POLYSACCHARIDE CONTENT AND GROWTH RATE OF *LESSONIA VARIEGATA* J. AGARDH: INVESTIGATING ITS POTENTIAL AS A COMMERCIAL SPECIES.

By

Lynaire Jane Abbott

A Thesis

Submitted to the Victoria University of Wellington

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Marine Biology

Victoria University of Wellington

2011

Abstract

The endemic brown alga *Lessonia variegata* has recently been shown to be four separate lineages. To determine differences between the four morphologically similar lineages, the economically valuable polysaccharides alginate and fucoidan were extracted and yields from each of the lineages were compared. In order to determine seasonal patterns in the yield of alginate and fucoidan, and the growth rate within *L.variegata*, polysaccharides were extracted and the growth rate measured on a monthly basis from March 2010 until February 2011 on plants from the Wellington lineage. The alginate and fucoidan yields were obtained via stepwise extraction with dilute acid and sodium carbonate as per the previously published methods of Usov *et al*. (1985). The growth rate of *L. variegata* from the Wellington lineage was assayed using the hole punch technique first described by Parke (1948). The yield of alginate within the Wellington lineage of *L. variegata* fluctuated seasonally with the highest percent occurring in spring and summer 2010. The yield of fucoidan in the Wellington lineage was at its highest in mid-autumn and late spring 2010. Two different growth rates were detected for the Wellington lineage of *L. variegata.* There was a period of significantly high growth from late winter 2010 until late summer 2011.The Wellington lineage had the lowest yield of alginate and the highest yield of fucoidan compared to the Northern lineage, the Kaikoura lineage and the Southern lineage. Based on the findings of this study, an appropriate harvest period for the Wellington lineage of *L. variegata* would be in early to mid-summer when polysaccharide yields and growth rates are high and the alga is vegetative.

Acknowledgements

I'd like to thank the following people who have helped me to complete my masters' dissertation:

Joe Zuccarello, my fabulous supervisor; for his patience, guidance, editing assistance, discussions and questioning which have all helped me to develop my skills in phycology.

Ceridwen Fraser for organising for the collection of algae from the Southern lineage.

Richard Taylor for organising for the collection of algae from the Northern lineage.

Leigh Tate and Paul South for the collection of algae from the Kaikoura lineage.

Pat Abbott, Lachlan Dixon and Aida Gulbransen for their assistance with collecting samples along the Wellington coast.

The Wellington Botanical Society for their grant to assist with field work.

Erasmo Macaya for his advice and help with locating reproductive samples of *Lessonia variegata* from the Wellington lineage.

Peter Martin for his valuable discussions and assistance with molecular work.

Joe Bucchanan for his valuable discussions and assistance with oral presentations via constructive advice.

Wendy Nelson for her assistance with locating information concerning both Lessonia variegata and Surface Sea Temperature.

Alan Hoverd, Chris Thorn, Mairead Murphy, Leslie Millicich and John van der Schmon for their assistance with locating equipment and for their technical advice.

Pat Abbott, Ray Abbott, Campbell Dixon and Lachlan Dixon for proofreading thesis drafts and giving useful feedback.

Table of Contents

List of Figures

List of Tables

Introduction

Historically the word 'kelp' has either referred to the ashes of brown seaweed, used for the purpose of glass and soap manufacturing; or to the large brown seaweeds of the orders Laminariales or Fucales (Onions 1936; Chapman 1970). Confusion over the use of the word 'kelp' to describe Laminariales occurs frequently, perhaps due to members of the genus *Durvillaea* (Fucales, Phaeophyceae) being commonly referred to as 'bull-kelp' (Fraser *et al*. 2009). Kelp are members of the order Laminariales (Bolton 2010) and phycologists (specialists in the study of algae) use the term 'kelp' to refer to members of the order Laminariales.

Kelp (Laminariales, Phaeophyceae) are important to the ecology of the subtidal environment. They provide a 3-dimensional habitat utilized by a rich variety of fish and invertebrate species (Tala and Edding 2005; Villegas *et al*. 2008). Commercially, kelp are important mainly for their use as fodder in the mariculture of urchins and abalone; for the use of polysaccharide extracts such as alginate for food and pharmacological products (Nelson 2005; Tala and Edding 2005; Gutierrez *et al*. 2006; Westermeier *et al*. 2007; Dhargalkar and Verlecar 2009) and fucoidan for nutraceutical products (Wijesekara *et al*. 2011). As the harvesting of kelp in the wild has increased, concerns have grown about the sustainability of kelp (Nelson 2005; Westermeier *et al*. 2007; Dhargalkar and Verlecar 2009). Methods of farming Laminariales, such as *Macrocystis* spp. and *Lessonia* spp. (Edding and Tala 2003) have been explored to enable commercial farming of kelp to meet consumers' needs without depleting wild stocks (Gutierrez *et al*. 2006; Westermeier *et al*. 2006; Buschmann *et al*. 2008).

A widespread subtidal species of kelp in New Zealand that is of commercial interest is *Lessonia variegata* J. Agardh (Nelson 2005). The commercial interest is due to its bioactive compounds and its suitability as fodder for farmed abalone (Nelson 2005). Bioactive compounds, such as the polysaccharides alginate and fucoidan, are found in the walls or mucilage of the cells of *L. variegata* (Chapman and Chapman 1980; Nelson 2005; Skriptsova *et al*. 2010). Alginates are used in the food industry as thickening agents and in the pharmaceutical industry as binders (Zvyagintseva *et al*. 1999), gelling agents and wound absorbents (South and Whittick 1987; Fenoradosoa *et al*. 2010). Fucoidan is of use to humans as it is known to be an anti-viral agent (Witvrouw and De Clercq 1997; Zvyagintseva *et al*. 1999), to have anticoagulant properties (Chandía and Matsuhiro 2008; Wang *et al*. 2009; Costa *et al*. 2010), to inhibit the leukocyte movement into tissues and to modulate the transmission of disease (including cancer) from one part of the body to another (Wang *et al*. 2009).

Lessonia variegata consists of four evolutionary lineages (Martin 2011). It is of interest to this study to identify a lineage of *L. variegata* with increased amounts of fucoidan and/or alginate and to identify a season within the Wellington lineage with increased levels of fucoidan, alginate or growth. Knowledge pertaining to a lineage or a season of increased amounts of fucoidan, alginate, or growth could be commercially important for informing decisions related to the best time and place to harvest *L. variegata* for exploitation of these bioactive compounds.

Taxonomy and evolution

Molecular clock analysis suggests that the order Laminariales (Phaeophyceae) diverged from other brown algae 16 to 30 million years ago (Saunders and Druehl 1992). Laminariales are characterized as follows: having a diplohaplontic life cycle, with an alternation between highly differentiated macroscopic diploid sporophytes and microscopic haploid gametophytes (Boo and Yoon 2000); the lack of an eyespot in meiospores; the use of the sexual pheromone lamoxirene (Lane *et al*. 2006) and a unique flagellation in the sperm whereby the chloroplasts are not associated with the flagella base (Kawai and Sasaki 2000). The Laminariales consist of three families; Alariaceae, Laminariaceae, and Lessoniaceae (Lane *et al*. 2006). Lane *et al*. (2006) have proposed a new family, Costariaceae, be included within the Laminariales based on molecular data. Traditionally the family Lessoniaceae was characterised morphologically by the 'splitting' of the original blade into two, with the division extending through the transition zone (Bold and Wynne 1978). The Lessoniaceae were polyphyletic due to the polyphyletic origin of the character of meristem splitting (Saunders and Druehl 1993; Lane *et al*. 2006), and presently consists of the genera *Ecklonia, Eckloniopsis, Egregia, Eisenia* and *Lessonia* (Lane *et al*. 2006).

The genus *Lessonia* is wide-spread in central and southern Peru; Chile; Southern Argentina; the Falkland Islands; Tasmania, Australia; and New Zealand (Hay 1987). It is the only kelp genus confined to the southern Hemisphere (Lane *et al*. 2006).

Historically, species of Laminariales were defined based on their morphological characteristics (Lane *et al.* 2006). Use of molecular data has shown that the identification of species based on a morphological basis alone has limitations. The family *Alariaceae* is comprised of twelve recognised species which are difficult to recognise based on

morphological characters (Lane *et al.* 2006). Lane *et al.* (2006) used DNA barcoding, using the 5' end of the mitochondrial gene encoding cytochrome c oxidase 1 (cox 1-5') to demonstrate the value of the use of molecular data to discriminate between morphologically similar species. Some species of Laminariales that are not morphologically distinguishable have been shown to be separate species, such as *Lessonia nigrescens* Bory (Tellier *et al*. 2009). Conversely, molecular evidence has identified species of Laminariales that are morphologically plastic (and therefore previously thought to be more than one species) as one species (such as *Macrocystis pyrifera* (Linnaeus) J. Agardh, (Macaya and Zuccarello 2010)). Previously *M. pyrifera* was thought to be four species (*Macrocystis angustifolia* Bory, *Macrocystis integrifolia* Bory, *Macrocystis laevis* C. H. Hay *and Macrocystis pyrifera)* based on holdfast (the attachment of the thallus to the substrate) and blade morphology (Macaya and Zuccarello 2010). Other species vary morphologically among their populations, but are one species, such as *Lessonia trabeculata* Villouta and Santelices in northern Chile (Villegas *et al*. 2008). One population appears shrub-like with the total mass distributed among many stipes with high flexibility and the other is arborescent where the total mass is allocated to a very few stipes with limited flexibility (Villegas *et al.* 2008). The morphology of the populations relate to their environment as the shrub-like population exists in areas with high levels of turbulence (Villegas *et al*. 2008).

