was prohibited. In order to maintain optimal production, the water temperature must stay within the optimal range for vegetative growth for the particular *Ulva* cultivar.

2.1.4 Water Volume Exchange Rate

Water volume exchange (VE) is vital to maintain healthy production. The exchange renews vital nutrients and gases which reduce the diffusion boundary layer of the thalli.

Reduction of the diffusion boundary layer is necessary to maintain optimal uptakes rates (Bidwell, 1995). The exchange also removes waste products to reduce their diffusion boundary layer. Nutrient concentrations, pH, temperature, salinity, gaseous concentrations are all a function of VE rates. Water flow rate is a function of stocking density, nutrient uptake rates, and medium (seawater) influx composition.

However, within an integrated system, the extractive components must remove the excess nutrient influx from the fed component. Without the use of temporary storage reservoirs, the extractive component must deal with the nutrient concentrations at the rate output from the fed component. Robertson-Andersson (2003), found that 12 VE d^{-1} was adequate for growth, 20 VE d^{-1} was the best rate for a number of different sized tanks. However, a rate of 4 VE d^{-1} was insufficient to maintain the correct parameter balance and resulted in poor crop performance. The increase in VE rate has one major drawback. The larger the VE rate, the decrease in nutrient removal efficiency of the system. Many investigations have previously neglected to refer to, and record VE in tank based seaweed cultivation research.

2.1.5 pH Variation

The photosynthetic action of macro-algae within the marine environment increases the pH levels (Falkowski and Raven, 2007), this occurs when CO_2 balance is skewed negatively. The reduction in CO_2 leads to a reduction in Carbonic Acid (HCO_3) as a result of the reaction between CO_2 and water. The result is fewer positive hydrogen ions. However, large changes in concentrations are needed to alter the pH levels. These events can occur during the long light periods of the summer months when algae densities are high, water movement is reduced, and photosy,nthetic action is at a peak. *Ulva* displays an adaptation to pH fluctuation as it does with many environmental parameters (Mvungi *et al*. 2012). Although, the pH range is species specific, overall a pH of 9 and above inhibits *Ulva* performance (Kandjengo, 2000). The pH of a cultivation tank must be maintained within a smaller range at approximately 7 to 8.5 for optimal growth performance, particularly if the *Ulva* is co-cultivated with more sensitive organisms (Lobban & Harrison, 1997). Co-cultivation of *Ulva* within shrimp (*Penaeus latisulcatus*) tanks maintained a good pH balance. The shrimp monoculture results in a drop in pH, and the presence of *Ulva* balances the pH again. Slightly elevated pH levels are more acceptable within an aquaculture system than, acidic conditions, particularly in shellfish cultivation.

2.1.6 Optimal Stocking Density

Stocking density of the cultivation system determines the level of competition the individual thalli must endure for light, space, inorganic nutrients, and dissolved gases. Ergo, the density must not exceed a defined competition limit; maximum yield is attained through the optimal stocking density (Neish and Knutson, 1978, Pereira *et al*., 2008, Abreu *et al*., 2011). The primary factor in seaweed cultivation is light due to the dimensionality of the medium (McLachlan, 1991). The morphological nature of *Ulva* thallus lends itself to the close knitted formation of “*mats*” (Malta *et al*. 2003) wherein stacked layers of thalli cause “self-shading” to those below. Mat formation is also determined by aeration and water exchange rates. Obviously, growth of the stock results in higher stocking densities, in order to reduce the demand on the system and risk system inefficiency, regular harvests must be carried out to return the system to the initial optimal stocking density (McLachlan, 1991).

In commercial production, yield and growth rates can become competing functions of stocking density. In the case of IMTA, optimal stocking density can only be calculated once the primary goal of the extractive inorganic component is determined. Higher growth rates result from lower initial densities, in order to maximise the system's seaweed production. However, if nutrient removal is the main aim, initial and renewed stocking densities must be higher.

Financial constraints factor into developing the optimal stocking density. The tank depth, design, and aeration system all determine the maximum volume of seaweed that can be maintained in circulated suspension. An increase in density increases the energy requirements of the aeration system; for example, DeBusk *et al*. (1986) found > 6kg m^{-2} to be beyond the capability of an aeration system to maintain sufficient circulated movement.

The range of optimal stocking densities used in previous macro-algal cultivation systems varies, 1kg FW m^{-2} (Mysura *et al*., 2008), 2-6kg FW m^{-2} (Shpigel *et al*., 1993), 4kg FW m^{-2} (Bruhn *et al*., 2011).

2.1.7 Epiphytes, Competition, and Marine Herbivores

The presence of non-productive species within an aquaculture system, leads to competition, crop degradation, and disease. Epiphytic growth on various substrates within the system, including the thalli of the crop alongside the presence of competitive algae within the water column leads to competition for light, nutrients, and space within the system. Within almost all aquaculture systems natural epiphytic growth can be beneficial to the nitrification process. To reduce costs, the specification of bead filters for a recirculation systems are based on the very premise of up to 40% in-situ nitrification by bacteria and micro-algal growth integral within the system (Aquaculture Systems Technologies, Pers. Comm.) However, within macro-algal cultivation, non-productive algal species compete for light, nutrients, dissolved carbon, increase the mechanical drag as epiphytic growth of the thallus, and also physical damage to the thalli surface through the penetration of rhizoids and the production of allelochemicals (Fletcher, 1995). Finally, presence of bacteria, viruses, and marine fungi can cause devastation to crops within aquaculture systems, as monoculture provides an unlimited host substrate, and the environmental parameters suitable for an outbreak are constantly maintained (Fletcher, 1995).

Solutions to mitigate the problem of non-productive species are determined by system design, input sources, finances, and intensity of the issue. Robertson-Andersson (2003), highlights three methods of mitigation, physical, chemical, and biological.

The physical method involves:

(i) Physical removal of the non-productive species from the host and/or the system. This method is very laborious and not cost effective.

(ii) Control the environmental parameters within the system to favour the cultivar or at a detriment to the non-productive species. Due to the physiological adaptability of *Ulva*, this can be achieved through the exposure of Ulva to air, via tank drainage or elevation of the thalli. Along a similar vein, flooding the system with freshwater can also help remove detrimental species. Robertson-Andersson (2003), achieved epiphyte control with shade cloths to reduce light levels, *Ulva* was less susceptible to lower light levels than the problem species.

(iii) Cleanse and sterilise system infrastructure prior to cultivation.

(iv) Screen cultivar input, especially if harvested from wild populations.

(v) Filter and screen water supply with biological, chemical, and/or physical barriers.

(vi) Maintain the optimal density and suspended circulation of *Ulva* to prevent epiphytic growth via thallus abrasion between individuals and tank wall (Lignell *et al*. 1987).

Ugarte and Santelices (1992), found the use of hypochlorite solution to sterilise the seawater and infrastructure prior to use. The chlorine compound then needs to be neutralized before the seaweed is added to the tank. Robertson-Andersson (2003), used Copper chloride as a preventative method for elimination of *Enteromorpha* and

Ectocarpus outbreaks within the system. However, the chance of contamination of the production crop remains the largest risk in the use of chemical methods.

The biological method of non-production species control involves the integration of isopods, amphipods, caprellids, gastropods, and opisthobranchs, and herbivorous fish species (Brawley and Fei, 1987) within the cultivation tanks. These species tend to feed naturally on the epiphytes amongst the beds of the host seaweed species. Ideally, the cultivation of commercially valuable species as a herbivorous epiphytic control system would generate extra revenue.

2.1.8 Nutrient Concentration and Composition

Nutrient concentrations are the fundamental parameters that determine the optimal growth rates of *Ulva (*Lobban and Harrison, 1997). Numerous investigations have highlighted the importance of nutrient concentration and *Ulva* production, many with particular reference to integrated cultivation systems (Ryther *et al*. 1975, Harlin, 1978, Muthuvelan *et al*. 1998, Shpigel et al., 1993, Jimenez del Rio *et al*. 1996, Copertino *et al*. 1998, Neori *et al*., 1998, Pinchetti *et al*. 1998, Naldi et al. 2002, Msuya *et al*. 2008, Ale *et al*. 2010, Liu *et al*. 2010, Yokoyama *et al*. 2010, Van Khoi *et al*. 2011).

De Boer, (1981) lists the range of nutrients and trace elements required for marine algal cellular function. C, H, O, N, P, Mg, Fe, Cu, Mn, Zn, Mo, S, K, Ca, V, Se, Si, Cl, Co, and B are all essential elements required for various metabolic functions within the cells. In natural seawater or artificial aquatic medium, such as Provasoli's E.M. Medium, the majority of essential elements are present and non-limiting, however, nitrogen, phosphorus, and carbon can be in limited supply Hansiak (1983), particularly within intensive cultivation. The limitation of one of these critical elements will dramatically reduce productivity. Björnsäter & Wheeler, (1990) provided evidence that the addition of nitrogen and phosphorus to nutrient poor waters significantly increased algal growth rates.

Nitrogen the primary focus of the majority of nutrient uptake based investigation (Ryther *et al*. 1975, Harlin, 1978, Muthuvelan *et al*. 1998, Shpigel et al., 1993, Jimenez del Rio *et al*. 1996, Copertino *et al*. 1998, Neori *et al*., 1998, Pinchetti *et al*. 1998, Naldi et al. 2002, Msuya *et al*. 2008, Ale *et al*. 2010, Liu *et al*. 2010, Yokoyama *et al*. 2010, Van Khoi *et al*. 2011). In nature, the general trend is algal productivity is a function of nitrogen supply, however, other parameters factor into the equation.

Seaweeds differ in their capacities for using different sources of nitrogen. Nitrate first has to be reduced by the algae before it can be utilized in the cells. For this reason, ammonium uptake is better in some species as it can be used immediately in the plant metabolism and can be taken up both actively and passively (DeBusk *et al*. 1986). Neither light intensity differences nor temperature fluctuations affect the uptake of ammonium in Ulva sp. (DeBusk *et al*. 1986; Duke *et al*. 1986b; Duke *et al*. 1989; Vandermeulen & Gordin, 1990), nor does changing the stocking density (Cohen & Neori, 1991). Ammonium uptake is regulated by tissue nitrogen and DIN (Duke *et al*. 1989).

Nitrogen limitation within an aquatic system alters the biochemical composition and cellular activity of the algae. In terms of macro-algal cultivation for consumption this can be detrimental. Nitrogen limitation results in increased Carbon:Nitrogen (C:N) ratio, due to the increase in carbohydrate synthesis (Lahaye *et al*. 1995). A higher protein content is preferred, thus the provision of unlimited nitrogenous compounds leads to more protein synthesis, resulting in a lower C:N ratio. This in turn increases the nutritional value of the product (Pinchetti *et al*. 1998). Pinchetti *et al*., (1998) also found that nitrogen enriched water causes an increase in the photosynthetic capacity of *U. rigida*. The same study highlighted the fatty acid composition of the thalli varied with nitrogen availability. The nitrogen enriched seawater samples showed a significantly higher proportion of poly-saturated fatty acids (PUFA), opposed to saturated and mono-saturated fatty acids (Pinchetti *et al*. 1998). Nitrogen enrichment (for example, fed component of IMTA effluent) of cultivation medium increases the nutritional value of the seaweed cultivated.

Harlin (1978) identified the high nutrient uptake capacity of marine algae. Rhyther *et al*. (1975) proved the capability of *Ulva* as an extractive inorganic bio-tool. Nitrogen uptake capacity of seaweeds (Vmax) is a direct function of Surface area : Volume (SA:V) ratio. However, nitrogen storage capacity varies approximately inversely with SA:V (Rosenberg & Ramus, 1989).

Ulva has one of the highest nitrogen uptake capacities of any marine macro-algae (Ale *et al*., 2011). The investigation by Naldi *et al*. (2002), supports Rosenberg and Ramus

(1989), in that Ulva has a higher nitrogen uptake capacity in comparison with other algal species due to the higher SA:V ratio, nitrate release (storage capacity) is also a function of the SA:V ratio and is significantly higher in, for example *Gracilaria pacifica* than in *U. fenestrata.* However, Rees (2003) suggests that there is no correlation between the half-saturation constants (K_m) and the SA:V ratio. Taylor *et al*. (1998) presents evidence that the uptake of ammonium differentiates dependent on geographical location and environmental factors opposed to SA:V ratio. Contrary to this Teichberg *et al*. (2010) found that nitrogen and phosphorus was universally a limiting growth factor, and that enrichment of nitrogen and phosphorus elicited a growth response in *Ulva* species.

In order to optimise the nutrient removal capacity of the extractive component, a starvation period is necessary, to prevent the saturation of the thalli with nutrients (Neori *et al*. 1998, 2003). *Ulva* uptakes nitrogen far in excess of that required for growth (‘luxury’ uptake), the storage capacity or starvation period can be as much as 10 days (Fujita, 1985). Consequentially, throughout the duration of the starvation period, a secondary support nutrient uptake system is required (i.e. another cultivation tank run in parallel) to maintain the integrity of the system.

The investigation by Ale *et al*. (2011) shows that *Ulva* has a preference for ammonium as a source of nitrogen. Ammonium is a reduced state inorganic nitrogen based compound as opposed to nitrate. Thus, the uptake and metabolic assimilation of ammonium is more efficient. Nitrate based nitrogen sources require a reduction nitrate to nitrite with nitrate reductase enzyme. The nitrite then requires further breakdown to ammonium, which requires more ferrodoxins, therefore more energy (Ale *et al*. 2011). The ammonium preference of *Ulva* increases its capability of a candidate for IMTA systems, as ammonium accumulation within an aquatic system (open or closed) can result in toxicity towards various aquatic organisms (Randall and Tsui, 2002). Ammonium is a product of a reaction between the water and ammonia released by the fed vertebrate component of an aquaculture system. The ideal form of integration and efficiency of removal is demonstrated in a study by Khoi and Fotedar (2011) whereby *U. lactuca* is directly grown with shrimp *Penaeus latisulcatus*. The ammonium is directly taken up by the thalli within the tank. This allows maximum nutrient concentration for the thalli, and reduces the chances of toxicity from ammonia-N compounds from the effluent of the shrimp.

Msuya and Neori (2008), found the performance of *U. lactuca* is a primarily a function of nutrient concentrations. Under nitrogen limitation, other factors such as temperature, salinity, illumination, agitation and aeration become more influential for optimal growth. Biomass yield and protein content depended only on nutrient loading. Indoor low light irradiances were sufficient for good growth with high nutrient loading. Increasing the nutrient load also increased the protein and phosphorus content, and decreased ash content proportionately.

Like nitrogen, phosphorus is an essential element that is required in relatively high amounts to maintain optimal growth. The uptake of phosphates in Ulva is lower than nitrates and ammonium (Rees, 2003), however, under highly intensive cultivation conditions, reduced phosphate levels can become the factor that limits optimal growth.

Carbon is usually dealt with separately with regard to nutrient uptake kinematics in seaweeds. Gordillo (2012) believes this to be a retrospective approach linked to studies on terrestrial based plants, as carbon is taken from the air, and other nutrients through the root system. However, carbon sequestration in seaweeds is similar to other nutrients, in that there are specific transporters in the cell surface determined by the diffusive boundary layer, intracellular carbon storage (i.e. photosynthesis can be continued in the absence of dissolved inorganic carbon (DIC) up to six hours in *Ulva* (Kock, 1993), and its utilisation requires photosynthetic energy via the Calvin-Benson cycle and ribulose biphosphate carboxylase oxygenase (RuBisco) (Gordillo, 2012). The uptake of DIC via direct CO_2 or the dehydration of HCO_3^- is insufficient to maintain carbon demands (Gordillo, 2012). The majority of DIC need in algae is met through carbon concentration mechanisms (Giordano, 2005). The addition of CO_2 to cultivation systems is prohibitively expensive, and is reserved primarily for pharmaceutical grade microalgae cultures.

2.1.9 Salinity

As an intertidal genus, *Ulva* is capable of withstanding the broadest saline fluctuations (Taylor *et al*, 2001). Lobban and Harrison (1997), note that Ulva may withstand salinities as weak as 3ppt to as much as 115ppt. Ulva maintain high internal osmotic pressures, thus, water loss is reduced and turgidity is maintained (Loban & Harrison, 1997). This tolerance adaptation can be exploited as an epiphytic or grazer mitigation trait, the tanks can be periodically flooded with freshwater to increase the osmotic stress on less tolerant epiphytic and grazer species. In a study of *U. fasciata* by Mantri *et al*. (2010), optimal growth rates were achieved when salinity was maintained between 20 – 35ppt, significantly higher than reduced salinities. Salinities below 15ppt exhibited considerable reduction in growth rates.

2.2 Site Description

The outdoor aquatics unit at the University of Victoria, Victoria, British Columbia, Canada, were the facilities used in this investigation. The facility has a 68’000 litre capacity saltwater recirculating aquaculture system. It was decided to run the experiments at the facility due to the high level control capability over the experiments. Utilisation of the in-house facility also supports the continuation of the useful research facility.

The facility is centrally located within the University of Victoria campus; the facility has no direct pipeline to source natural seawater. Fresh natural seawater is collected by truck from Cattle Point, Victoria, B.C. as required. Existing and new seawater is mixed and held within a large holding reservoir during regular system top-ups. Initially water is pumped through two propeller washed bead filters, PBF-10 Aquaculture Systems Technologies, New Orleans, Louisiana. Each of these filters allow for nitrification of 100 gallons per minute of water, the system allows for 30% in-situ nitrification. In-situ nitrification is the nitrification that occurs naturally on the tank walls, within the pipework, and the water column. Water is then passed through a foam (protein) fractionator, RK2 Systems (PE Series), San Diego, California. The system is also equipped with an Ozone delivery system, RK1000MG, RK Systems, San Diego, California. The water is passed through a 600W UV Steriliser, COM6600HO, Emperor Aquatics, Pottstown, Pennsylvania. Finally, the sterilised seawater is passed through a temperature control system, Danfoss Chillers, Type RT, Mississauga, Ontario. The chillers are set to 10.5°C; the water entering the main tanks is maintained at a temperature of 10.5 – 11.5 °C. Water is then distributed as necessary to the various tanks and aquatic systems.

The wolf-eel, *Anarrhichthys ocellatus* holding tanks are 4’500 litre circular fibreglass constructs. The stocking density of the juvenile *A. ocellatus* is approximately 250 individuals per tank, the body weight ranged between 180-220g ± 10 throughout the duration of the experiment. Lower irradiance levels are maintained in the tank. The twice daily feed regime consisted of 600g of 3.5mm Black Cod pellets, Taplow, Chilliwack, B.C., unless the animals are undergoing treatment. The majority of solid waste is collected in the settlement sump at the bottom of the tank. The remaining effluent water is normally discharged back into the recirculation system for treatment. Throughout the duration of the investigation, effluent water from the *A. ocellatus* tanks is piped untreated directly into the *Ulva* tanks.

2.3 Experiment Design

The *Ulva* growth tanks were custom equipped U-shaped 680 litre opaque polyethylene tanks, water depth was 60 cm, to allow sufficient light attenuation (figure 3.1), manufactured by Premier Plastics, Delta, B.C. Two inch diameter regulated inflow pipe positioned two inches above the three inch outflow to allow for smooth through-flow action, and prevent overflow. Additional inflow water drop increased passive water movement within the tank. The outflow was covered by a custom built extended mesh trap to prevent *Ulva* escape and blockages. Six *Ulva* tanks were built, and plumbed to allow equal flow rates to each tank, and positioned

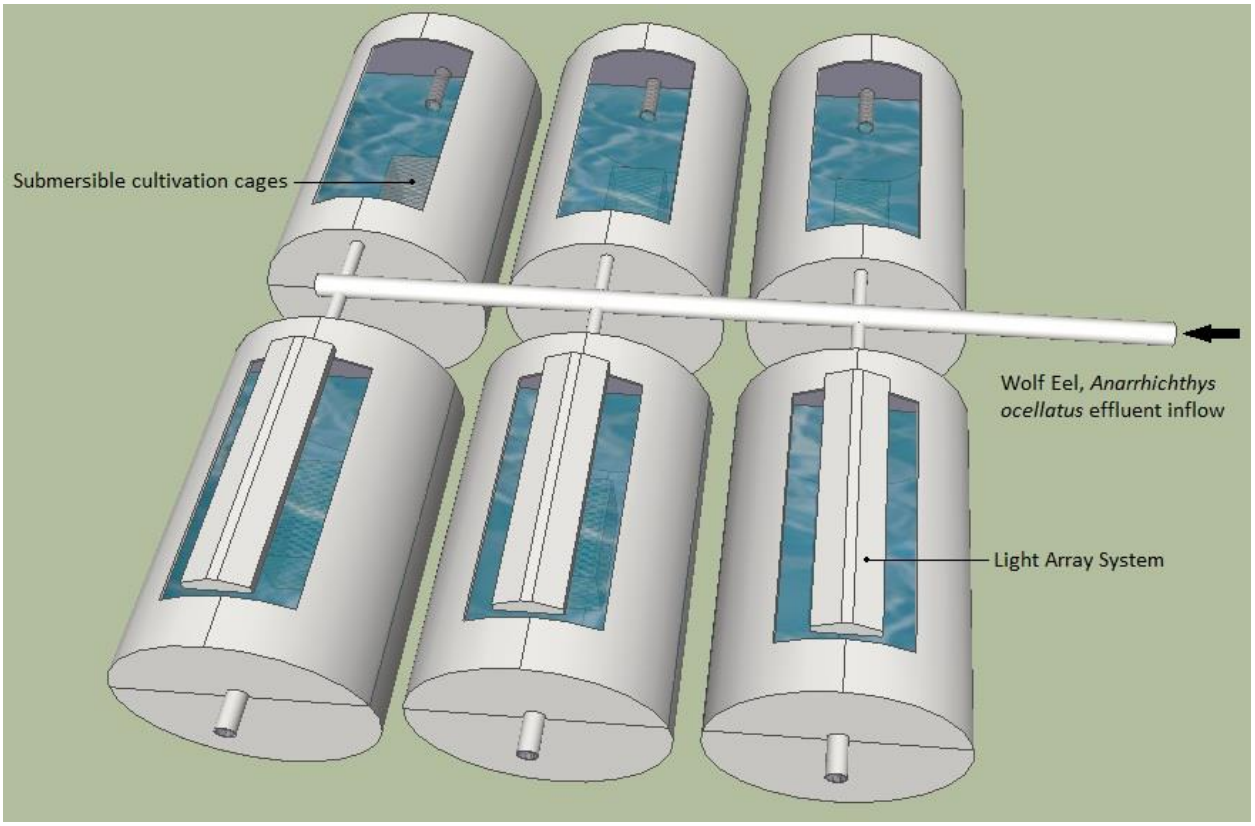

Figure 2.1. Growth experiment system design for the cultivation of *Ulva lactuca* in a recirculation aquaculture system with Wolf Eel, *Anarrhichthys ocellatus*.

within the facility to allow maximum natural light. The regulated inflow could be altered in accordance with the required water volume exchange. The maximum flow for all tanks was set at 10 l $^{-1}$min^{-1} or approximately 24 complete volume exchanges per day. The maximum flush-time for all six tanks equated to the necessary water exchange required in the *A. ocellatus* tanks to maintain integral water quality parameters. Reduction in *Ulva* tank inflow rate meant excess *A. ocellatus* effluent was discharged back into the facilities recirculation system.

The lighting array systems were designed for three of the six tanks. Lighting ballast systems for each tank included four Philips F32T8 32W full spectrum bulbs, Philips, Canada. The arrays were suspended nine inches above each of the designated tanks. The lighting system gave off no discernible heat. Temperature taken at water surface did not vary between the illuminated and natural light tanks. Additional artificial light intensity was 60 µE/ m^2s^{-1} at the surface during non-daylight hours. Light attenuation coefficient within the tank varied in accordance with turbidity levels of the effluent water. The average light illumination at the bottom of the artificially illuminated tank was 8.4 ± 1.3 µE/ m^2s^{-1} during non-daylight hours. Natural daylight hours were measured with Apogee MQ200 Quantum Light Meter, Apogee Instruments, Apogee Instruments, Logan, Utah.

Tumble cultivation as described in Vandemeulen, (1989), and later in successful *Ulva* cultivation attempts by Dr. Robertson-Andersson in South Africa, is necessary for successful tank cultivation of free floating macro-algal species. The water movement causes the *Ulva* to circulate around the tank, reducing epiphytic growth through friction, and exposes all the *Ulva* stock within the tank to an even distribution of illumination and nutrients. In this investigation, tumble culture was generated by using a Hydor Seltz L-40 pump, HD10141, Hydor USA, Sacramento, California. A pump was positioned in the tank, and moved 740 gallons of water per hour. In the initial set-up, smaller pumps were used to reduce energy consumption; however, the decrease in water movement was insufficient to tumble a fully stocked tank.

2.4 Ulva Collection Site

The *Ulva* material was collected from the rocky intertidal area at the southern end of Willows Beach, Oak Bay, Victoria, Canada at various dates in June during low tidal periods. Oak Bay is located on the south-eastern coast of Vancouver Island as part of the Salish Sea (Juan de Fuca Strait). Chihara (1969) noted that the intertidal area at this location was dominated by *U. fenestrata*. The material used in this investigation was morphologically identified as *U. fenestrata* (a heterotypic synonym of *U. lactuca*) (Guiry and Guiry, 2012). However, it was beyond the remit of this investigation to confirm the samples with molecular analysis. Possible contamination of samples with *U. californica, U. lobata, U. pertusa*, or *U. rigida*. The bio-geographical conditions of the intertidal zone of Oak Bay are ideal *Ulva* habitat, a mixture of exposed rocky substrate interspersed between sandy sediment. The Bay is relatively sheltered, with a tidal range up to between 1.7 - 4.2 metres (Fisheries and Oceans Canada, 2012). The water temperature for Oak Bay ranges between 6°C to 15°C throughout the year (Fisheries and Oceans Canada, 2012). Only healthy fronds with obvious basal portions were taken as growth samples. The collected samples were acclimatised in holding tanks for a couple of days in the recirculating system with effluent from the *A. ocellatus* tanks.

2.5 Growth Experiment 1

To investigate the variation in growth rates between the random thallus fragments cut from the apical region and the use of specifically cut basal portions of the thallus. The six cultivation tanks were set-up as previously described. Each tank had an inflow rate of 10 l min^{-1} or approximately 24 complete volume exchanges per day. As this experiment was carried out in July, artificial lighting was not set-up for the system. The initial stocking density of each cultivation tank was 1kg m^2 of acclimatised *Ulva lactuca* thallus fragments. Three of the tanks in the series were chosen at random and stocked with fragments of the basal portion of the thallus. The remainder were stocked with fragments of approximately the same size cut indiscriminately from any portion of the thallus. The fragments were kept in suspension with an aeration system provided by perforated bubble-tubes along the central base of the tank. All the fragments were collected and removed from each tank every three days. The seaweed mass was centrifuged in a domestic salad spinner for 60 seconds to remove any superficial surface water and to mitigate damage to the thallus. The entire mass was weighed on a precision balance Ohaus Pioneer PA214, Parsippany, NJ. The trials were continued for three weeks.

2.6 Growth Experiment 2

To determine the difference in growth rates between artificially illuminated tanks and naturally lit tanks in winter conditions. This trial was conducted in November. Light arrays as previously described were positioned at random over three of the tanks. The light system was set for an extended day length period of 16 hours. Due to the low initial stocking density and the cage set-up, no additional aeration system was required in this investigation. Each tank had an inflow rate of 10 l min-1 or approximately 24 complete volume exchanges per day. For this investigation an alteration to the cultivation technique used in growth experiment one, was applied. Fifty 30mm square fragments were precisely cut from the basal portion of healthy acclimatised thalli with a scalpel. The fifty fragments were placed within a plastic mesh cage (mesh size = 1cm^2), cage dimensions were 460x260x270mm. Every three days the fragments were removed and individually measured with calipers.