Lessonia variegata is an example of a species in which molecular analysis was used to identify four evolutionary lineages (Martin 2011) in what was previously thought to be one species (Adams 1994). The evolutionary lineages are morphologically similar, except for variegation in the blades of the Wellington lineage, but each lineage is easily detectable via molecular analysis.

The distribution of Lessonia

Lessonia are distributed in the Southern Hemisphere (Hay 1987) between latitudes of 17°S and 56°S (Tellier *et al*. 2009). Their distribution suggests that they are under the influence of the Antarctic Circumpolar current (west wind drift), the current system around the southern oceans that is believed to play a major role in the distribution of marine taxa (Tynan 1998; Waters 2008). The distribution of *Lessonia* is along wave exposed coasts of Central and Southern Peru, Chile, south Argentina, Falkland Islands, Kerguelan Islands, Heard Island, Tasmania and New Zealand (Hay 1987, Tellier *et al*. 2009). Four species, (*Lessonia nigrescens* Bory*, Lessonia trabeculata, Lessonia flavicans* Bory*, Lessonia vadosa* Searles) are found on the South American coast; one species, (*Lessonia corrugata* Lucas*)* is found in Tasmania, Australia; and four species, (*Lessonia variegata, Lessonia adamsiae* C.H. Hay*, Lessonia brevifolia* J. Agardh and *Lessonia tholiformis* C.H. Hay) are found in and around New Zealand (Hay 1987).

Lessonia are thought to be poor dispersers (Hay 1987, Tellier *et al*. 2009) and the gametophytes are thought to only travel metres before settling onto the substrate. Sporophytes of *Lessonia* are not buoyant and they are thought to only be capable of dispersing long distances by becoming entangled in rafts of buoyant Laminariales species such as *Macryocystis* (Hay 1987).

Lessonia variegata is a sub-tidal species endemic to mainland New Zealand. It is widespread along the wave exposed coast of the North and South Islands, and Stewart Island at depths from 2-20 m (Nelson 2005). The four evolutionary lineages of *L. variegata* have mostly non-overlapping ranges (Martin 2011). The ‘Northern’ lineage is distributed from outer South Head around North Cape to East Cape. The ‘Wellington’ lineage is

distributed from East Cape across Cook Strait to White Bay in Marlborough Sounds. The 'Kaikoura' lineage is distributed between Cape Campbell and Goose Bay and the 'Southern' lineage is distributed from Tumbledown Bay to Stewart Island (Fig. 1). The genetic distance between *L. variegata* lineages is of equal magnitude to the genetic distance between known species (Martin 2011).

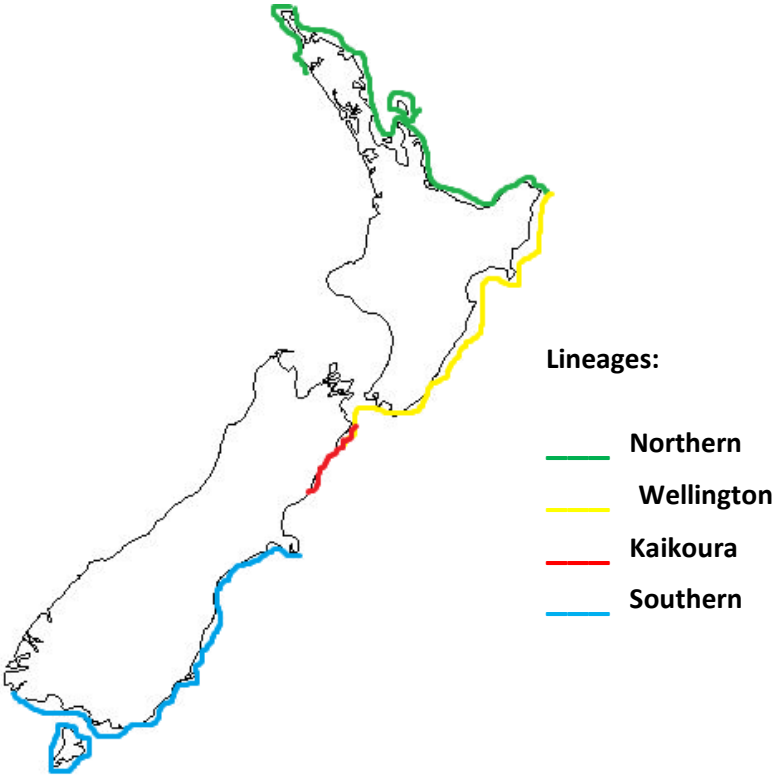

Fig. 1. Map of New Zealand highlighting the approximate distribution of each of the 4 evolutionary lineages of *Lessonia variegata.* Note: Coastal areas on the map not coloured indicate areas where *Lessonia variegata* is not present or areas not surveyed.

Lessonia variegata has a heteromorphic life cycle, alternating between the sporophytic meiospore-bearing phase, and the microscopic gametophytic phase (Nelson and Schwarz 2005). When *L. variegata* is fertile, the sori (clusters of sporangia) become raised, changing to a darker colour (personal observation). Schwarz *et al*. (2006) found blades of three populations of *L. variegata* in the Wellington lineage to be mature from April through to September. The highest percentage of sori mature from late winter to early

spring when day length is increasing and water temperatures are at their lowest mean annual levels (Nelson 2005). My study of the Wellington lineage found sori to mature in late autumn to early winter in the observed population during the year 2010 (personal observation).

In anticipation of environmental problems caused by overexploitation of natural algal beds of Laminariales species, mariculture techniques have been developed to grow sporophytes (the diploid macroscopic life cycle stage) in culture for possible future transplanting for farming purposes and for regenerative seed stock of depleted wild stocks (Nelson 2005; Westermeier *et al*. 2006; Shan and Pang 2009). Potential uses and commercial interest that may cause population declines in *L. variegata,* in particular, include harvesting wild stocks for the extraction of bioactive compounds such as fucoidan and alginate for human use, and for fodder for the aquaculture of abalone (Nelson 2005).

Ecological Importance

Kelp are of ecological importance to the coastal environment. They ameliorate the effects of wave action on the shore (Raimondi *et al.* 2004), provide protection to organisms from harmful UV radiation (Bruno *et al*. 2003), are an ideal nursery ground for fish larvae due to their complex structure (Camus 1994); and are a food source to a variety of organisms (Gaylord *et al*. 2004; Contreras *et al*. 2007; Tala and Edding 2007).

Lessonia variegata provides shelter, food and habitat for fish, algae and invertebrates (Hawes *et al.* 2004; Nelson and Schwarz 2005). *L. variegata* is of such importance in New Zealand that it is one of seven genera of algae proposed by the Ministry of Fisheries to be introduced into the Quota Management System (QMS), the system used to manage New Zealand's commercial fisheries (Nelson 2005). To date, only one of the

seven genera of algae has been included in the QMS. *Macrocystis pyrifera* was included into the QMS in October 2010 with catch limits set for the east coast of the South Island (QMS area KBB3G) and the Chatham Islands (QMS area KBB4G) at 1238 tonnes and 274 tonnes respectively (Heatley 2010).

Commercial application of kelp

Historically, brown seaweed were initially harvested commercially for the production of soda utilized mainly in the manufacturing of glass (Chapman 1970) and later kelp were harvested as a source of iodine for the medicine industry (Chapman 1970). At one stage kelp were harvested for the production of potassium carbonate required to make gunpowder in World War 1 (Neushul 1989). In present times they are harvested mainly to be used as fertilizer; stock feed; or for the extraction of the large amounts of cell constituents, such as the alginates and/or fucoidans (Fenoradosoa *et al.* 2010) utilized in industry and in the food or nutraceutical markets.

Laminariales represent an important economic resource in Chile for alginate production, bioremediation and as forage for maricultured urchins and abalone (Nelson 2005; Gutierrez *et al*. 2006; Westermeier *et al.* 2007). *Lessonia* also contain fucoidan of which there is a growing interest within the nutraceutical field (Wijesekara *et al.* 2011).

The major structural polysaccharide of brown algae is alginate (Chandía *et al*. 2001), which is linear anionic polysaccharide with a structure of 1,4-linked α-L-guluronate and β-D-mannuronate blocks (Storz *et al.* 2009) (Fig.2). It occurs in exploitable quantities in Laminariales and Fucales and is commercially extracted mainly from *Ascophyllum nodosum* (Linnaeus) Le Jollis*, Laminaria* spp, *Lessonia nigrescens, Ecklonia maxima* (Osbeck) Papenfuss*, Macrocystis pyrifera* and *Durvillaea antarctica* (Chamisso) Hariot; where it can

reach up to 40% of the dry weight (Fenoradosoa *et al.* 2010). Alginates are employed mainly in the food industry for their thickening characters and for their gel-forming abilities (Fenoradosoa *et al*. 2010); they are also utilized extensively in the cosmetic and pharmaceutical industries as emulsifiers and gelling agents (South and Whittick 1987). *Lessonia trabeculata* is the main source of alginate along the Northern Chilean coast with the average alginic acid from its blade, stipe and holdfast being 15.3 – 21.3% of dry weight (Dhargalkar and Verlecar 2009).

The chemical structure of alginates varies between species. Alginate is composed of blocks of mannuronic and guluronic acid, referred to as M and G blocks, which are assembled in various sequences of MM, MG and GG blocks (Fig.2). The elasticity of the alginate is dependent on the chemical structure of the alginate. A higher M/G ratio is synonymous to a more elastic alginate (Miller 1996) which has a higher freeze and thaw stability (Sriamornsak and Sungthongjeen 2007) and a lower M/G ratio is synonymous to a more brittle alginate (Miller 1995) which is heat stable (Sriamornsak and Sungthongjeen 2007). *Lessonia variegata* has been shown to have an M/G ratio of 1.95 (Miller 1996) which is quite a high M/G ratio equating to an elastic gel.

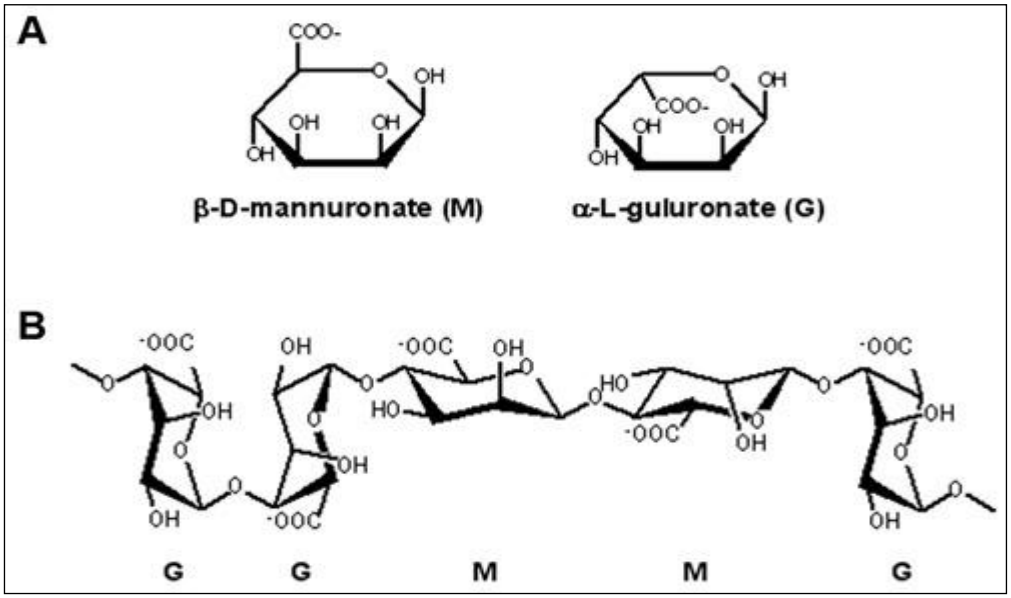

Fig.2. Structural data for alginates: (a) the monomers in alginate; (b) the alginate chain, in this instance the fragment: GGMMG (G = guluronic acid; M = mannuronic acid) is shown (Sriamornsak and Sungthongjeen 2007).

Variation in the composition of alginate has been recorded for the same species at different locations; whereas composition of alginate for the same species, at the same location but for different years has been shown to be the same (Craigie *et al.* 1984). Craigie *et al.* (1984) extracted alginate from a number of orders of Phaeophyceae from Nova Scotia, Canada, and found that the alginate composition varied between members of the same species at different locations; but did not vary between members of the same species at the same location in different years. This implies that alginate composition is not random but may be related to environmental influences such as wave action, temperature, and sunlight hours; which in turn may be controlled by the enzymes involved in alginate production and used within the cells (Miller 1996).

The life history of kelp (alternation of generations) makes farming of alginophytes expensive and time consuming compared to the cost of harvesting and transporting wild seaweed (Dhargalkar and Verlecar 2009) therefore algae utilized for the extraction of alginate is harvested from wild stocks. Most cultivated algae are cultivated for the purpose of human consumption (Shan and Pang 2009), except for *Laminaria japonica* Areschoug which is cultivated in China, mainly as a food source but also for an alginate source (Dhargalkar and Verlecar 2009).

Fucoidans are sulfated polysaccharides found mainly in various species of brown seaweeds. They were first discovered and named fucoidin by Kylin (1913), but they have since had a name change to fucoidan in accordance with IUPAC rules (Li *et al.* 2008). Sometimes fucoidans are referred to as fucans, fucosans or sulfated fucans. They are located within the inter-cellular mucilage and the cell walls of Phaeophyceae and their composition and quantity fluctuate with season, maturity (Chapman and Chapman 1980; Wang *et al.* 2009; Skriptsova *et al.* 2010), species (Zhuang *et al.* 1995) and part of the plant (Honya *et al*. 1999).

Fucoidans have been shown to be helpful in the prevention and treatment of cardiovascular disease by having anticoagulant properties (Chapman and Chapman 1980; Costa *et al*. 2010). They also have the potential to be a reactive oxygen species (ROS) scavenger (Witvrouw and De Clercq 1997; Wang *et al*. 2009) as well as being of value due to their antithrombotic, antiviral (Hemmingson *et al.* 2006), antitumour (Yang *et al.* 2008) and antioxidant properties (Boo and Yoon 2000; Wang *et al.* 2009). In Japan and the United States of America the health benefits to humans from the oral intake of fucoidans is well known; for example, fucoidans are included in mainstream foods in Japan such as yoghurts

and fruit juices; and in the USA, South Pacific derived fucoidan-based liquid preparations are increasing in popularity (Fitton *et al.* 2008).

Fucoidans have high concentrations of L-fucose and sulfate, together with minor amounts of monosaccharide including xylose, galactose and mannose (Li *et al.* 2008; Kim *et al.* 2010) (Fig.3). Fucoidans from various species of algae have different chemical structures, different composition and varying molecular weights (Zhuang *et al*. 1995; Honya *et al.* 1999) which are all thought to contribute to the spectrum of biological action (Yang *et al*. 2008; Costa *et al.* 2010; Skriptsova *et al*. 2010). There is also variation in the structure and quantity of fucoidans extracted from different parts of the same plant (Honya *et al*. 1999; Skriptsova *et al.* 2010) and from plants of different ages (Zvyagintseva *et al.* 2005). Extensive research projects are being undertaken to elucidate the relationship between the structure and activity of the fucoidans (Li *et al*. 2008). Publications have reported that the correlation between fucoidans and seasonality show that the amount of fucoidans increase five-fold with the production of sori (Honya *et al.* 1999; Skriptsova *et al.* 2010). It was thought that the production of sori caused a subsequent effect of the synthesis of fucoidans perhaps via enzymatic activity, leading to an increase in the amounts of crude fucoidans in the sporophylls (blades bearing sporangia) (Skriptsova *et al*. 2010). Therefore, knowledge about the fluctuating levels and structure of fucoidans from a particular species of kelp could lead to planned harvesting at different times of the year depending on the required structure and thus the desired pharmacological properties (Skriptsova *et al.* 2010).

The structure of the fucoidan from *Lessonia variegata* has not been published but the structures from some other species of *Lessonia* have been reported, for example a 1 →3 linked α-L-fucan from *Lessonia vadosa* (Chandía *et al.* 2005; Chandía and Matsuhiro, 2008).

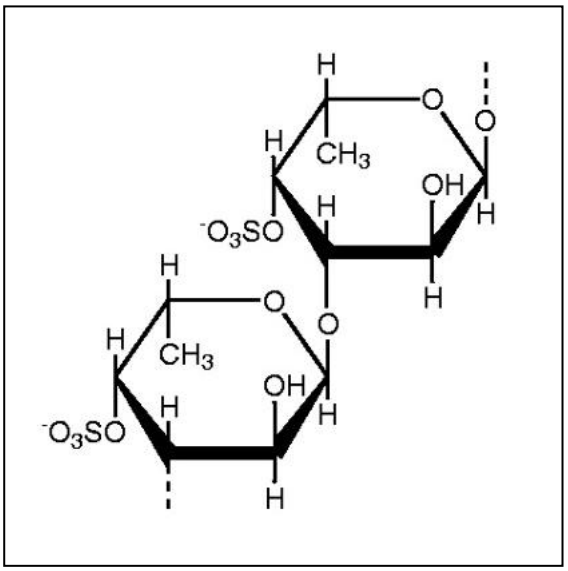

Fig 3. 1 → 3 linked α-L-fucopyranose 4-sulfate, as found in some algal fucoidans (Bearteau and Mulloy 2003).