2.7 Nitrate Uptake Experiment 3

To investigate the viability to measure nitrate uptake rates of local *U. lactuca* strain in a temperate recirculation aquaculture system with the use of an in-situ real-time nitrate sensor, Satlantic ISUS V3 Nitrate Sensor, Satlantic, Halifax, NS. The sensor was calibrated prior to each deployment with de-ionized water as per the instruction manual. The sensor is powered by a 12V Deep Cycle Motive Power Battery, Battery Direct, Calgary, Alberta. The deployment experiments were conducted in December and January. Data collection began at 17:00 Pacific Time for each deployment, and collected data at 1 hour intervals for a period of 10 seconds throughout the 72 hour period. 10 litre glass containers were used for the nitrate sensor experiments and were kept outdoors in temperature baths regulating the temperature at 10°C. Each treatment was carried out

10 times, the mean for each treatment was recorded. Effluent from Wolf Eels was taken just prior to treatment to reduce the breakdown of Ammonia-N and other nitrification occurring in holding tanks. Effluent water was sieved through plankton net, (mesh size = 50 microns) to remove particulate matter to reduce interference with the sensor.

The sensor deployment treatments were as follows:

1. Control – No *U. lactuca* in tank.
2. Basal fragments of nutrient starved, vegetative *U. lactuca* stocked in tank at density equivalent of 3kg/m^3 (30g Wwt.).
3. Basal fragments of nutrient saturated, vegetative *U. lactuca* stocked in tank at density equivalent of 3kg/m^3 (30g Wwt.).
4. Fragments of matured thalli of *U. lactuca* (>50% thalli surface undergone maturation "*ghost tissue*" at a density equivalent of 3kg/m^3 (30g Wwt.).

As per Pederson (1994), nitrate uptake rate was calculated with the following equation:

$$V = [(S_0 \times vol_0) - (S_i \times vol_i)] \div (t \times B)$$

Where:

V = nitrate uptake rate (µm N gDW^{-1} hr^{-1})

S_0 = nitrate concentration at start (µm), S_i = nitrate concentration at end (µm)

vol_0 = water volume at start (litres), vol_i = water volume at end (litres)

t = time elapsed between samplings (hours)

B = dry weight biomass of *U. lactuca* (g)

Wet to dry weight ratio was found to be 7.44: 1 (±0.7). Initial nitrate concentrations varied between trials between 99 to 85µm.

2.8 Statistical Analysis

All growth rate data will be analysed with SPSS version 19.0 for Windows, IBM Software. Data will be tested for normality and homogeneity. The Sharpio-Wilk test for normality will be employed due to the relatively small sample size, and Levene's Test will be used respectively. Parametric and non-parametric analysis will follow as appropriate.

Chapter 3 - Results

3.1 Growth Experiment 1 - Basal and thallus fragment growth rates

Over the three week period the average daily growth rate for the basal fragments is 3.67gDW.m^{2}.d^{-1} ± 0.7 (Cf. 95% = 0.565), the average daily growth rate for the randomly selected apical portions of thallus is 1.00gDW.m^{2}.d^{-1} ± 1.7 (Cf. 95% = 1.358). Over a three week growth period a significant ($p<0.05$) difference has been identified between the basal growth rates and that of apical thallus fragments. However, within the first week, the average growth rates for basal and random thallus fragments were 6.5g DW.m^{-2}.d^{-1} ± 1.08 (Cf. 95% = 0.86), 8.51gDW.m^{2}.d^{-1} ± 2.25 (Cf. 95% = 1.8) respectively. There was no significant difference found between the first week growth rates ($p>0.05$), although, the p value 0.056 was extremely close to the critical value. A larger sample size may have resulted in a significant difference. The initial burst of growth (3 days) for both fragment types resulted in very high growth rates, of up to 17.43% specific daily growth rate. Figure 4.1 shows the average daily growth rates of the two fragments types and shows a clear pattern emerge to highlight the variation in fragment growth in terms of crop maintenance.

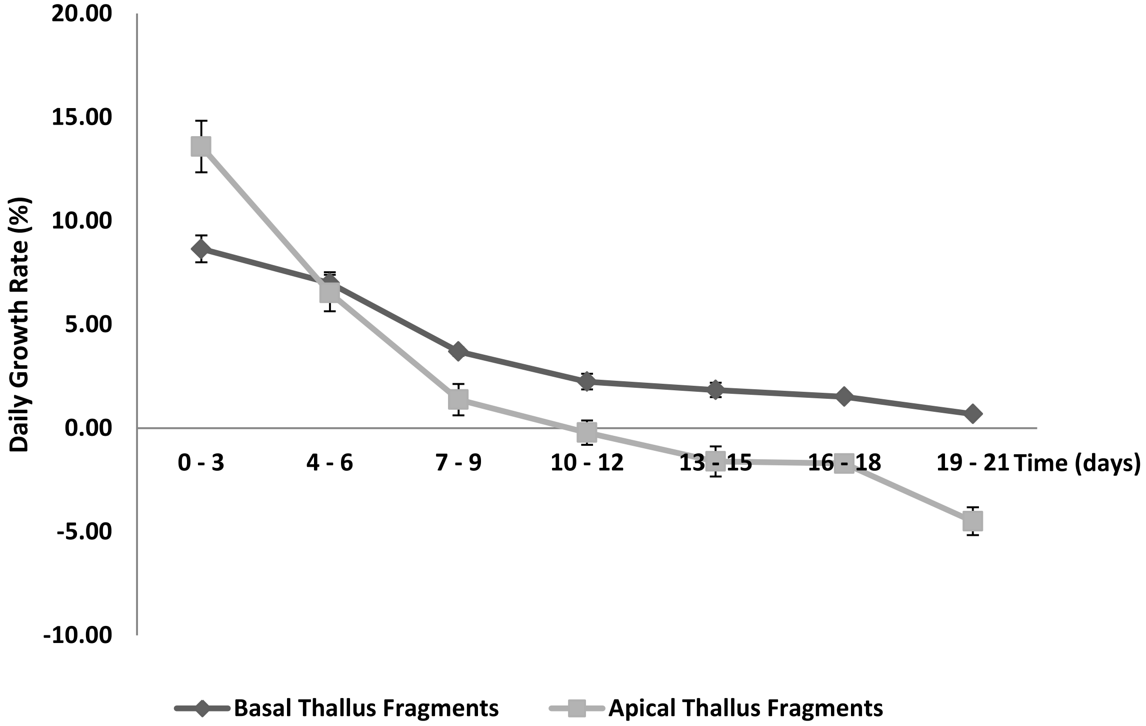

Figure 3.1. Average daily growth rates of basal and apical thallus fragments of *Ulva lactuca* grown over 21 days in a recirculation aquaculture system outdoors in July/August.

3.2 Growth Experiment 2 - Artificial illumination versus natural winter light

A maximum percentage daily growth rate of 4.26% was achieved under artificially illuminated conditions. However, no significant difference was found between the thallus growth rates for artificially illuminated tanks and naturally lit tanks for the first two week growth period ($p>0.05$, n = 150) (Fig. 4.2), although the p-values show a declining trend over the two week period. By the third week, the thallus growth in the artificially illuminated tanks is significantly greater than the naturally lit tanks ($p<0.01$, n = 100).

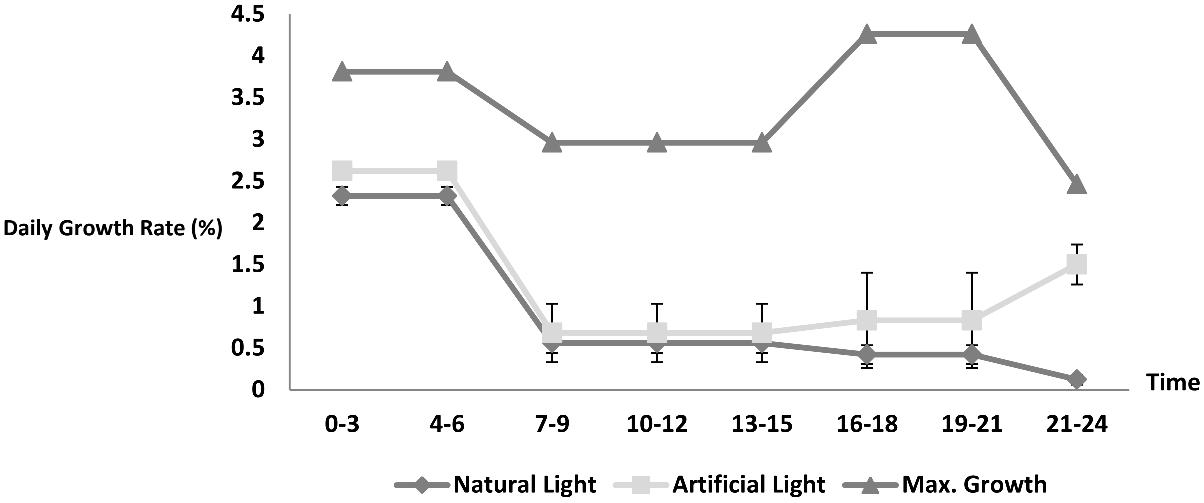

Figure 3.2. Daily growth rates of *Ulva lactuca* over 24 days in a recirculation aquaculture system outdoors in November/December. Variation in growth rate determined by the use of artificial lighting.

The growing disparity and increases in standard deviation and confidence intervals for the two treatments over the growth period highlight the variability in growth rates of individual *Ulva* thalli. This differentiation appears to be magnified by the additional artificial illumination as the standard deviation of the samples is consistently greater than the naturally lit tanks. Furthermore to verify the variation in individual thallus growth rates is the comparison between the average growth and the maximum growth rate observed by an individual thallus. The average difference between the maximum growth rate and average growth rate was 19%, one individual thallus attained a 39% difference in growth rate above the average growth rate in an artificially illuminated tank. There was no significant variation between the maximum and average growth rate differences with artificial or naturally lit treatments ($p>0.05$, n= 6).

The observed rate of thallus maturation was greater in the artificially illuminated tanks than the naturally lit tanks (Figure 4.3). After the 21 day period the mean percentage maturation rate was 46% in the natural light only tanks and 58.33% in the artificially lit tanks. However, the difference between the treatments is not significant ($p>0.05$).

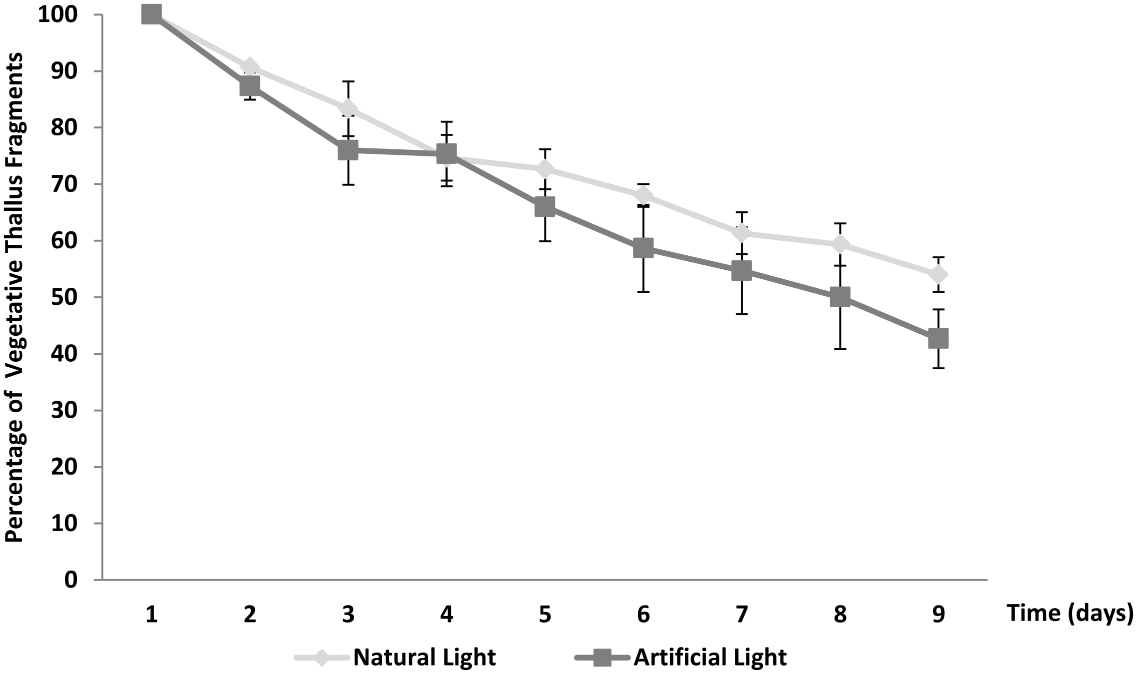

Figure 3.3 Variation in maturation rates in response to cultivation of *Ulva lactuca* under artificial light, expressed in percentage of thalli that displays vegetative growth only.

3.3 Nitrate Uptake Capacity Experiment

The results demonstrate a clear difference in the uptake capacity between the trials as shown in figure 4.4. The starved vegetative *U. lactuca* thalli exhibit no immediate variation in nitrate uptake (slight downward trend), after 16 hours the nitrate levels begin to drop rapidly during daylight hours. During first dark period, the nitrate levels plateau slightly, which indicates a reduction in nitrate uptake. The next daylight hours bring another period of more rapid level of nitrate uptake. The level then begins to decrease more steadily, although still exhibiting a difference between light and dark.

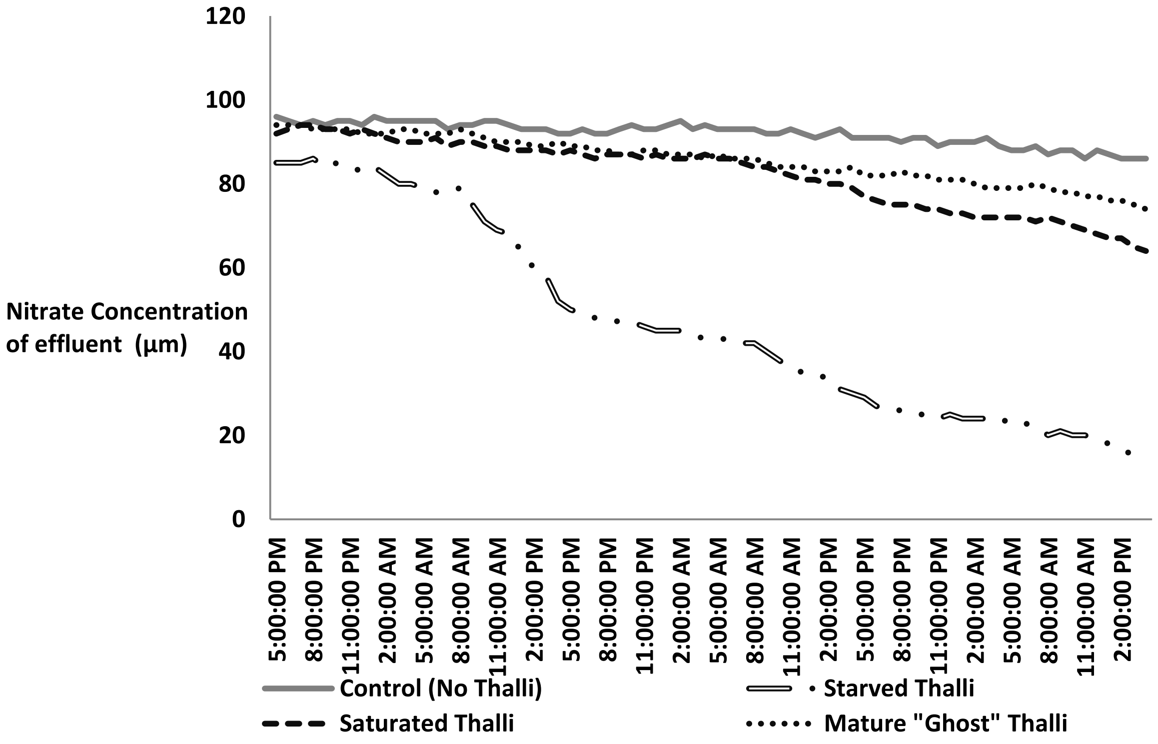

Figure 3.4. Figure 3.4. Nitrate uptake capacity of different states of Ulva lactuca represented by nitrate drawdown from tank seawater using a Satlantic ISUS Nitrate Sensor over 3 days. Uptake in starved thalli was significantly higher than the other treatments after the initial 24-hour period.

The saturated vegetative *U. lactuca* displayed a reduced nitrate capacity throughout the 72 hour period. The presence of a slight increase in nitrate levels within the effluent at the onset of the experiment suggests perhaps a nitrate release from the saturated thalli in the presence of ammonia-N. The level of uptake appears to increase after a period of 36 hours which suggests the ammonia-N and tissue based-N reduced enough to allow for the utilisation of nitrates as the dominant source of nitrogen.

The significant difference ($p < 0.05$) between the control and the other three trials underscores the obvious yet necessary observation that it is indeed the *U. lactuca* thalli that are removing the nitrate from the medium. However, there is some slight reduction in nitrate levels in the control which is to be expected of in-situ nitrification by other organisms within the effluent.

The trials with thalli undergoing greater than 50% (surface area) sporulation and gametogenesis showed significant signs of reduced nitrate uptake in comparison with vegetative thalli. This result supports other findings that suggest a reduced N requirement during maturation phase, thus a reduced uptake capacity.

Discussion

3.4 Growth Experiment 1

Basal fragments tend not to display signs of maturation. Basal fragments grow slower than the apical thallus portions (Chihara, 1969); however, apical cells rapidly mature under optimal conditions, i.e. higher salinity, warmer temperature, light, and high nutrient concentration. This cell maturation results in thallus breakdown for the apical portions of the thalli. Basal fragments in this investigation displayed slightly slower growth rates in the first week in comparison with apical portions. Longer term growth rates were significantly higher for basal thallus portions opposed to apical thalli portions over a three week period as the apical portions were subject to thallus deterioration as a result of gametogenesis /sporulation. Thalli deterioration in conjunction with the prohibition of cell growth due to maturation results in the reduced growth rates exhibited by the apical thalli portions. The decayed "*ghost*" tissue weighed considerably less than the functional cells, by a reduction of approximately 90% (Pers. observ.).

3.5 Growth Experiment 2

The minimal difference in growth rates between the naturally lit treatments and the artificial treatments during the first week of growth substantiate the claim that nutrient concentration is the key growth parameter. The initial pulse of growth occurs due to high nutrient concentration, followed by thallus saturation. Post-saturation thalli response to artificial light was greater as exhibited by the remainder of the growth experiments whereby a significant difference began to occur between the natural light and the supplemental artificially lit system. Economically, the proportionate increase in growth attained under the influence of artificial lighting arrays does not match the cost benefit analysis with the necessary increase in logistics, infrastructure and energy consumption.

3.6 Nitrate Uptake Capacity

The data for the starved thalli reveals the following trends. Initial onset of the experiment shows little variation in nitrate removal from the effluent. Followed by a series of pulse declines during light periods and reduced uptake during dark periods followed by a plateauing after three days. The nitrate uptake trend aligns with previous *Ulva* based nutrient extractive data (Jimenez del Rio *et al*. 1996, Naldi *et al*. 2002, Copertino *et al*. 2008, Liu *et al*. 2010, Ale *et al.* 2011).

An explanation for the initial delay period is likely as a result of *Ulva*’s preference for ammonia base nitrogen opposed to nitrate. Naldi *et al*. (2002) identified the disparity in ammonia-N and nitrate uptake, with initial preference towards ammonia-N over nitrate. Once ammonia levels had been depleted nitrate uptake begins, it was also suggested that the utilisation of tissue nitrogen occurs prior to nitrate uptake.

The variation in nitrate uptake rates during light and dark periods coincides with data collected by Harlin (1978), Jimenex del Rio *et al*. (1996) and Liu *et al*. (2010). This investigation was not as in depth as Harlin's work. Harlin found during the first hour of darkness nitrogen uptake reduced 65%, the second hour was reduced to 10%, with negligible levels been recorded until daylight returned. The work further describes the possibility of system development in conjunction with *Chondus crispus* cultivation, as *C. crispus* demonstrates a reversal of nitrate uptake strategy in that it removes nitrates during dark periods opposed to light periods as with *Ulva*. However, this data is in contrast with Liu et al. (2010) where approximately only 30% reduction of nutrient uptake capacity of *U. pertusa* was recorded.

The nitrate uptake capacities and uptake capacity patterns of *U. lactuca* in these experiments are similar using the Satlantic ISUS V3 nitrate sensor to other nitrate uptake experiments (Copertino *et al*. 2008, Ale *et al*. 2011). Preliminary experiments showed that ammonia levels were undetectable and proved unreliable when trying to establish ammonia-N uptake capacity.

The uptake rates were considerably lower than the work done by Harlin (1978). A possible reason for this could be how temperature directly affects nitrate uptake. At 15°C V_{max} for nitrate uptake was 129 um g DW h^{-1}. At 5°C, the uptake rate was 19% of that of 15°C uptake rate. At 0°C nitrate uptake ceased altogether. These experiments were conducted in water cooler than 10°C, this could account for the reduced nitrate uptake capacity.

However, in contrast to this, Robertson-Andersson (2003) found that temperature had very little effect on uptake rates on a µmN g^{-1} h^{-1} basis. However, that increased vegetative growth rates affect uptake rates through increased biomass accumulation. The results from this investigation imply that lower temperatures do illicit lower nitrate uptake rates, although direct causation between temperature and uptake, vs. growth rate and uptake rate could not be concluded.

The investigation showed that thalli undergoing mass sporulation and gametogenesis (over 50% of thalli surface) showed significant reduction in V_{max} for nitrate. Copertino *et al*. (2008) stated the primary causes for nutrient uptake reduction was saturation and maturation, which is clearly demonstrated in these results.

An important issue raised in Liu *et al*. (2010) is the recognition of the difference in stocking material and uptake capacity. It was found that material cultivated in nutrient enriched water had higher uptake capacity than wild harvested stock or stock cultivated in medium lower in nutrient content. Unfortunately, is this experiment the stocking material was all wild harvested material and therefore this relationship could not be examined. Although, in the development of a commercial system that will require intensive stock replenishment, it is pertinent information to include.

In summary, the primary factors involved in inorganic extractive capacity of an Ulva component are:

- Nutrient uptake of *Ulva* is reduced in dark periods.
- Stocking material cultivated or acclimatised in N-enriched water has a higher uptake capacity.
- Ammonia preference over nitrate, once ammonia is used, and tissue nitrogen is utilised, nitrate uptake begins. Nitrate release by *U. fenestrata* in presence of high levels of ammonia.
- Initial high uptake period when not saturated, plateau to lower constant level of uptake once saturated.
- Maturation reduces nutrient uptake capacity.
- Vegetative growth increases nutrient uptake capacity.

3.7 Overall Experiment Discussion

Growth rates obtained in this investigation were considerably lower than some of the other investigations; however, the temperatures were also considerably lower. When compared with growth rates at similar temperatures to those found in this investigation, the growth results coincided with that of this investigation. Although, the increased temperatures would have the detrimental effect of high maturation rates, which reduces the nutrient uptake efficiency and increases labour and energy with additional harvest and processing time, not to mention, the economic unfeasibility of additional heat and refrigeration of the water in aquaculture systems.

It was observed with use of the light meter that turbidity of the Wolf Eel, *A. ocellatus* effluent was considerably higher than that of normal seawater, a similar conclusion was reached by Robertson-Andersson (2003), whereby, the turbidity of Turbot effluent was higher than the Abalone effluent and resulted in lower yield due to reduced photosynthesis, even though the proportion of nutrients was higher in the Turbot effluent. The impact of effluent generated turbidity will have less of an impact in open systems, than in closed or semi-closed systems. However, in series after the organic extractive component, the turbidity would be reduced to allow more light penetration.

Another possible reason that could result in reduced growth capacity and continued crop longevity is a limitation in other essential nutrients, for example, phosphate, carbon or other essential trace elements. Secondary nutrient limitation is an issue widely acknowledged in freshwater aquaponic systems and also in recirculating marine systems (Robertson-Andersson, 2003, Paul and Tseng, 2012). Identification of specific nutrient limitations of a system is essential. Nutrient limitation tends to be a function of cultivation intensity and is of particular relevance to monoculture or large scale intensive IMTA production.

3.8 Experiments Conclusion

Robertson-Andersson (2003) recognised that optimal stocking density is system specific and is dependent on primary cultivation goal. Thus, specifying a generic stocking density would be futile. However, a stocking density range between 1kg to 4kg Wwt. m^{-2} is advised. The lower end of this spectrum achieves higher growth rates and potential yields. The alternative attains a higher inorganic extractive capacity. The low value of *Ulva* as a standalone product and higher infrastructure costs would imply the uses of higher stocking densities are more suitable to the development of West Coast IMTA systems.

Artificial light allows additional growth, but the high nitrate content of the effluent caused the greatest growth rate flux at the beginning. The results suggest that nutrient concentration is the principal parameter to determine initial growth rate in thalli, with light acting as an important secondary factor once the thalli are saturated with nutrients. The use of additional artificial light throughout the winter months to promote growth and increase nutrient uptake is not economically viable. The low value of the crop, in combination with only slight increases in growth does not justify the initial investment in additional infrastructure or the continual energy requirements.

An indirect association with these results and previous research highlights the importance of temperature on growth rates. Temperature appears to be critical to obtain optimal growth, however the increase in temperatures also increase the rates of maturation. Cultivation of local strains of *U. lactuca* exhibited sporulation/gametogenesis at 5°C contrary to previous research of Northern latitude *Ulva* strains (Kalita and Tytlianov, 2003). However, levels of sporulation/gametogenesis appeared to be considerably less than in +10°C water. Some thalli in holding tanks have displayed continued vegetative growth throughout the winter period when temperatures have not exceeded 10°C. In open IMTA systems, temperature regulation is not an option. For the coastal waters of British Columbia, the relatively consistent cooler temperatures of finfish farm sites between 6°C - 12°C will limit the optimal growth rates achieved by *Ulva*. Lower temperatures have the additional benefit of maturation reduction. In closed/ semi-closed systems increased temperatures are possible and indeed likely during the summer period. However, due to the requirements of other components of the IMTA systems, additional cooling is required, and thus increases the cost significantly.

In the majority of literature on *Ulva* based growth and nutrient uptake experiments the specifics about the type of material used to stock the experiments was often insufficient, with the exception of Hiraoka and Oka (2008). It appears that the majority of previous growth research stocked the tanks with randomly picked wild harvested vegetative thalli. The resultant stock thalli experienced a rapid initial growth phase, followed by maturation of the majority of thallus surface area. Consequently the degraded material produced progressively lower production levels until negative levels were reached due to the decay of the matured thalli portions. Unless cultivated at a uniform rate, the uneven distribution of growth stages would result in a poor harvestable crop, with a large proportion of thallus in a state of degradation, with little to no nutritional value. The samples used in this investigation were collected from wild stock. However, fragments from the basal portion of vegetative thalli were selected for cultivation. That said, the results still exhibit a broad range of growth rates. In order to cultivate a viable uniform crop, the tanks need to be stocked with young individual equally sized thallus derived from germling based production as per Hiraoka and Oka (2008). The alternative would be to find a local sterile strain of *U. lactuca* and stock utilising the fragmentation process as per Kosaka *et al*. (2008).

Chapter 4 - SWOT Analysis of Ulva IMTA Potential for the Coast of British Columbia

The aim of this chapter is to evaluate the use of local *Ulva* species within IMTA systems on the West coast of Canada with the use of a project evaluation tool – the S.W.O.T. analysis. (Bolton, 2009) used S.W.O.T. analysis with Ulva in South Africa. Utilise data from previous *Ulva* cultivation research over the past four decades, alongside data and experiences from my own growth experiments at the University of Victoria aquatics facility. The S.W.O.T. analysis is discussed under the four terms:

- ***Strengths*** - Examine the current internal advantages of growing *Ulva* species as an inorganic extractive component within an IMTA system on the coast of British Columbia (Fig. 6.1)
- ***Weaknesses*** - Review the existing weaknesses in Ulva species cultivation in both monoculture and IMTA systems (Fig. 6.2).
- ***Opportunities*** - Explore the external factors and potentialities that could benefit the development of *Ulva* IMTA on the coast of British Columbia (Fig. 6.3).
- ***Threats*** - Identify the external factors and pitfalls that could risk the potential success of *Ulva* IMTA on the coast of British Columbia (Fig. 6.4).