Information about the seasonal growth rates of kelp along with information about the reproductive timing is useful for determining an appropriate season for the harvesting of kelp while still maintaining the recruitment of juvenile plants (Schwarz *et al*. 2006). Parke (1948) identified two distinct rates of annual growth for Laminariales, a slow rate and a rapid rate. Identification of the pattern of growth for *Lessonia variegata* coupled with information relating to enhanced levels of fucoidan and alginate could be of high importance for determining an appropriate time to harvest this species.

In the present study, I investigated the following aspects of *Lessonia variegata*:

1) Monthly variations in the yield of fucoidan and sodium alginate within the Wellington lineage;

2) Monthly variations in the growth rate of blades within the Wellington lineage;

3) Variation in the yield of fucoidan and sodium alginate between the Wellington lineage and each of the other lineages.

Aims

This study aimed to investigate seasonal changes within the Wellington lineage of *Lessonia variegata* to better determine its pattern. The Wellington evolutionary lineage was selected as a study species due to the convenience of access to the study site on a regular basis over the course of a twelve month period. The characters that were measured and compared (monthly and for a period of a year) were the yield of alginate, the yield of fucoidan and the growth rate. Each of these characters is potentially of commercial interest and the detection of a season with superior qualities in one or more of these traits could be of economic importance.

Evolutionary lineages of *Lessonia variegata* are different to each other genetically and in their geographic distribution (Martin 2011). Morphologically, however the four evolutionary lineages of *L. variegata* are similar. It is of interest to this study to explore other differences between the lineages such as differences in the cell wall chemistry between *L. variegata* lineages.

In order to determine differences in the cell wall chemistry of *Lessonia variegata* lineages, alginate and fucoidan were extracted from *L. variegata* from each of the four evolutionary lineages (Northern, Wellington, Kaikoura and Southern); and the yields were compared between lineages. The identification of an evolutionary lineage of *L. variegata* with a higher yield of alginate and/or fucoidan could be beneficial for the selection of a species of *L. variegata* for commercial exploitation.

This study aimed to look at changes within *Lessonia variegata* to better determine its pattern. Knowledge pertaining to its patterns could be of commercial importance to fisheries when planning for commercial harvesting of *L. variegata. Lessonia variegata* is one

of the seven genera of algae that are proposed by the Ministry of Fisheries to be included in the QMS. When *L. variegata* is included into the QMS, knowledge pertaining to its seasonal patterns of growth rates, and yields of alginate and fucoidan could be important for the Minister of Fisheries to inform decisions made regarding total allowable catch (TAC) relevant to quantity, area, and season of harvest.

Materials and Methods

Study site

The Wellington study site (Fig. 4), close to the old quarry near Owhiro Bay on the South Coast, was selected based on its easily accessible location within a well established population of the study species, *Lessonia variegata* 'Wellington lineage'. The study site (GPS 41° 34.930 S, 174° 73.961 E) is located outside of the Taputeranga Marine Reserve, therefore allowing for the sampling of flora. It is in a relatively sheltered bay which is protected from the predominant northerly winds but exposed to strong southerly winds.

Samples of *Lessonia variegata* that were collected from the Southern, Kaikoura and Northern species (Fig. 4) were collected at locations convenient to the collectors. Blades from the Southern population were collected by Ceridwen Fraser at (GPS 45° 73.609 S, 170° 59.712 E) on 21 March 2010. Samples from the Kaikoura population were collected at South Bay on 13 April 2010 by Leigh Tate and Wairepo Bay on 21 April 2010 by Paul South. The collecting of samples from the Northlern population was organised by Dr. Richard Taylor. They were collected near Mokohinau Islands (GPS 35° 54.70S, 175° 06.13E) on 5 October 2010.

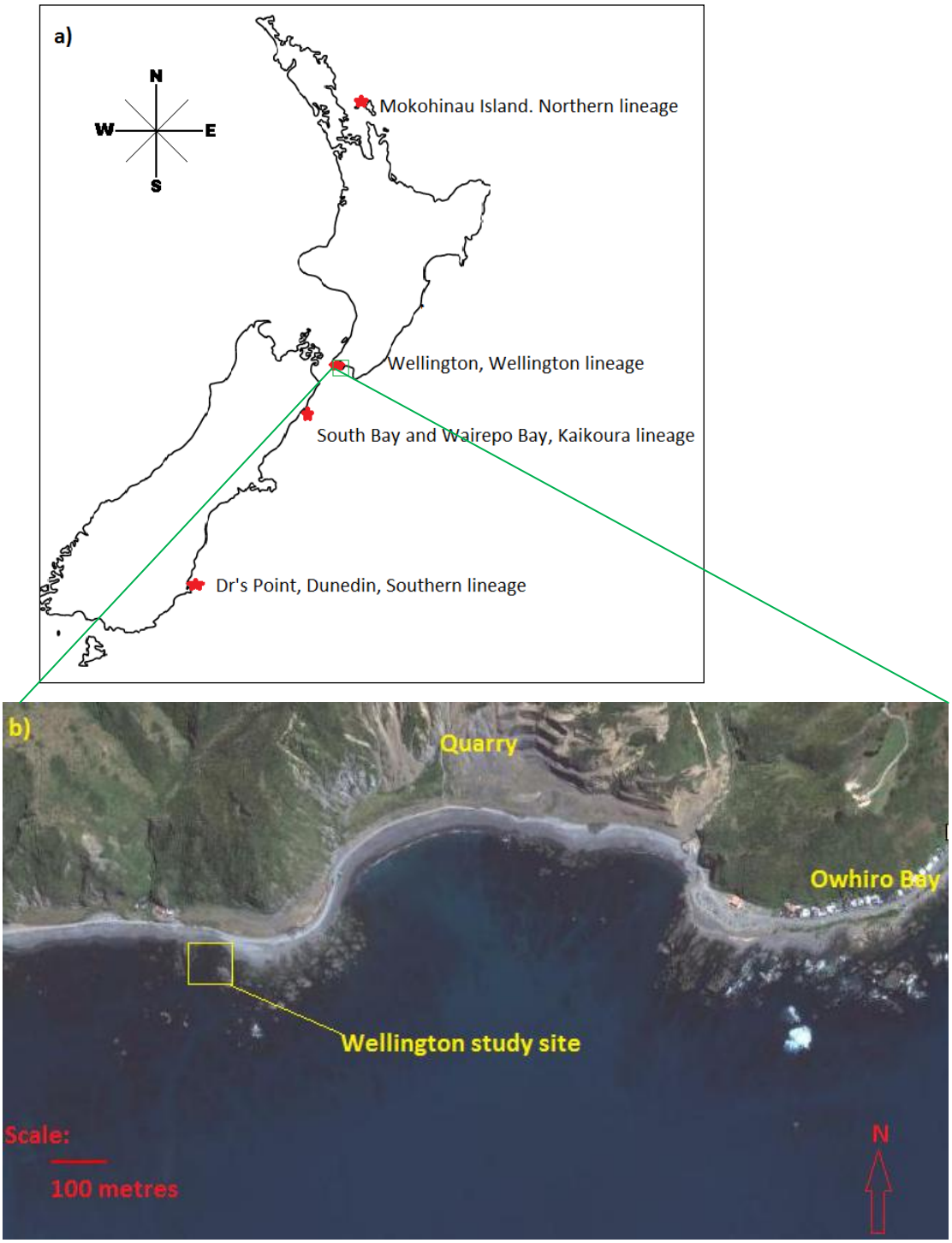

Fig. 4. The location of sampling a) for each of the four lineages of *Lessonia variegata*; Northern, Wellington, Kaikoura, and Southern lineages; b) for the Wellington lineage, on the south coast of Wellington (Google Earth 5.2.1.1329).

Polysaccharide extraction

Ten individuals of *Lessonia variegata* growing at the Wellington study site (Fig 4) were randomly selected each month from the *L. variegata* plants that were growing across the width of the study site. Collection of the plants began in March 2010 and ended in February 2011. The selected plants had a minimum of 100 blades and were growing at a depth of >1 metre at low tide. Two blades from each of these ten *Lessonia variegata* plants were harvested. The two blades were combined to make one sample from each plant. The self-imposed size restriction for selected plants (100 blades) was thought to be a good indicator of mature plants within the population at this site. Plants with fewer than 100 blades appeared to be smaller and could be adversely affected by the harvesting of their blades, whereas the plants with more than 100 blades were hypothesised to not be adversely effected by having two of their blades removed. The blades were wrapped in paper towels and taken to the laboratory within 2 hours of harvesting. A small piece of tissue (~30 mm x 20 mm) was cut from each blade and stored in a sealed plastic bag with silica beads for future DNA analysis, if needed. The harvested blades were washed with 70% ethanol and then rinsed with tap water to remove epiphytes and other contaminants. The blades were oven dried at 60°C overnight. The basal portion, near the stipe, of each blade was then milled to <1mm particles using a mortar and pestle. The basal portion was chosen to be used for the polysaccharide extraction because it is the youngest tissue and it was thought to be a good representation of the growth material for that month. Using the same portion of the blade each month allowed for some standardisation of the measurements in order to be able to make comparisons between different months.

Two blades from each of ten individuals of *L. variegata* were harvested from each of the three other lineages (Southern, Kaikoura and Northern). These blades were rinsed with ethanol and then with water; and dried in an oven at 60° overnight before being sent to Wellington. They were then milled in the same way as the Wellington lineage.

The methods used for the extraction of the polysaccharides were modified from the previously published methods of Usov *et al*. (1985) (summarized in Fig.5).

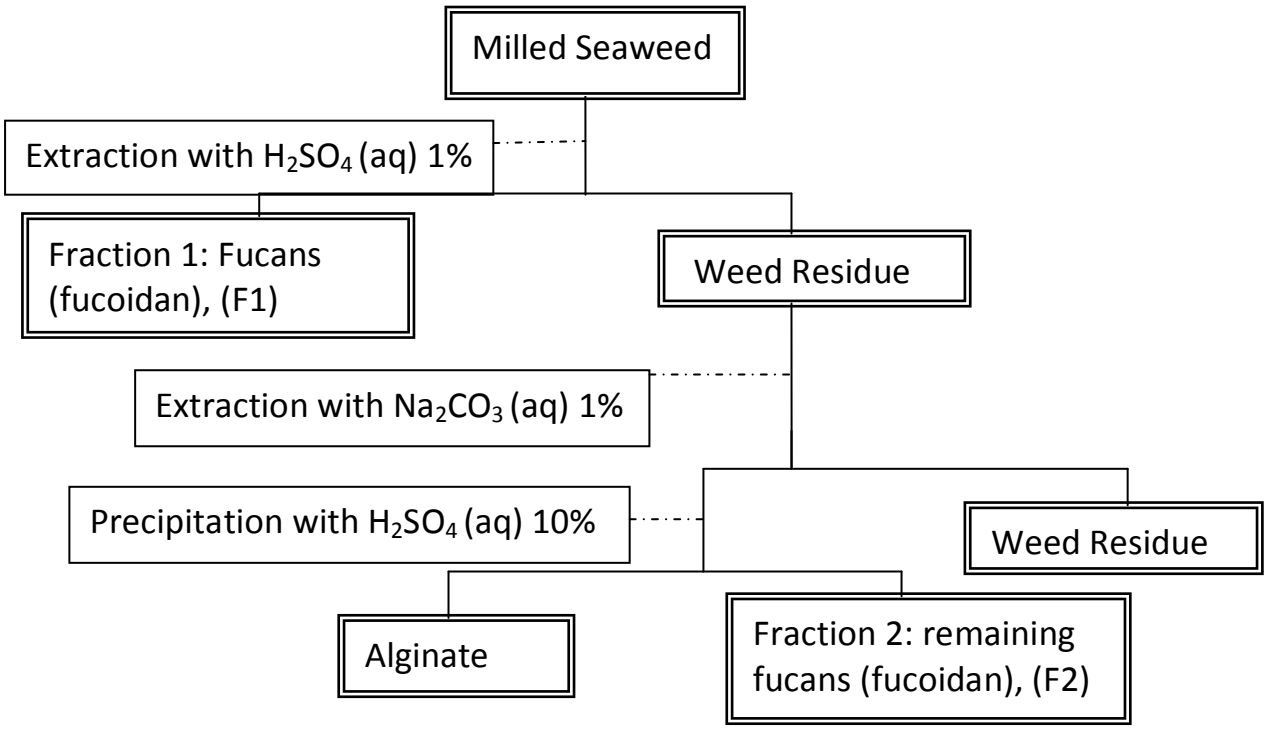

Fig. 5. Schematic diagram depicting the extraction of the polysaccharides fucoidan and alginate from the alga using selective solvents.

First Fucoidan extraction (Fraction 1; F1)

Monthly and for each of ten *Lessonia variegata* plants, 300 milligrams of the milled blades was stirred with 15 millilitres of a 1% solution of sulphuric acid (H_2SO_4) on a magnetic stirrer for four hours at room temperature. The suspension was centrifuged (2150 x g) for twenty minutes. (Hereafter whenever centrifuging is mentioned, a Sigma 2-16P is used at 2150 x g for 20 minutes). The supernatant was decanted and stored at 4°C. The residual seaweed was stirred with 15 millilitres of a 1% solution of H_2SO_4 on a magnetic stirrer overnight at room temperature. The suspension was centrifuged and the supernatant decanted and pooled with the first supernatant. The pooled supernatants were neutralized using approximately 2.2 millilitres of a 10% solution of sodium hydroxide (NaOH), using a handheld pH meter to check when pH 7.0 had been reached. The sample was then dialysed in 44 millimetre wide, flat dialysis tubing with a molecular weight cut-off of 14 kilo Daltons (MW 14 kDa) for 72 hours against running tap water and then overnight against distilled water to remove salts. The dialysed extract was condensed in an oven at 70° C for approximately four hours until 10 millilitres of the fucoidan extract remained. The fucoidan extracts were lyophilised and the dried extract was weighed and recorded as a percentage of the original dry weight of the milled seaweed.

Alginate extraction (Alginate Fraction)

To extract the alginate from the seaweed, the weed residue from the fucoidan extraction above was combined with 15 millilitres of a 1% solution of sodium carbonate (Na_2CO_3) and extracted for three hours at room temperature. The suspension was centrifuged and the supernatant was decanted and stored at 4° C. The seaweed was further extracted with 15 millilitres of a 1% solution of Na_2CO_3 for an additional three hours after

which the suspension was centrifuged and the supernatants pooled. The seaweed was extracted again with 15 millilitres of a 1% solution of Na_2CO_3 overnight, followed by the suspension being centrifuged and the supernatants pooled. The weed residue from the extraction was washed with 4 millilitres of distilled water and centrifuged.

The washings from the weed residue were decanted and added to the pooled supernatants. The pH of the pooled supernatants was adjusted to pH 1.6 by adding a 10% solution of H_2SO_4 to the supernatants. The supernatants were then centrifuged to separate the precipitated alginate from the suspension. The supernatant was decanted and the remaining alginate was washed twice with 4 millilitres of distilled water, centrifuged each time and washed with 4 millilitres of acetone, centrifuged, decanted and air dried on the laboratory bench for 48 hours. The dried precipitated alginate was weighed and the weight was recorded. The distilled water and acetone washings were discarded.

The Second Fucoidan Extraction (Fraction 2; F2)

Following the extraction of the alginate from the solution (above), the decanted supernatant was neutralized with a 10% solution of NaOH. It was then dialysed in 43 millimetre wide flat dialysis tubing (MW 14 kDa) for 72 hours against running tap water and then overnight against distilled water to remove salts. The dialysed extract was condensed in an oven at 70°C for approximately eight hours until 10ml of the fucoidan extract remained. The fucoidan extracts were lyophilised and the dried extract was weighed, added to the weight of the initial fucoidan extracted and recorded as a percentage of the original dry weight of the milled seaweed.

Purification of sodium alginate

20.66 millilitres of 1% Na_2CO_3 was added to 6.2 milligrams of the alginate fraction from one randomly selected sample and stirred for 16 hours at room temperature. The sample was centrifuged to separate the precipitate which was then discarded. The supernatant was dialysed for 72 hours in running tap water and then overnight with distilled water. 20.66 millilitres of 2% calcium chloride ($CaCl_2$) was added to the solution which was then centrifuged to separate off the calcium alginate. The precipitate was washed with distilled water and centrifuged to separate the precipitate from the supernatant. The precipitate was stirred with 62 millilitres of 1N HCl for 2 hours. The solution was centrifuged and the supernatant was decanted. The precipitate was washed with 0.5 N HCl, centrifuged and then washed seven times with distilled water. It was centrifuged after each wash. The precipitate was suspended in distilled water and brought into solution by the careful addition of 1 N NaOH. The solution was dialysed for 72 hours against running tap water and then overnight against distilled water. It was then lyophilized to produce a purified, colourless sample of sodium alginate.

Residual seaweed

The solids that were left after the alginate extraction were washed twice with 5ml of distilled water. The solution was centrifuged each time and the supernatants were discarded. The remaining seaweed residue was oven dried at 50°C overnight and the dried residue was weighed and recorded.

Measuring the growth rate of Lessonia variegata from the Wellington lineage

Twenty plants at the Wellington study site (Fig. 4) were selected for the growth study which commenced with tagging plants on 1st March 2010 and ended with the final collection of plants on 21 February 2011. For the selection of plants, the study site was divided into two areas (sites a and b, Fig. 6). Each study area was divided into an inshore area and an offshore area in order for the plants selected to be spread out rather than clustered into one small area. Plants displaying evidence of extensive herbivory were avoided because extensive herbivory has been reported to be a cue for the production of additional defensive chemicals in brown algae, which could lead to reduced growth (van Alstyne 1988). Different plants to the ones used in the polysaccharide extractions were used for the growth study to avoid possible chemical changes within a plant that may have occurred as a result of hole punching.