4.1 Strengths

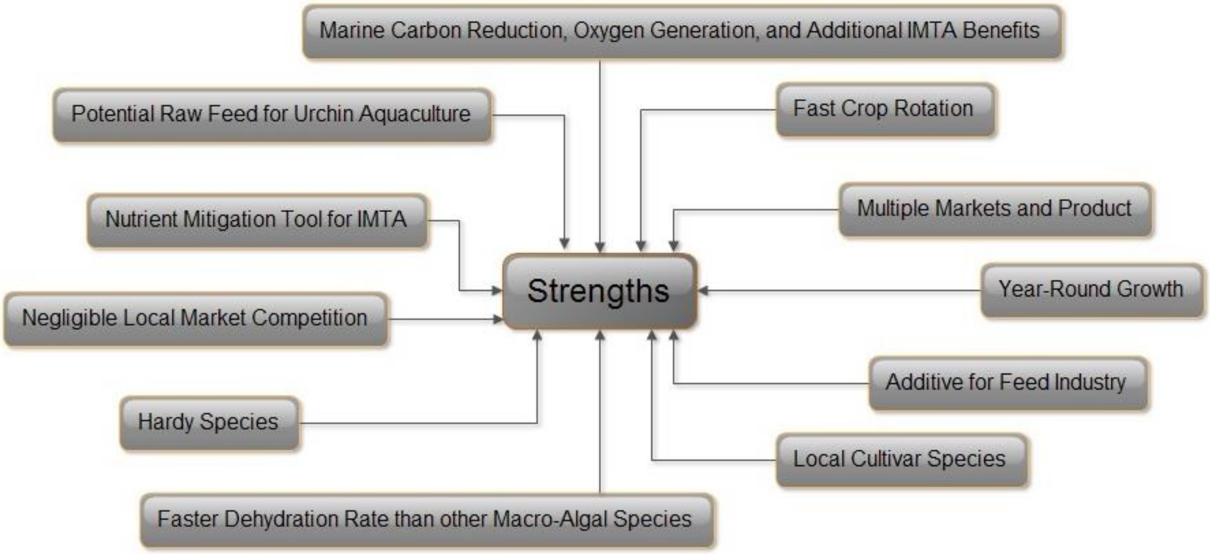

Figure 4.1. SWOT analysis (Strengths) for the potential development of *Ulva lactuca* cultivation as an inorganic extractive component for a West coast Integrated Multi-Trophic Aquaculture system.

4.1.1 Nutrient Mitigation Tool for IMTA

The primary strength and focus of *Ulva* cultivation within an IMTA system is the nutrient uptake capacity of this genera. *Ulva* has some of the highest uptake rates of inorganic nutrients in comparison with other macro-algal species. *Ulva* has a particular preference towards the utilisation of ammonium. Accumulations of dissolved inorganic nitrogenous compounds can become toxic to other aquatic organisms (Ale et al. 2011). In commercial terms, the nitrogen flux from a marine farm results in a 67-71% net loss of nitrogen into the environment (Jimenez del Rio *et al*. 1996). This percentage equates to approximately 95-102kg of nitrogen per tonne of fish produced (Jimenez del Rio et al. 1996). At uptake rates of 2g DIN m^{-2} d^{-1} found in the work of Jimenez del Rio et al. (1996), an IMTA system requires 130-153m^2 of *U. lactuca* cultivation area to mitigate the DIN production from one tonne of farmed finfish. The work done by Copertino *et al*. (2008) resulted in uptake rates of 0.4g DIN $m^{-2}d^{-1}$, this is considerably less and would require 657-822m^2 to mitigate the DIN production from one tonne of farmed finfish. Liu et al. (2010) approximate the need for 846m^2 (at a stocking density of 3kg m^{-2}) of *U. pertusa* cultivation area to process the 2000 tons of wastewater (34µmol/l DIN d^{-1}) from their fish hatchery.

4.1.2 Marine Carbon Reduction, Oxygen Generation and Additional IMTA Benefits

As a result of photosynthesis, the mass cultivation of *Ulva* would act as a carbon sink, provided the crop is not used as a biofuel or for methanogenesis. It is now accepted that the anthropogenic effects of increased inorganic carbon in the marine environment and the associated reduction in pH will have catastrophic implications for oceans of the world (Orr et al., 2005). Abreu et al. (2011) found that *Gracillaria vermicullophylla* at a density of 3kg m^{-2} removed 347.8± 17.8 g Cm^{-2} $month^{-1}$. pH levels were shown to increase in IMTA systems with seaweed cultivation tanks (Robertson-Andersson *et al*. 2008, Abreu et al. 2011). *U. lactuca* has higher photosynthetic productivity than *G. vermiculophylla* of 5.06 mg carbon g h^{-1} (Littler & Littler, 1983). When grown at a density of 3kg m^{-2} then the equivalent monthly carbon removal would be 1093 g Cm^{-2} $month^{-1}$.

The benefits of carbon removal could economical as well as environmental; carbon removal figures could be offset against the overall carbon footprint of the farm.

The integration of *Ulva* cultivation within enclosed IMTA systems increases the O_2 levels in tank systems in light periods. However, the increased respiration levels will increase O_2 consumption during dark periods. Zou *et al*. (2007). Supplementary lighting could reduce the dark periods, and allow a consistency of raised O_2 levels in closed containment systems. Thus, improve the economic viability of closed containment systems as the need for artificial oxygen supplementation would be mitigated, supplemented oxygen is one of the key prohibitive factors in the development of closed containment farms (Boulet *et al*. 2010).

4.1.3 Multiple Markets and Products

A major benefit of *Ulva* cultivation is the numerous and various applications for *Ulva* based products. The variety of uses for *Ulva* was discussed earlier. Product diversification is critical in current market conditions for successful business development. The current markets in British Columbia for Ulva are cosmetics, nutritional, domestic and agricultural fertilisers, and animal feeds.

4.1.4 Negligible Local Market Competition

There is currently very little local market competition for *Ulva*. The seaweed industry in British Columbia is relatively small. 18 seaweed based companies currently operate in British Columbia. Of those, only two have *Ulva* based products. At present all the local seaweed marketed in British Columbia is harvested from the wild. The growth of the industry will lead to environmental pressures on wild stocks and the organisms that depend on them. Cultivation of *Ulva* will allow for a more consistent and higher quality product over wild harvested *Ulva* and other associated seaweeds. The majority of *Ulva* used in Canada is imported from Asia. In British Columbia in particular, there is a growth in the preference for Canadian produced products. Fresh, local products command a higher price than imported counterparts.

4.1.5 Potential Raw Feed for Urchin Aquaculture

The demand for alternative aquatic products in British Columbia has expanded over recent years. Urchin cultivation is one such industry that is expected to grow as demands on wild harvested Urchins is detrimental. The use of *Ulva* as food for integrated Urchin cultivation on-site within the IMTA system would dramatically increase the value of both products. Firstly, a controlled diet is required to optimise the quality of the roe. Secondly, farmed seaweeds can attain the consistency necessary for optimal roe quality. The seaweed would not have to be processed and could be fed directly to the Urchins, which would significantly reduce costs. Thirdly, as noted by Robertson-Andersson (2008), an in-situ farmed diet reduces wild harvest pressures and costs. Finally, IMTA systems allow mutually beneficial production dynamics which reduce energy and costs in transportation.

4.1.6 Additive for Aquatic Feed Industry

In addition to Urchin diets, *Ulva* has proven beneficial as an additive in aquatic feeds. *U. rigida* as a supplementary diet for Carp *Cyprinus carpio*. L and *Oreochromis niloticus*, showed peak growth performance with a 5-15% diet inclusion (Diler *et al*. 2007, Soyutu *et al*. 2009). Research has shown that *Ulva* is more beneficial for algivorous finfish species than carnivorous finfish. However, further dietary investigation is required for local marine farmed finfish species.

4.1.7 Fast Crop Rotation

The productivity of *Ulva* is a positive for the cultivation of Ulva within an IMTA system. Daily growth rates of up to 17% were achieved in this study for local strains of *U. lactuca*, with similar growth rates (16-18%) been achieved for Northern latitude *U. lactuca* cultivation in other investigations (Ale *et al*. 2011, Bruhn *et al*. 2011). It is possible for continual harvest to be initiated within a few weeks after initial seeding of the cultivation system. With the exception of plankton, very few aquatic or even terrestrial crops have such high production rates.

4.1.8 Year-Round Growth

There is a dramatic temporal variation in the *Ulva* growth rates throughout the year. However, growth can be maintained all year round. Lower temperatures in winter are more conducive to vegetative growth. Year round productivity allows *Ulva* to maintain nutrient mitigation abilities, albeit at lower levels. A positive response to the reduced growth levels would allow for higher stocking densities. This would reduce the area required for complete system nutrient mitigation.

4.1.9 Faster Dehydration Rate than other Macro-Algal Species

Due to the morphologically distromatic nature of *Ulva* thalli, the resultant dehydration rate is considerably reduced in comparison with other seaweeds. Reduced thalli drying times result in lower processing costs.

4.1.10 Hardy Species

Ulva is a robust and hardy species, as previously stated in the introduction it can withstand a number of environmental parameter pressures and variances. *Ulva* species have some of the broadest tolerances of any cultivated aquatic species. Although, many of these environmental parameter variations are not conducive to optimal growth, it is unlikely that variations within the cultivation system would result in loss of stock as with other aquatic organisms. An example of the parameter tolerance of *Ulva* IMTA opposed to other macro algae is the tolerance for brackish water. This is of particular relevance to coastal farms with larger freshwater lens influence that dramatically reduce the viability of other marine algae.

4.1.11 Local Cultivar Species

U. lactuca is found along the entire coastline of British Columbia (Druehl, 2001). Cultivation of only local strains is necessary for the development of sustainable aquaculture practices. The result of farming non-native species has had direct negative impacts on both the environment, through ecological disruption, and public opinion of aquaculture on the coast of British Columbia.

4.2 Weaknesses

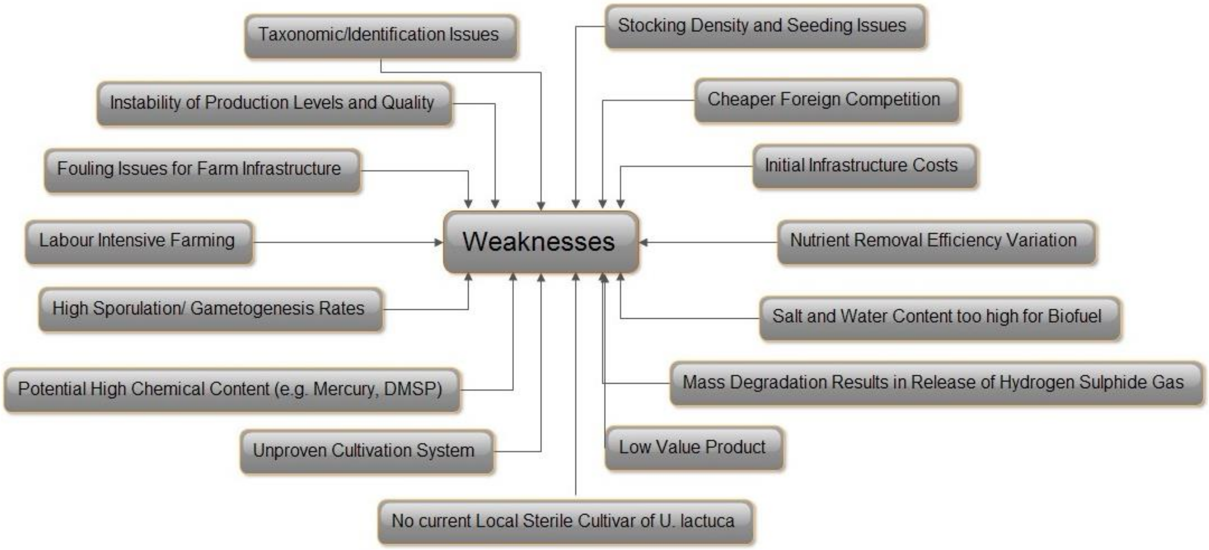

Figure 4.2. SWOT analysis (Weaknesses) for the potential development of *Ulva lactuca* cultivation as an inorganic extractive component for a West coast Integrated Multi-Trophic Aquaculture system.

4.2.1 Potential High Chemical Content (E.g. Mercury, DMSP)

The counterbalance to beneficial nutrient absorption properties of Ulva is the inherent ability to absorb heavy metals and other substances (Zeroual *et al*. 2003, Suzuki *et al*. 2005, Zakhama *et al*. 2011). *Ulva* species have a high absorption and retention capacity of various elements. For example, 67 mg g^{-1} of Ni, 112 mg g^{-1} of Cu, 127 mg $g^{-1}$of Cd, and 230 mg g^{-1} of Pb (Zakhama *et al* 2011). Absorption and retention capacity is positively correlated with the pH level of the seawater, rates of Mercury absorption are 27.24, 84.74 and 149.25 mg g^{-1} at pH levels of 3.5, 5.5 and 7 respectively (Zeroual *et al*. 2003).

Another compound that has been found to negatively affect the use of *Ulva* within IMTA systems is Dimethylsulphoniopropionate (DSMP) levels (Smit *et al*., 2007). Smit *et al*. (2007) identified that the high concentrations of DMSP found in *Ulva* when fed to Abalone *Haliotis midae* resulted in bad tasting and odorous meat.

4.2.2 Low Value Product

Ulva in various forms is readily available. The costs quoted for a kilogram of dried *Ulva* (few sources can confirm exact species) are between \$0.5 – 12 kg^{-1} of 100% pure *Ulva* product, dependent on form, grade, and volume ordered (www.alibaba.com, online quotes: 13th February 2013). Prices for *Ulva* are comparatively cheap in relation to other seaweed products on the market; Kelp Powder commands values of between \$10 – 600 kg^{-1} (www.alibaba.com, online quotes 13th February 2013). "*Green Tides*" generate huge wild harvestable sources where some areas actually pay to clear up the excess biomass.

The high costs of harvest, processing, transportation and marketing all have to be assessed prior to the confirmation of a positive or negative economic feasibility outcome.

4.2.3 Salt and Water Content to High for Biofuel

Interest in *Ulva* as a potential biofuel was deemed economically unviable by Ryther *et al*. (1984). Further investigation into anaerobic digestion to produce methane and direct combustion were found to be possible. However, the ratio of energy production versus processing costs was not feasible (Morand *et al*. 1999, Bruhn *et al*., 2011). The primary reasons for the additional processing were high moisture content, alkalinity, sulphur content, and salt levels (Morand *et al*. 1999, Bruhn *et al*., 2011).

4.2.4 Mass Degradation Results in Release of Hydrogen Sulphide Gas

The high levels of sulphur found in *Ulva* species results in the production of Hydrogen Sulphide gas during decomposition. The toxicity of mass aggregations of rotting *Ulva* biomass has resulted in several fatalities in Europe. There is a possible link in the interaction between sulphur, arsenic, and high nitrate concentrations (Charlet and Sposito, 2012), the interaction is hypothesized that *Ulva* acts as an arsenic reservoir in areas of increased nitrate levels.

4.2.5 Unproven Cultivation System

A variety of Ulva cultivation approaches currently exist, on experimental levels with some small scale commercial scale operations. This paper approached the development of a local Ulva integrated IMTA system with the use of the tank system. This method would be appropriate for use both in open cage farming with the correct infrastructure development and closed containment/ land-based systems. The cultivation method for local strains is not proven. The data collected in this investigation proved the necessity for further research into the commercial scale cultivation methods of Ulva on the B.C. coast. Previous research for Ulva cultivation has shown an array of disparities in the cultivations conditions required to attain optimal productivity. For example, both Neori *et al*. (1998) and Robertson-Andersson *et al*. (2008) noted that as cultivation volume increased, total yield was reduced along with growth rates. Different flow rates and the necessity of aeration are to in contention. Copertino *et al*., (2009) attained good growth rates with a single daily volume exchange, opposed to a static volume exchange every four days. However, Robertson-Andersson *et al*. (2008) used 12 volume exchanges per day. Khoi and Fotedar (2011) used approximately 8 volume exchanges per day in their *Ulva* integrated IMTA system. Msuya and Neori (2008) determined that aeration was a sub-parameter for growth rates in the presence of high nutrient levels. Jimenez del Rio *et al*. (1996), found that two volume exchanges gave significantly lower growth rates, than four or more volume exchange rates, after 4 volume exchange rates, no significant growth rate variation occurred. *Ulva* cultivation research displays enormous variation in system design, maintenance, growth rates, locality, nutrient uptake capacity, stocking density, and stocking method. Semi-commercial scale *Ulva* integrated IMTA production has been successfully achieved in warmer climates Israel, Neori *et al*. (1998), South Africa, Robertson-Andersson *et al*. (2008), Brazil, Copertino *et al*. (2009), Australia, Khoi and Fotedar (2011).

4.2.6 No Current Local Sterile Cultivar of *U. lactuca*

Previous research and commercialisation of *Ulva* cultivation has required the identification of a local sterile vegetative cultivar strain for optimal production (Migita, 1985, Hirata, 1993, Muthuvelan, 2002, Robertson-Andersson, 2003, Sato *et al*. 2006, Wang *et al*. 2007, Kosaka *et al*. 2008). Sterile portions of the thalli are found to have the highest levels of photosynthetic activity and nutrient uptake capacity (Kolmakov *et al*. 1990, Voskoboinikov *et al*. 1991). Non-sterile thallus will undergo sporulation within 5-14 days at temperatures above 8°C and dependent on other conditions. Continuous sporulation events cause a loss of biomass, and reduce the nutrient uptake capacity of the IMTA inorganic extractive component. Constant harvesting and re-stocking would be required to maintain the capacity and productivity of the system.

4.2.7 High Sporulation/Gametogenesis Rates

Luning *et al*. (2008) noted that the semilunar (bi-weekly) gamete discharges for Ulva species coincided with spring tides. As mentioned above the infraradian rhythm for *U. lactuca* of 5-14 days is detrimental to the nutrient capacity and the total crop productivity of the seaweed component. Copertino *et al*. (2009) amongst other highlight the high levels of sporulation/gametogenesis in a cultivation system and the sub sequential negative impacts.

4.2.8 Fouling Issues for Farm Infrastructure

Ulva is considered an epiphytic pest species for various sectors of marine industries. Bio-fouling research often uses Ulva spores as a subject for anti-fouling substances and mechanisms (Pettitt *et al*. 2004, Hopkimeier-Wilson *et al*. 2004, Shin, 2008). The settlement potential as a result of mass spore and gamete generation from the cultivation system could dramatically increase bio-fouling on the farm infrastructure and surrounding environment. This additional bio-fouling demand could increase maintenance costs substantially.

4.2.9 Instability of Production Levels and Quantity

A full analysis of *Ulva* cultivation research and practice has revealed a large range of productivity variation. Temporal and spatial variations occur in production levels. Large seasonal trends in productivity are well documented. As an example, Copertino *et al*. (2008) found that in Brazil over the summer that the specific growth rates and biomass yield were variable, subsequently ranging from 0 to 30% and from 0 to 70 g FW d^{-1} respectively. Jimenez del Rio *et al*. (1996) attained a growth rate, yield, variation of 3.6-12.6% d^{-1}, 12.2-40.2g DW $m^{-2}d^{-1}$ respectively. Temporal yield variation is acceptable in stand-alone crops as a seasonal market can be developed. However, as a bio-engineering tool (inorganic nutrient extraction), productivity has to be relatively constant in order to facilitate the effectiveness and requirements of the component.

4.2.10 Labour Intensive Farming

An issue inherent in the cultivation of macro-algae is the high levels of labour intensity required to generate commercial volumes. Cultivation of algae within developed nations requires some degree of mechanisation within the process to reduce the labour requirements. The total labour costs would be determined by the cultivation, processing, transportation, and marketing approaches.

4.2.11 Taxonomic/Identification Issues

The taxonomic and identification of *Ulva* species and strains is another weakness in the potential cultivation development. Many studies have shown that the morphological characteristics can be highly variable within species, varying with age, reproductive state, wave exposure, tidal factors, temperature, salinity, light, and biological factors such as grazing (Guiry and Guiry, 2012). To attain maximum growth rates, yields, and nutrient uptake capacities, correct species and strain identification is paramount to cultivation parameter design.

4.2.12 Stocking Density and Seeding Issues

The primary premise for the cultivation of Ulva as an inorganic extractive component is the nutrient removal efficiency. The optimal stocking approach to achieve maximum nutrient removal efficiency includes; a relatively high stocking density, the use of non-saturated thalli, and the use of larger thalli. However, high stocking densities considerably reduce the overall yield (Robertson-Andersson, 2003), that majority of research with larger scale Ulva cultivation have opted for high yield/growth rates, thus stocking densities have ranged between 1-2kg m^{-2} of *Ulva*. 1kg m^{-2} of *U. lactuca* (Shpigel *et al*. 1993), 1.1kg m^{-2} *U. rigida* (Jimenez del Rio *et al*. 1996), 1.5kg m^{-2} of *U. lactuca* (Neori *et al*. 1998), 2kg m^{-2} of *U. clathrata* (Copertino *et al*. 2009), 2kg m^{-2} of *U. lactuca* (Robertson-Andersson *et al*. 2008).

A considerable cultivation concern that is not fully addressed in the literature, and significantly affects the production capacity is the stocking material. The majority of previous cultivation attempts used wild harvested material. As shown in this investigation, there is a considerable spectrum in the growth rates attained by wild harvested individual thalli fragments. Wild harvested thalli samples have been used in the majority of cultivation experiments, most of which neglect to mention the portions or sizes of fragments used in the cultivation process. This investigation also highlighted the significant difference between cultivation of the basal and apical portions of the thallus as per Chihara (1969). The cultivation of wild genotypes is generally considered detrimental to productivity capacity of a system (Dunham, 2012). Strain selection is required to develop strains with traits suited to artificial cultivation systems, strain will be system design and site specific.

4.2.13 Initial Infrastructure Costs

The overall initial infrastructure costs are very dependent on system design, scale of system, and site location. Regardless of actual final total, the development of infrastructure for an *Ulva* cultivation component will incur some initial financial outlay. Initial costs would include but may not be limited to: design, development, and siting of the cultivation system; equipment for maintenance and harvesting of the crop; equipment and energy requirements to move water volumes, processing plant and equipment; equality assurance tests; transportation, packaging, and marketing costs; and fees or licences to cultivate *Ulva*. An additional cost factor to be considered is the extra nutritional supplements required to maintain growth rates and meet nutrient balance requirements (Robertson-Andersson, Bruhn *et al*. 2011). This could have an additional negative complication of exacerbating the nutrient flux from the system, thereby negating the very purpose of the inorganic extractive component.

4.2.14 Cheaper Foreign Competition

From an economic stand point the primary weakness for the development of a cultivated seaweed market in British Columbia is the competition for seaweed based products from the international market. With low labour costs, and very well established markets, some competitors, particularly in Asia, produce vast quantities of seaweed.

4.2.15 Nutrient Removal Efficiency Variation

A key weakness in the inorganic extractive potential of *Ulva* is the nutrient uptake rate (V_{max}) variation. Nutrient removal efficiency decreases in dark periods, for example *U. linza* V_{max} reduces 65% in the first hour, and then by 10% in the second (Harlin, 1978). Nitrate removal capacity is also reduced by decreases in temperature. For *U. linza*, a decrease between 15°C and 5°C results in a drop in V_{max} of 81%, with a further reduction as temperatures continue to decline (Harlin, 1978). At 0°C nitrate uptake becomes undetectable. Copertino *et al*. (2009), found there was a considerable temporal V_{max} variation after the initial stocking, after 15 hours dissolved inorganic nitrate (DIN) uptake rate was 7.83mg g DW d^{-1}, after 3 days, the DIN was 3.09mg g DW d^{-1}, after 10 days, the DIN was 1.79mg g DW d^{-1}. Reduction in nitrate uptake efficiency is directly correlated with an increase in percentage of thallus sporulation. Jimenez del Rio *et al*.

(1996), indicates that VE rate is a parameter that affects the V_{max}, DIN removal efficiency increases with flow rate, the DIN removal efficiency was increased by approximately 40% with an increase of 2VE to 4VE. However further increases in VE resulted in modest increases of DIN removal efficiency of 4% between 4VE to 8VE, and from 8VE to 12VE.

4.3 Opportunities

Figure 6.3. SWOT analysis (Opportunities) for the potential development of *Ulva lactuca* cultivation as an inorganic extractive component for a West coast Integrated Multi-Trophic Aquaculture system.

4.3.1 Feed Potential for Onsite Herbivores (E.g. Abalone)

The potential economic, social, and environmental success of *Ulva* integrated IMTA systems in British Columbia appears to be dependent on the use of *Ulva* as an on-site feed source for other aquatic species. Robertson-Andersson (2003), Bolton *et al*. (2008), Robertson-Andersson *et al*. (2008), identify the many direct and indirect economic benefits of integrating *U. lactuca* as an inorganic extractive component and direct feed for abalone *Haliotis midae*. Cruz-Suarez *et al*. (2010) system allows full integration within same tanks. Shrimp, *Litopenaeus vannamei* and *U. clathrata* are co-cultured together within the same tank. *U. clathrata* provided supplemental food and nutrient removal. Shrimp cultivated in this system increased growth rate by 60% and found an increased in quality (carcass, DHA levels) with a pellet diet supplemented with *U. clathrata*.

4.3.2 Local Market Development and Increase Local Public Opinion on Aquaculture Credibility

At present, there are two companies that provide *Ulva* based products in British Columbia. With the increased awareness of the potential benefits of marine algae both as nutritional supplements and garden fertilisers, the market has the potential for expansive growth. Marketing strategies and increased local aquatics products awareness, such as the Aquatic Foods Initiative will help promote the use of British Columbia produced aquatic products. Currently, all seaweed based companies use wild harvested seaweed in British Columbia. Harvesting wild seaweeds reduces local algal populations, and reduces habitat and grazing. Farmed seaweed can be marketed as a better alternative option to wild harvested. The inorganic extractive benefits of *Ulva* alongside the general sustainable approach of West coast IMTA can help to alleviate the current negative public perception of aquaculture in British Columbia and increase market potential. Market development will benefit on the capitalisation of current market trend towards the use of local high quality produce opposed to high mileage imported product. Further marketing is required to steer market consensus away from intensive Atlantic salmon, *Salmo salar* farming towards sustainable aquaculture practices.

4.3.3 Development and Identification of Local Cultivar Strains with Beneficial Cultivation Traits

As with any agriculture or aquaculture practice, cultivars need to be developed through selection to provide the beneficial traits required to optimise productivity and inorganic extractive capacity. In the case of *Ulva*, sterility, high vegetative growth rate, high nutrient uptake rate, and increased protein content are possible beneficial traits to be cultivated. Luning *et al*. (1992), identifies the difference between the portions of the Ulva thalli, the upper portion grows more vigorously than the lower. However, basal discs showed only vegetative growth after 8 days, whereas apical discs showed complete sporulation/gametogenesis (Luning *et al*. 2008). Strattmann *et al*. (1996) documented the existence of a longitudinal protection system within the different location on the thallus, based on a gradient of hormonal sporulation inhibitors with the highest concentration in the basal portion, that inhibit the Ulva thallus from breaking down through gametogenesis. Thus, the continuity of the benthic Ulva population on the substratum is secured. Growth and nutrient removal efficiency is reduced with sporulation/gametogenesis so vegetative growth is the ideal.

Sterile mutants of other *Ulva* species have been found, those that continue to demonstrate vegetative growth with no maturation of thallus. For successful cultivation of *U. lactuca* in British Columbia, a local strain would need to be found. A sterile strain would also have the additional benefit of reduced bio-fouling or environmental population dynamic impacts. In order to cultivate a uniform product, the production of spores to facilitate mass cultivation would be required. The work by Hiraoka and Oka (2007) demonstrates a simple cultivation method for the mass production of "germling" spores of *U. prolifera* to facilitate mass cultivation; the same could be repeated for local *U. lactuca* strains.

Cultivated *Ulva* grown in nutrient enriched waters has been shown to be far superior to wild Ulva crops in regards to nutritional composition (Pinchetti *et al*. 1998, Msuya and Neori, 2008). Nitrogen starvation promotes saturated and mono-unsaturated fatty acid levels in Ulva as found in wild stocks. In contrast, nitrogen enrichment promotes the levels of poly-unsaturated fatty acids, including Docosahexaenoic Acid, and reverses the concentration of the other respectively (Pinchetti *et al*. 1998). Msuya and Neori, (2008) found that protein levels in *U. lactuca* increased from 14.2% dry weight under very low nitrogen loaded seawater to 43.6% dry weight in very high nitrogen loaded medium, carbohydrate and ash decreased proportionately.