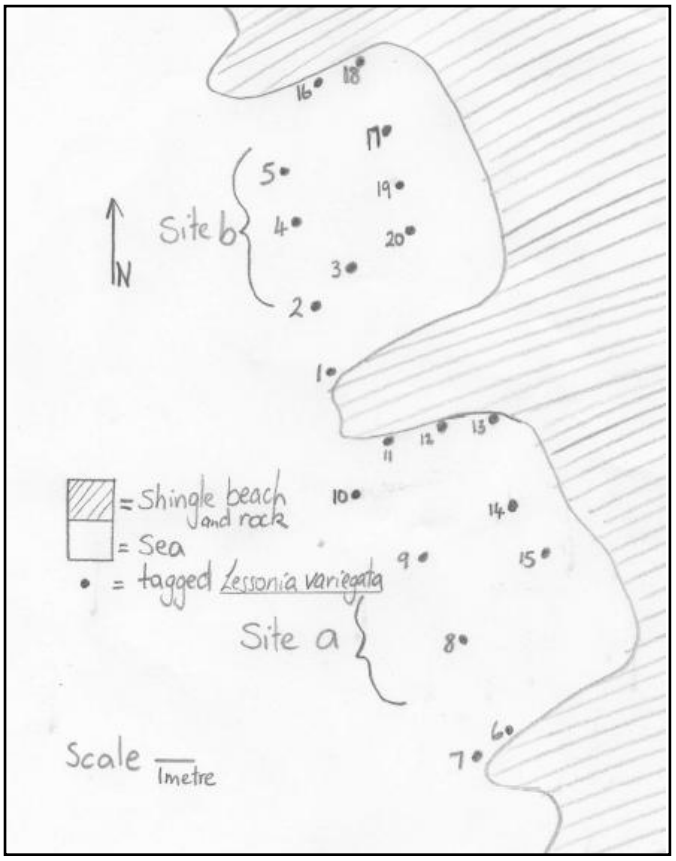

Fig. 6. Field sketch of the study site on the south coast of Wellington. The dots numbered 1-20 represent the location of individual *Lessonia variegata* plants used in the growth study.

Plants were marked for identification by tagging using a small plastic tag cut from the lid of an ice-cream container. The tags were marked with a number from 1 to 20 etched and written on them (Fig. 7). Each tag had a 5mm hole made using a hole punch and was attached to the stipe of one of the blades of the plant using a cable tie.

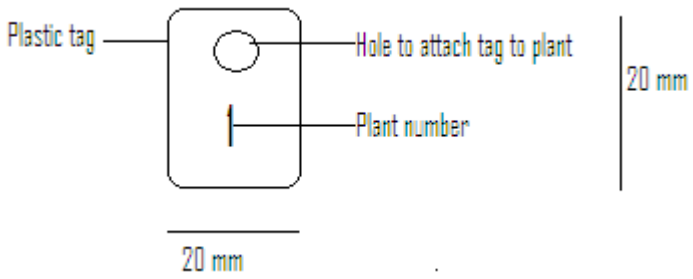

Fig. 7. Diagram of the plastic tag used to identify plants for growth experiments.

A map was drawn of the study site, marking the location of the plants (Fig. 6, Table 1). Different coloured cable ties were used to mark the blades of the plants within each area so that if the number tag was lost, the plant could still be identified using the colour of the ties and the map for reference. Five different colours of cable ties were used to allow for each plant at each location to be tagged using a different colour. This made it easier to identify individuals in the field.

Table 1. Location of individuals of *Lessonia variegata* tagged for monthly growth measurements.

Study Site	Offshore Plants (tag #)	Inshore Plants (tag #)
Site a	6, 7, 8, 9, 10	11, 12, 13, 14, 15
Site b	1, 2, 3, 4, 5	16, 17, 18, 19, 20

Two blades from each of the 20 plants were tagged using a cable tie attached to the base of the stipe for identification. Each blade had a 5 mm hole made in the blade with a hole puncher. The holes were punched 50 mm along the blade from the meristem at the stipe/blade junction, in an effort to avoid the transition zone (meristematic tissue) of the blade (Parke 1948) (Fig. 8).

The blades were left *in situ* for approximately one month. The length of time varied depending on the sea conditions and the time of low tide (Table 2). Harvesting of blades and hole punching additional blades were usually within the period of the lowest tides each month.

The hole punched blades were harvested monthly and the position of the hole was remeasured from the stipe/blade junction to the base of the hole (Fig. 9). The initial distance of 50 millimetres from the stipe/blade junction to the hole was deducted from the final length (FL) to ascertain the blade elongation in millimetres for the month. This distance was divided by the amount of days (d) to express the growth rate (GR) as millimetres per day (mm/d^{-1}) (Table 2).

$GR = (FL – 50)/d$

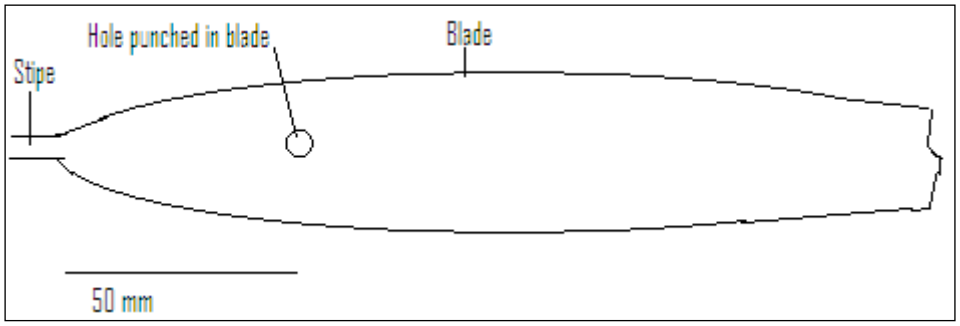

Fig.8. Diagram of the blade of *Lessonia variegata* showing the initial position of the hole punched in the blade that is used to measure the growth of the blade.

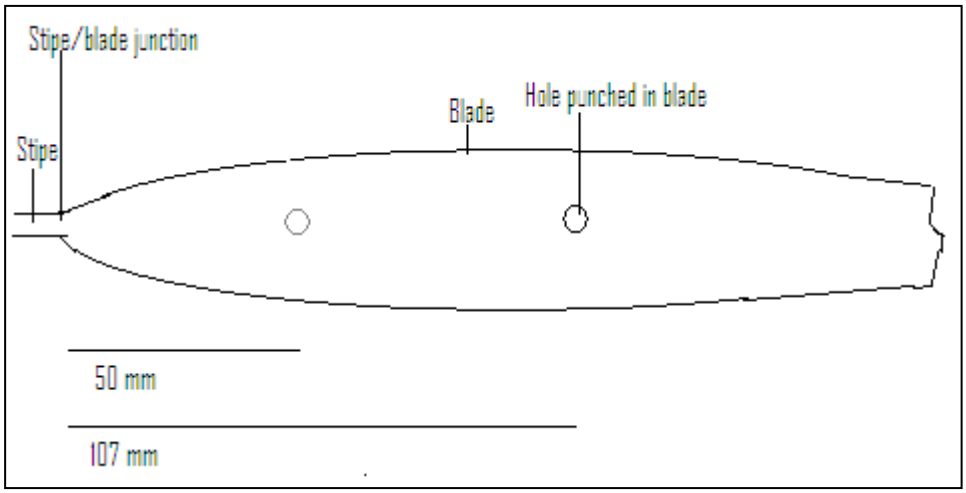

Fig. 9. Diagram of a blade of *Lessonia variegata* showing the initial position of the hole for measuring growth (left) and the final position of the hole (right)

Table 2. Table showing the collection dates and location for all lineages of *Lessonia variegata,* and the dates between tagging and collecting blades for the growth analysis.