4.3.4 Potential Technological Development for Pollution Removal and Bio-indicator Tool

Ulva could be developed as part of a carbon negative environmental biosorption agent for the uptake of excess nutrient and heavy metal loading from estuarine or coastal environments. After deployment, the "loaded" *Ulva* could be removed from the water body, then be subjected to extraction processes to remove heavy metals or utilised as soil additives in agriculture if contaminant levels are deemed safe. These could also be deployed in areas prone to algal blooms to reduce “green tides”. Suzuki *et al*. (2005) found that pre-treatment of nonliving Ulva increased the sorption capacity for heavy metals, Cadmium, Zinc, Chromium, Nickel and Copper. Several studies have presented the successful potential of algae as heavy metal biosorbent material (Chong and Volesky, 1995, Davis *et al*. 2003, Gonzalez *et al*. 2006).

4.3.5 Nutrient Mitigation Monetary Incentives

The environment cost and indirect economic costs of nutrient loading by aquaculture and agriculture practices in the coastal marine environment can be high. The impacts of “green tides” and toxic algal blooms have detrimental effects for the marine ecosystem. Many suggest a policy change towards environmental mitigation technology (Neori *et al*. 2007). Holdt *et al*. (2006) suggests an associated bio-mitigation cost of $44 per kilo of remediated nitrogen. *Ulva* has high photosynthetic productivity of 5.06 mg Carbon g h^{-1} (Littler & Littler, 1983), thus, acts as a carbon sink, if the material is removed and correctly disposed. The marine carbon reduction is useful to mitigate further acidification that is threatening the world’s oceans. Carbon mitigation could be used to potentially offset the carbon footprint of the farm. The development of bio-mitigation reimbursement by the government funded for by the creation of environmental taxation for nutrient and pollution generating industries, would allow IMTA and environmentally sound bio-technological advancements the competitive edge and force a positive change for all global industry.

4.3.6 Development of Potential New Pharmaceutical/Industrial Compounds

Research into potential novel biomaterials is an ongoing practice. A move away from oil derived products towards natural sustainable products is driving research to investigate naturally occurring compounds and substances. The global distribution and mass availability makes Ulva an exciting potential for product research. Cultivated *Ulva* would produce a more consistent quality of product, with strains developed to produce beneficial traits as required. Various products and uses have currently developed from Ulva (Lahaye, 1991, Lahaye *et al*. 1993, Ohno *et al*. 1993, Bixler, 1996, Bodin-Dubigeon *et al*. 1997, Ortiz *et al*. 2006, Harder *et al*., 2004, Abd El-Baky *et al*. 2008, Bruhn *et al*. 2011). Any potential development of new products or uses will increase the market value.

4.3.7 Standard Crop Uniformity will Increase Value over Wild Harvested Crops

Cultivation will aim to produce a superior, more consistent product with aquaculture for market. The production environment and strain selection can be varied to produce predominant traits and required constitution for the intended purpose. A standard product can increase the efficiency of the cultivation, harvest, and processing. Msuya and Neori (2008) highlight another benefit of *Ulva* grown within an IMTA system opposed to wild harvested or monoculture based *Ulva* product, in that protein levels of the thalli increase from 14.2% (dry weight) in very low nutrient loaded medium to 43.6% (dry weight) in very high nutrient loaded medium. Pinchetti *et al*. (1998) support this protein enhancement and further provide evidence that nitrogen deficiencies in the thallus promote the cellular accumulation of saturated and mono-unsaturated fatty acids. Whereas, consistent organic nitrogen enriched growth medium (as experienced in an IMTA system) promotes the accumulation of beneficial poly-unsaturated fatty acids, including Docosahexanoic Acid (DHA), and reverses the concentration of the other respectively.

4.4 Threats

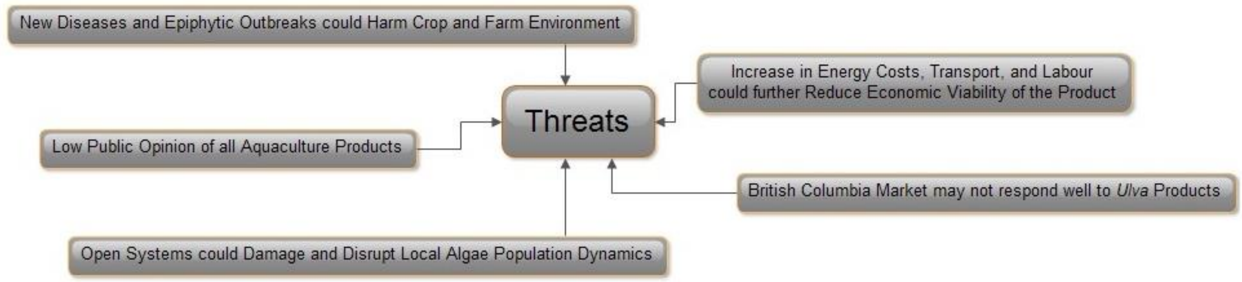

Figure 6.4. SWOT analysis (Threats) for the potential development of *Ulva lactuca* cultivation as an inorganic extractive component for a West coast Integrated Multi-Trophic Aquaculture system.

4.4.1 New Diseases and Epiphytic Outbreaks could Harm Crop and Environment

As with any intensive cultivation practice, the potential for pathogenic or epiphytic outbreak is increased. Robertson-Andersson (2003) had considerable issues with a brown algal epiphyte, *Myrionema strangulans*. It was found that *M. strangulans* caused a decrease in specific growth rate and caused a breakup of infected thallus. Infections of *M. strangulans* devastate entire crops of *Ulva* (Robertson-Andersson, 2003). Without sanitising the whole system the infestation will re-emerge. Other unknown outbreaks could emerge that are specific to the coast of British Columbia, and therefore infect potential systems. There is very little that could be done in open systems, Robertson-Andersson (2003), found that physical shading with the use of shade cloth mitigated the growth of *M. strangulans*. Any infestations that occur within an open system could potentially affect the in-situ natural ecosystem.

4.4.2 Increase in Energy Costs, Transport, and Labour could further reduce the economic viability of the products

The inevitable increase in aquaculture overhead costs, such as energy, transport, and labour could have a negative or positive impact on the cultivation of macro-algae in British Columbia. Operational viability may be compromised for the commercial farms with an increase in costs, if the market does not support the increase in product cost. However, overhead costs are increasing globally which could lead to a balance in the economics of production between currently competitive foreign markets and local companies.

4.4.3 British Columbia market may not respond well to Ulva products

The overall public opinion of aquaculture in British Columbia, particularly on the coast is negative. However, the misguided bias towards the intensive cage culture of Atlantic salmon, *S. salar*, is the primary reason for this opinion. The negative impacts of some early phases of open cage farming of this non-native species have marred the entire industry. The development of algal cultivation sites will also interfere with other water uses, which can result in negative connotations. The low cost of imported seaweed products could sway potential market outlets, especially in the current economic climate.

4.4.4 Open Systems could Damage and Disrupt Local Algae Population Dynamics

The excess production of sporophytes and gametophytes into environment if produced in open systems would lead to variation in local algal populations. This increase in base level production would lead to disruption within the natural phytoplankton populations, and at later stages the macro-algal population structures. The increase demand on nutrients and oxygen could lead to eutrophication and nutrient depletion. Large algal blooms can self-perpetuate further seasonal algal blooms due to the nutrient release from existing biomass which is retained in the environment (Morand and Briand, 1999).

4.4.5 Low Public Opinion of Aquaculture Products

As mentioned previously, the general public consensus in British Columbia is negative towards aquaculture. Farmed aquatic products are generally seen as inferior to wild harvested products (Pers. Comm. Michelle Patterson, Aquatic Foods Initiative). The market for seaweed is relatively small in British Columbia, and in many areas. The majority of sectors are still under developed. A recently developed market is extremely susceptible to public opinion and can be swayed by relatively small events, for example, negative media coverage.

4.5 SWOT Discussion

Over the last forty years, the potential inorganic extraction capabilities and commercial scale production of *Ulva* has been explored in various locations. Integrated cultivation has been implemented successfully in some locations, which include South Africa, Israel and Brazil. However, no ongoing success has been achieved in other geographical locations, particularly Europe and North America. Overall the potentialities of *Ulva* as an inorganic extractive species in IMTA systems outweigh the drawbacks, with one major exception. The economic feasibility for the cultivation of *Ulva* is simply unrealistic unless grown simply as an organic extractive component coupled with an onsite use for the *Ulva.* Other species of macroalgae are far more economically suited to cultivation within an open cage IMTA system in British Columbia if the macroalgae is to be used offsite as a standalone product.

Chapter 5 - Conclusion

In consideration of the feasibility of developing *U. lactuca* as a sustainable option for an inorganic extractive component species for IMTA systems on the West coast of British Columbia, the current outlook is low. Primarily for economic reasons, as a standalone product, the value of the product cannot justify the production, logistical and processing costs. However, if situations changed, for example, if permission was granted for the cultivation of Abalone, *Haliotis kamtschatkana* and *Ulva* could be used fresh onsite, as a feed source. The outlook could become more favourable. In the latter case, the author would like to suggest a number of recommendations in order to maximise the feasibility of an *Ulva* based inorganic extractive component.

The recommendations for this development and further work to include:

- Cultivars to be grown in high nutrient loaded medium for developing a higher nutrient uptake capacity and affinity.
- An onsite system for continual stock replenishment needs to be established utilising the “germling” production method as previously described.
- On-site use of the *Ulva* product as fresh feed for an algivorous component species within the IMTA system.
- *U. lactuca* is grown in conjunction with a dark period uptake extractive species such as *C. crispus*. In closed systems *U. lactuca* cages can be withdrawn from the

water during dark periods to allow for reduced oxygen consumption and reduced epiphyte growth.

- Inorganic extractive component design should be based on a rotational, light-weight float cage system, submerged up to 1.5 metre below the surface (dependent on annual water turbidity). The rotational cage system would save costs on aeration requirements, and reduce initial start-up costs associated with tanks and pumping systems. The individuals cages would be small enough to easily handle to allow easy removal from the water body to reduce fouling, epiphytic growth, disease, prevent nutrient saturation, and allow for easy res-stocking and harvest. Cage growth within tanks on land based systems also prevents clumping in tank corners, and reduces harvest time. Initial grow out on inoculated lines to prevent clogging of filters and nets with tiny thalli.
- To facilitate necessary inorganic extractive efficiency there are two options.

  (1) Routine switches of material within the system to allow passive reduction of nutrient saturation levels within original material, to enable uptake capacity.

  (2) Or the system must allow for the containment of enough inorganic extractive material to inhibit component saturation. However, if growth and yield is more important than optimal nutrient removal, Neori et al. (1991), found that yield was increased by 38% when the crop was fed a continuous effluent supply opposed to pulse fed, which would support the latter approach.

- The farm must actively participate in increasing local public awareness about the potential benefits of *Ulva* and seaweed as a low cost, nutritionally beneficial, local, and organic product.
- As *Ulva* grows faster in the presence of ammonia opposed to nitrate (Ale et al. 2011), the *Ulva* component should be positioned as close as possible to fish effluent component to maximise ammonia-N volumes before it breaks down within the water column. In an open cage system, the *Ulva* cultivation component would be ideally situated in close proximity to the fed component again to allow maximum exposure to the ammonia-N. *Ulva* in series, *Ulva* in first tanks will in theory grow quicker (Naldi et al. 2002). If possible, cultivation of the *Ulva* within the fed component would result in the optimum nutrient exposure, and can benefit the fed species in terms of provision of cover and additional feed as per Khoi and Fotedar (2011). Although, in practicality purposes in terms of current open cage farming, this approach may not be feasible.

In summary, *Ulva lactuca* can indeed be utilised as a successful inorganic extractive species. However, in order to implement the successful commercialisation of an *Ulva* inclusive IMTA system, as stated a number of issues require further attention.

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Appendix

Table 1. Growth experiment one data (basic analysis) for two trial periods testing the difference in growth between basal and apical thallus fragments of *Ulva lactuca* in a recirculation aquaculture system in Wolf Eel, *Anarrhichthys ocellatus* effluent

Growth Experiment One - First Trial						
Wet weight (g)	**Tank 1 (B)**	**Tank 2 (A)**	**Tank 3 (B)**	**Tank 4 (B)**	**Tank 5 (A)**	**Tank 6 (A)**
July-15-12	2000.00	2000.00	2000.00	2000.00	2000.00	2000.00
July-18-12	2573.00	3046.00	2448.00	2680.00	2996.00	2840.00
July-21-12	3190.00	3686.00	3050.00	3275.00	3724.00	3487.00
July-24-12	3598.00	3918.00	3401.00	3573.00	3911.00	3910.00
July-27-12	3921.00	4003.00	3717.00	3837.00	3970.00	4098.00
July-30-12	4203.00	3811.00	4029.00	4113.00	3994.00	4061.00
August-02-12	4468.00	3445.00	4201.00	4310.00	3797.00	3842.00
August-05-12	4551.00	2701.00	4251.00	4478.00	3501.00	3404.00
(A) Apical Thallus Fragments (B) Basal Thallus Fragments						
% Daily Growth Rate						
15th to 18th	9.55	17.43	7.47	11.33	16.60	14.00
18th to 21st	7.99	7.00	8.20	7.40	8.10	7.60
21st to 24th	4.26	2.10	3.84	3.03	1.67	4.04
24th to 27th	3.00	0.72	3.10	2.46	0.50	1.60
27th to 30th	2.40	-1.60	2.80	2.40	0.20	-0.30
30th to 2nd	2.10	-3.20	1.43	1.60	-1.64	-1.80
2nd to 5th	0.62	-7.20	0.40	1.30	-2.60	-3.80
Total % Increase	127.55	35.05	112.50	123.90	75.05	70.20
Yield over 3 weeks (g wwt.)	2551.00	701.00	2251.00	2478.00	1501.00	1404.00
Yield over 1 week (g wwt.)	1190.00	1686.00	1050.00	1275.00	1724.00	1487.00