Tag/Collection Date	**Location**	**Collected By**	**Polysaccharide analysis**	**Growth analysis**	**Days between tagging**
1.3.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	No	No	0 (1st tags set)
21.3.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	20
21.3.2010	45° 73.609 S, 170° 59.712 E Dr's Point near Dunedin	C.Fraser	Yes	No	N/A
13.4.2010	South Bay, Kaikoura	L.Tate	Yes	No	N/A
19.4.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	29
21.4.2010	Wairepo, Kaikoura	P.South	Yes	No	N/A
31.5.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	42
28.6.2010	41° 34.930 S, 174°7 3.961 E	L.Abbott	Yes	Yes	28
27.7.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	29
11.8.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	15
8.9.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	28
5.10.2010	GPS 35° 54.70 S, 175° 06.13 E Mokohinau Island, Arch Bay	Divers from Leigh Marine Laboratory	Yes	No	N/A
8.10.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	30
8.11.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	31
6.12.2010	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	28
2.1.2011	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	27
28.1.2011	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	26
21.2.2011	41° 34.930 S, 174° 73.961 E	L.Abbott	Yes	Yes	24

Productivity of alginate and fucoidan in Lessonia variegata (Wellington lineage)

One blade from each of ten individual *Lessonia variegata* plants within the study site was harvested for the purpose of ascertaining a basic measure of productivity. Each blade was washed with 70° ethanol and rinsed with tap water. A ten millimetre long cross-section was cut from each blade at the point of the blade's maximum width. The section was weighed, and the width measured before being dried overnight in an oven at 50° C. The dried sections were weighed and an average weight of 30.625 mg per 10 mm section was calculated. These figures were used to calculate a measure of length per 1 gram of dry weight (32.653 cm), which was then used to calculate the amount of alginate and fucoidan produced per month based on the amount of growth. The monthly growth (mm/d^{-1}) was converted to centimetres and then multiplied by 30 to ascertain the centimetres of growth per month. The number 30 was used as a proxy for a month. Then this figure (centimetres per month) was multiplied by either the amount of fucoidan or alginate per centimetre of blade length to give a basic measure of productivity of these two polysaccharides in *Lessonia variegata* at the Wellington study site.

Environmental Factors

Data pertaining to environmental factors that were thought to perhaps be related to the percent alginate, percent fucoidan and/or the amount of monthly growth was collected throughout the study. The environmental factors analysed were the monthly sun hours and the surface sea temperature (SST) as it was likely that the amounts of polysaccharides and/or growth would be linked with changes in the amount of sun hours and/or the SST.

The amount of sun hours for each month for Wellington, and for the months of algal collection for each of the three other lineages, was obtained from the National Institute of

Water and Atmospheric research (NIWA) climate data available at www.niwa.co.nz/ourscience/climate/publications/all/cs/monthly.

Sun hours for the Wellington lineage were different to the other lineages for the months of algal collection. In an effort to standardise sun hours between the Wellington lineage and each of the other 3 lineages, the Wellington data set with similar sun hours to the lineage being compared was used. The data from the same calendar month of collection between Wellington and each of the other lineages was also compared in an effort to ascertain if sun hours was a significant factor affecting the alginate and fucoidan yields.

Average monthly SST was calculated using data collected from sensors located at Evan's Bay and Baring Head, Wellington (Fig. 10). Both data sets were provided by C. Stewart, oceanographer for NIWA. The sensor at Evan's Bay, Wellington is set at an approximate depth of 1 metre, beside NIWA. The sensor at Baring Head was a waverider set at an approximate depth of 1 metre in 50 metre deep water. Baring Head is located on the South coast of Wellington, and the data collection point at Evan's Bay is within the Wellington Harbour. The monthly SST average was calculated using the temperature at 1500 hours for each day of the month. There was not a full data set available from either of the Evan's Bay or Baring Head locations, therefore the data for March 2010 through to August 2010 is taken from the Evan's Bay data set and the data from September 2010 through to January 2011 is taken from the Baring Head data set. Temperatures at both sites were similar to each other for the months when there was data available from both sites.

Fig. 10. Map showing the location of the two sites for the SST sensors and the location of the Wellington study site (Google Earth 5.2.1.1329).

Statistical Analysis

Statistical analysis One-way analysis of variance (ANOVA) was performed using SPSS 18 to detect differences in the means for the monthly yield of alginate data for the Wellington lineage of *Lessonia variegata.* The data was tested for normality (Kolmogorov Smirnov) and homogeneity of variances using the Levene test. Tukeys Honest Significance Difference (HSD) post-hoc test was applied for comparison of the monthly means; p values less that 0.05 were considered statistically significant.

Due to the violation of the assumption of homogeneity of variance required for ANOVA, the Kruskal Wallis test (non parametric form of ANOVA, SPSS 18) was performed to test for significant differences in the samples for the monthly yield of fucoidan and growth data for the Wellington lineage of *Lessonia variegata.*

Correlation analysis were performed in order to determine the amount of significant variance that could be explained by the average sun hours or the surface sea temperature (SST) on observed variation in the amounts of alginate, fucoidan and/or growth for the Wellington lineage of *Lessonia variegata.* R values were calculated and graphs constructed in Microsoft Excel.

Multivariate and univariate analysis was performed to determine what effect the sun hours and the surface sea temperature had on the yield of alginate, yield of fucoidan and the growth data for the Wellington lineage.

Statistical significance of the alginate and fucoidan yield between evolutionary lineages of *Lessonia variegata* was determined using the Mann-Whitney U test (SPSS 18); p values less than 0.05 were considered statistically significant. The Mann-Whitney U test was used as it is the non-parametric form of Students'*t* test and due to the low number of samples the assumption of homogeneity of variance was violated.

Results

Monthly Polysaccharide Yield within the Wellington Lineage of Lessonia variegata.

Alginate

Alginate was extracted from blades of *Lessonia variegata* collected on twelve different occasions. The yield of alginate per sample of individual blades ranged from 10.8 % to 30.9 % of dry weight (Appendix 1, Table 5). In order to determine if there were significant differences in the yield of alginate extracted each month, one-way Analysis of Variance (ANOVA) was conducted. This test of means identified a significant difference ($\alpha = 0.05$) between the monthly means for alginate, $F = 5.750$, 11 d.f., $P < 0.001$. There was a trend in the data for increased yields of alginate from the Wellington study site in the months of November and December, and for the least yield of alginate in March over the period of this study, Fig. 11.

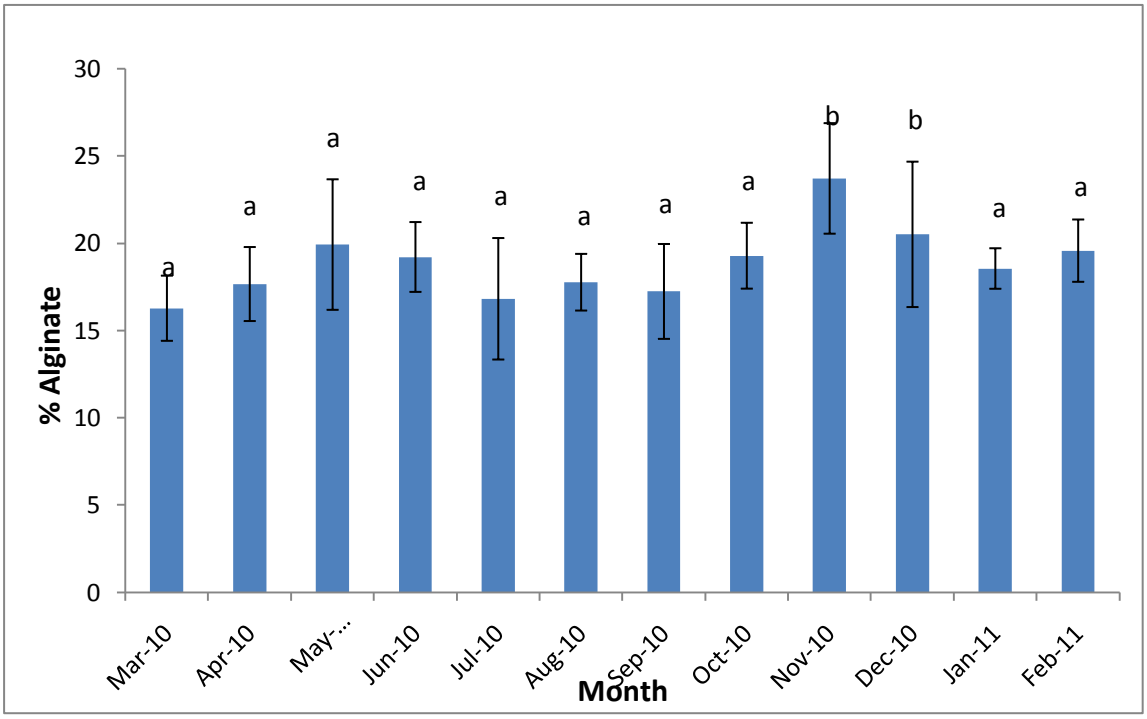

Fig.11. Histogram showing the monthly mean yield (± standard deviation, n= 9-10) for alginate (% dry weight) within the Wellington lineage of *Lessonia variegata* for the period of March 2010 – February 2011. Columns with the same letters are not significantly different at $P < 0.05$.

To determine which months were significantly different, Tukey HSD comparisons were performed. The mean yield of alginate for November 2010 was significantly higher than yields for March, April, June - October 2010, January and February 2011; and the yield of alginate for December was significantly different to the yield of alginate for March. There was no significant difference in the mean yield of alginate for each of the other months.

Correlation analysis was performed to detect any significant relationships between the factor alginate and other factors such as: fucoidan yield, growth, sun hours and surface sea temperature (SST). There was no significant correlation between alginate and any of the other factors tested (Appendix 2, Figs. 27 - 30).

In order to detect seasonal variation in the yield of alginate, the data was grouped according to season and analyzed using one-way ANOVA. The yield of alginate was similar for all seasons with only slight variation detected between autumn and spring; and winter and spring, P= 0.042, Fig. 12.