Growth Experiment One - Second Trial						
Wet weight (g)	**Tank 1 (A)**	**Tank 2 (B)**	**Tank 3 (A)**	**Tank 4 (A)**	**Tank 5 (B)**	**Tank 6 (B)**
August-06-12	2000.00	2000.00	2000.00	2000.00	2000.00	2000.00
August-09-12	2672.00	2504.00	2576.00	2762.00	2414.00	2492.00
August-12-12	3177.00	2909.00	2754.00	3408.00	2805.00	3060.00
August-15-12	3234.00	3197.00	2622.00	3553.00	3100.00	3445.00
August-18-12	3195.00	3454.00	2488.00	3335.00	3230.00	3528.00
August-21-12	3122.00	3559.00	2294.00	2875.00	3404.00	3594.00
August-24-12	3010.00	3698.00	2239.00	2737.00	3571.00	3703.00
August-27-12	2489.00	3746.00	1957.00	2441.00	3680.00	3736.00
(A) Apical Thallus Fragments (B) Basal Thallus Fragments						
% Daily Growth Rate						
6th to 9th	11.20	8.40	9.60	12.70	6.90	8.20
9th to 12th	6.30	5.40	2.30	7.80	5.40	7.60
12ht to 15th	0.60	3.30	-1.60	1.42	3.51	4.20
15th to 18th	-0.40	2.68	-1.70	-2.05	1.40	0.80
18th to 21st	-0.76	1.01	-2.60	-4.60	1.80	0.62
21st to 24th	-1.20	1.30	-0.80	-1.60	1.64	1.01
24th to 27th	-5.60	0.43	-4.20	-3.60	1.02	0.30
Total % Increase	24.45	87.30	-2.50	22.05	84.00	86.80
Yield over 3 weeks	489.00	1746.00	-43.00	441.00	1680.00	1736.00
Yield over 1 week	1177.00	909.00	754.00	1408.00	805.00	1060.00

Table 2. Growth experiment two data (basic analysis) from *U. lactuca* cultivation under different light treatments in a recirculation aquaculture system in Wolf Eel, *Anarrhichthys ocellatus* effluent

Tank 1 - Natural light only									
Days	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**
Average Thallus Size (mm)	30.00	32.05	34.10	34.60	35.20	35.82	36.00	36.24	36.54
Average Vegetative Only Size (mm)	30.00	32.20	34.40	35.20	35.80	36.20	36.80	37.00	37.03
Maximum Thallus Size (mm)	30.00	33.50	37.00	38.50	39.50	40.00	41.00	42.00	43.00
Percentage of thalli in vegetative state	0	90	74	68	66	64	54	52	50
Average number of Vegetative only thalli	50	45	37	34	33	32	27	26	25
Average number of partially mature/broken thalli	0	4	10	12	12	11	14	14	13
Average number of	0	1	3	4	5	7	9	10	12
% Thalli Daily Growth Rate	0.00	1.95	0.63	0.63	0.63	0.63	0.32	0.32	0.08
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.10	0.65	0.65	0.65	0.65	0.22	0.22	0.23
% Thallus Maximum Daily Growth Rate	0.00	3.33	1.01	1.01	1.01	1.01	0.50	0.50	0.98

Tank 2 - Natural light only									
Days	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**
Average Thallus Size (mm)	30.00	33.10	35.20	35.86	36.50	36.96	37.40	37.88	37.78
Average Vegetative Only Size (mm)	30.00	33.10	35.10	35.00	36.80	37.09	37.40	37.89	37.98
Maximum Thallus Size (mm)	30.00	34.00	38.00	39.50	40.50	41.50	44.00	47.00	47.00
Percentage of thalli in vegetative state	0	90	86	82	78	70	64	62	60
Average number of Vegetative only thalli	50	45	43	41	39	35	32	31	30
Average number of partially mature/broken thalli	0	4	6	7	8	10	12	11	12
Average number of	0	1	1	2	3	5	6	8	8
% Thalli Daily Growth Rate	0.00	2.48	2.48	0.62	0.62	0.62	0.50	0.50	-0.05
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.43	2.43	0.71	0.71	0.71	0.43	0.43	0.05
% Thallus Maximum Daily Growth Rate	0.00	3.81	3.81	1.15	1.15	1.15	2.65	2.65	0.00

Tank 3 - Natural light only									
Days	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**
Average Thallus Size (mm)	30.00	32.40	34.80	35.00	35.40	35.95	36.40	36.85	36.39
Average Vegetative Only Size (mm)	30.00	32.25	35.10	35.40	35.80	36.02	36.80	37.11	37.24
Maximum Thallus Size (mm)	30.00	34.00	38.00	38.50	39.50	40.00	41.00	42.00	43.00
Percentage of thalli in vegetative state	0	92	90	74	74	70	66	64	52
Average number of Vegetative only thalli	50	46	45	37	37	35	33	32	26
Average number of partially mature/broken thalli	0	2	3	4	4	4	9	8	14
Average number of	0	2	2	4	4	6	8	10	10
% Thalli Daily Growth Rate	0.00	2.29	2.29	0.41	0.41	0.41	0.50	0.50	-0.25
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.43	2.43	0.33	0.33	0.33	0.60	0.60	0.07
% Thallus Maximum Daily Growth Rate	0.00	3.81	3.81	0.66	0.66	0.66	1.00	1.00	0.48

Tank 4 - Light Array

Days	0	3	6	9	12	15	18	21	24
Average Thallus Size (mm)	30.00	32.60	35.20	35.90	38.00	39.00	38.90	38.81	46.03
Average Vegetative Only Size (mm)	30.00	32.75	35.50	36.50	38.40	39.39	40.80	43.28	46.07
Maximum Thallus Size (mm)	30.00	34.00	38.00	42.00	44.00	47.00	52.00	57.00	64.00
Percentage of thalli in vegetative state	0	92	84	82	58	52	48	38	34
Average number of Vegetative only thalli	50	46	42	41	29	26	24	19	17
Average number of partially mature/broken thalli	0	4	6	0	6	3	2	4	3
Average number of	0	0	2	9	15	21	24	27	30
% Thalli Daily Growth Rate	0.00	2.48	2.48	1.35	1.35	1.35	-0.10	-0.10	3.72
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.62	2.62	1.37	1.37	1.37	1.97	1.97	1.29
% Thallus Maximum Daily Growth Rate	0.00	3.81	3.81	2.96	2.96	2.96	4.26	4.26	2.46

Tank 5 - Light Array

Days	0	3	6	9	12	15	18	21	24
Average Thallus Size (mm)	30.00	33.45	35.45	35.90	36.40	36.78	36.90	37.22	38.87
Average Vegetative Only Size (mm)	30.00	33.90	35.90	35.90	36.50	36.78	37.00	37.31	39.65
Maximum Thallus Size (mm)	30.00	34.00	38.00	39.00	41.50	43.00	43.00	43.00	43.00
Percentage of thalli in vegetative state	0	84	80	80	78	74	70	68	52
Average number of Vegetative only thalli	50	42	40	40	39	37	35	34	26
Average number of partially mature/broken thalli	0	6	8	8	8	10	7	8	6
Average number of	0	2	2	2	3	3	5	8	18
% Thalli Daily Growth Rate	0.00	2.60	2.60	0.47	0.47	0.47	0.24	0.24	0.89
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.81	2.81	0.31	0.31	0.31	0.29	0.29	1.25
% Thallus Maximum Daily Growth Rate	0.00	3.81	3.81	1.64	1.64	1.64	0.00	0.00	0.00

Tank 6 - Light Array

Days	0	3	6	9	12	15	18	21	24
Average Thallus Size (mm)	30.00	32.30	34.65	34.90	35.40	35.96	36.20	36.32	38.86
Average Vegetative Only Size (mm)	30.00	32.30	35.10	35.20	36.00	36.12	36.40	36.55	40.20
Maximum Thallus Size (mm)	30.00	34.00	38.00	39.00	39.50	40.00	41.00	42.00	44.00
Percentage of thalli in vegetative state	0	86	64	64	62	50	46	44	42
Average number of Vegetative only thalli	50	43	32	32	31	25	23	22	21
Average number of partially mature/broken thalli	0	4	8	6	4	8	4	4	3
Average number of	0	3	10	12	15	17	23	24	26
% Thalli Daily Growth Rate	0.00	2.21	2.21	0.47	0.47	0.47	0.20	0.20	1.40
% Thalli Daily Growth Rate (Vegetative only).	0.00	2.43	2.43	0.36	0.36	0.36	0.24	0.24	2.00
% Thallus Maximum Daily Growth Rate	0.00	3.81	3.81	0.66	0.66	0.66	1.00	1.00	0.95

Table 3. Nitrate uptake experiment data. Nitrate readings taken from Satlantic ISUS V3 Nitrate Sensor

Dark Shading (Night)/Light Shading (Day)	Control (No Thalli)	Control (No Thalli)	Starved Thalli	Starved Thalli	Saturated Thalli	Saturated Thalli	Mature "Ghost" Thalli	Mature Thalli
Time	Nitrate Reading (µm)	Nitrate Uptake (µm N $gDW^{-1}h^{-1}$)	Nitrate Reading (µm)	Nitrate Uptake (µm N $gDW^{-1}h^{-1}$)	Nitrate Reading (µm)	Nitrate Uptake (µm N $gDW^{-1}h^{-1}$)	Nitrate Reading (µm)	Nitrate Uptake (µm N $gDW^{-1}h^{-1}$)
5:00 PM	96		85		92		94	
6:00 PM	95	2.5	85	0	93	-2.5	94	0
7:00 PM	94	2.5	85	0	94	-2.5	94	0
8:00 PM	95	-2.5	86	-2.5	94	0	93	2.5
9:00 PM	94	2.5	84	5	93	2.5	93	0
10:00 PM	95	-2.5	85	-2.5	93	0	93	0
11:00 PM	95	0	84	2.5	92	2.5	93	0
12:00 AM	94	2.5	83	2.5	93	-2.5	92	2.5
1:00 AM	96	-5	84	-2.5	92	2.5	92	0
2:00 AM	95	2.5	82	5	91	2.5	92	0
3:00 AM	95	0	80	5	90	2.5	93	-2.5
4:00 AM	95	0	80	0	90	0	93	0
5:00 AM	95	0	79	2.5	90	0	92	2.5
6:00 AM	95	0	78	2.5	91	-2.5	92	0
7:00 AM	93	5	78	0	89	5	92	0
8:00 AM	94	-2.5	79	-2.5	90	-2.5	93	-2.5
9:00 AM	94	0	75	10	90	0	92	2.5
10:00 AM	95	-2.5	71	10	89	2.5	91	2.5
11:00 AM	95	0	69	5	89	0	90	2.5
12:00 PM	94	2.5	68	2.5	88	2.5	90	0
1:00 PM	93	2.5	64	10	88	0	90	0
2:00 PM	93	0	60	10	88	0	89	2.5
3:00 PM	93	0	58	5	88	0	89	0

4:00 PM	92	2.5	52	15	87	2.5	90	-2.5
5:00 PM	92	0	50	5	88	-2.5	89	2.5
6:00 PM	93	-2.5	49	2.5	87	2.5	89	0
7:00 PM	92	2.5	48	2.5	86	2.5	88	2.5
8:00 PM	92	0	48	0	87	-2.5	88	0
9:00 PM	93	-2.5	47	2.5	87	0	87	2.5
10:00 PM	94	-2.5	47	0	87	0	87	0
11:00 PM	93	2.5	46	2.5	86	2.5	88	-2.5
12:00 AM	93	0	45	2.5	87	-2.5	88	0
1:00 AM	94	-2.5	45	0	86	2.5	87	2.5
2:00 AM	95	-2.5	45	0	86	0	87	0
3:00 AM	93	5	44	2.5	86	0	87	0
4:00 AM	94	-2.5	43	2.5	87	-2.5	86	2.5
5:00 AM	93	2.5	43	0	86	2.5	87	-2.5
6:00 AM	93	0	43	0	86	0	86	2.5
7:00 AM	93	0	42	2.5	85	2.5	86	0
8:00 AM	93	0	42	0	84	2.5	86	0
9:00 AM	92	2.5	40	5	84	0	85	2.5
10:00 AM	92	0	38	5	83	2.5	84	2.5
11:00 AM	93	-2.5	36	5	82	2.5	84	0
12:00 PM	92	2.5	35	2.5	81	2.5	84	0
1:00 PM	91	2.5	34	2.5	81	0	83	2.5
2:00 PM	92	-2.5	34	0	80	2.5	83	0
3:00 PM	93	-2.5	31	7.5	80	0	83	0
4:00 PM	91	5	30	2.5	79	2.5	84	-2.5
5:00 PM	91	0	29	2.5	77	5	82	5
6:00 PM	91	0	27	5	76	2.5	82	0
7:00 PM	91	0	26	2.5	75	2.5	82	0
8:00 PM	90	2.5	26	0	75	0	83	-2.5
9:00 PM	91	-2.5	25	2.5	75	0	82	2.5
10:00 PM	91	0	25	0	74	2.5	82	0
11:00 PM	89	5	24	2.5	74	0	81	2.5
12:00 AM	90	-2.5	25	-2.5	73	2.5	81	0
1:00 AM	90	0	24	2.5	73	0	81	0
2:00 AM	90	0	24	0	72	2.5	80	2.5
3:00 AM	91	-2.5	24	0	72	0	79	2.5

4:00 AM	89	5	23	2.5	72	0	79	0
5:00 AM	88	2.5	24	-2.5	72	0	79	0
6:00 AM	88	0	23	2.5	72	0	79	0
7:00 AM	89	-2.5	22	2.5	71	2.5	80	-2.5
8:00 AM	87	5	20	5	72	-2.5	79	2.5
9:00 AM	88	-2.5	21	-2.5	71	2.5	78	2.5
10:00 AM	88	0	20	2.5	70	2.5	78	0
11:00 AM	86	5	20	0	69	2.5	77	2.5
12:00 PM	88	-5	19	2.5	68	2.5	77	0
1:00 PM	87	2.5	18	2.5	67	2.5	76	2.5
2:00 PM	86	2.5	17	2.5	67	0	76	0
3:00 PM	86	0	15	5	65	5	75	2.5
4:00 PM	86	0	15	0	64	2.5	74	2.5