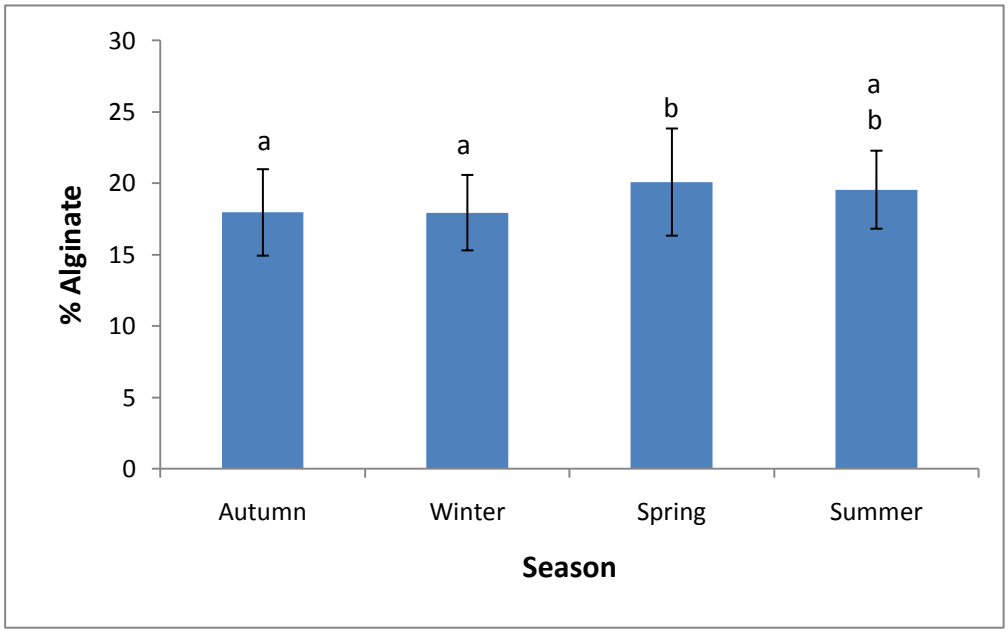

Fig. 12. Histogram showing the seasonal means (± standard deviation, n= 29-30) for alginate (% dry weight) within the Wellington lineage of *Lessonia variegata* for the period of March 2010 – February 2011. Columns with the same letters are not significantly different at $p < 0.05$.

Fucoidan

Fucoidan was extracted from blades of *Lessonia variegata* collected from the Wellington study site on twelve different occasions from March 2010 until February 2011. The yield of fucoidan per sample ranged from 6.0 % to 26.5 % dry weight (Appendix 1, Table 5). In order to determine if there were significant differences in the yield of fucoidan for each month, Kruskal-Wallis U test (the non-parametric equivalent to one-way ANOVA) was conducted. The yield of fucoidan was different across the months within the study period, H = 28.709, 11 d.f., P = 0.03. There was a trend in the data for increased yields of fucoidan from the Wellington study sight in the months of March, April and November 2010, Fig. 13.

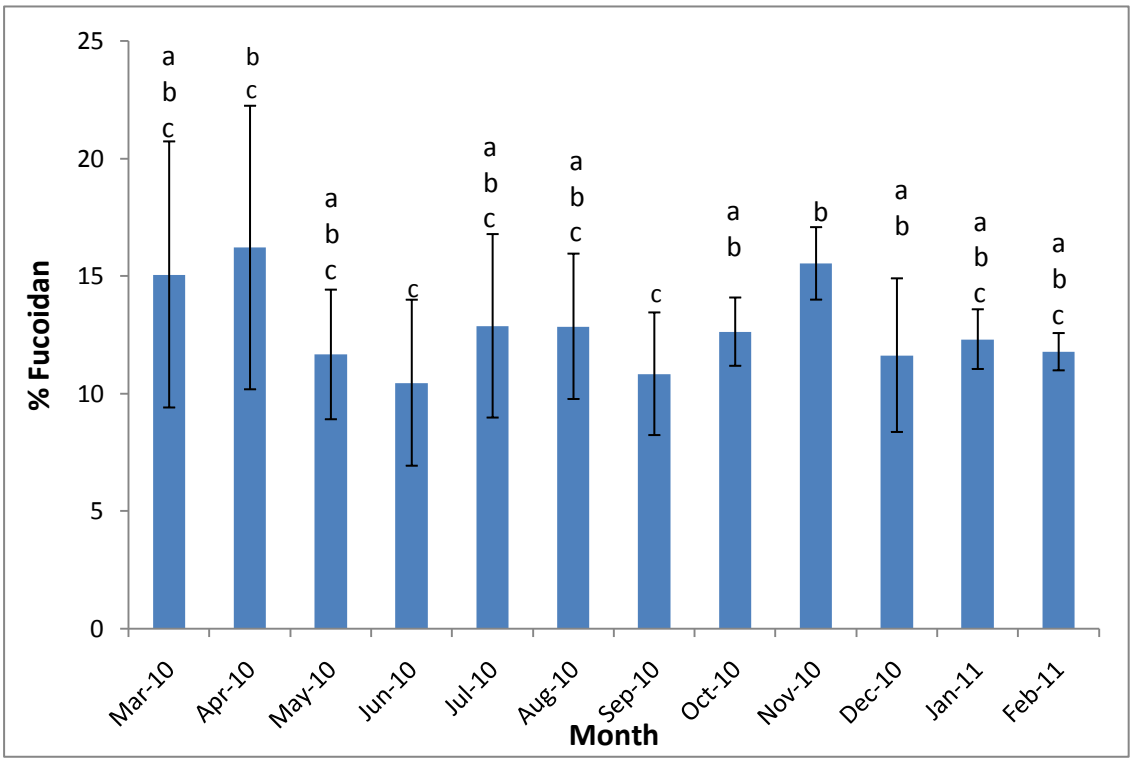

Fig. 13. Histogram showing the monthly means (± standard deviation, n= 8-10), for fucoidan (percent dry weight) for the Wellington lineage of *Lessonia variegata.* Columns with the same letters are not significantly different at $P < 0.05$.

Correlation analysis was performed to detect any significant relationships ($\alpha = 0.05$) between the factor fucoidan and other factors such as: growth, sun hours and SST. There were significant positive correlations between fucoidan and sun hours ($R^2 = 0.0682$, $P = 0.005$), Fig. 14; and fucoidan and SST ($R^2 = 0.0395$, $P = 0.045$), Fig. 15. As the sun hours increased, the levels of fucoidan also increased, as they did to a lesser extent with SST. There was no significant relationship between fucoidan and growth (Appendix 2, Fig. 31).

In order to detect seasonal variation in the yield of fucoidan, the data was grouped according to season and regression analysis was performed in SPSS 18. The yield of fucoidan was similar across all seasons, $H = 4.614$, 3 d.f., $P = 0.194$ (Appendix 2, Fig. 32).

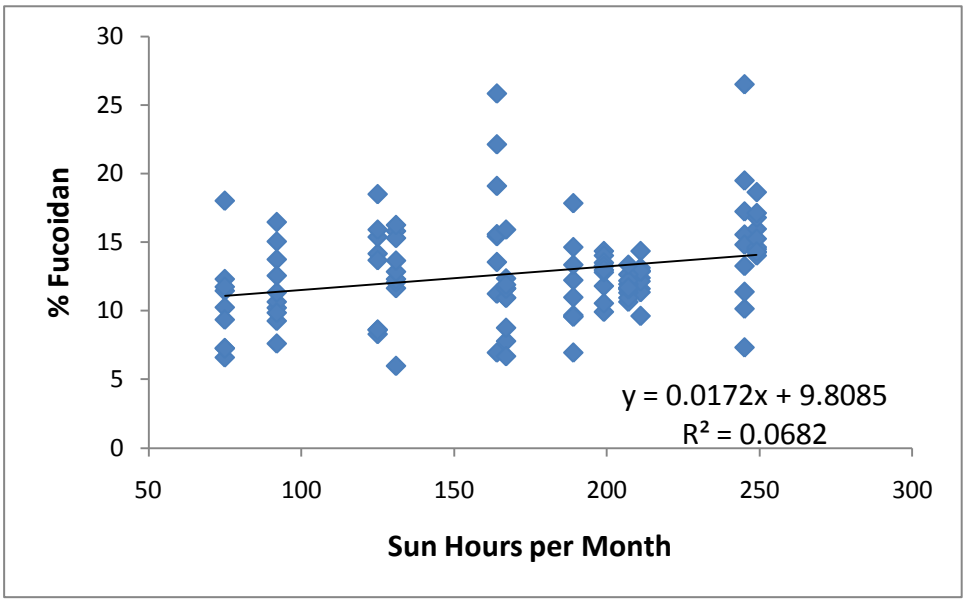

Fig. 14. Scatterplot of the relationship between the sun hours per month and the yield of fucoidan each month, for the period of March 2010 – February 2011.

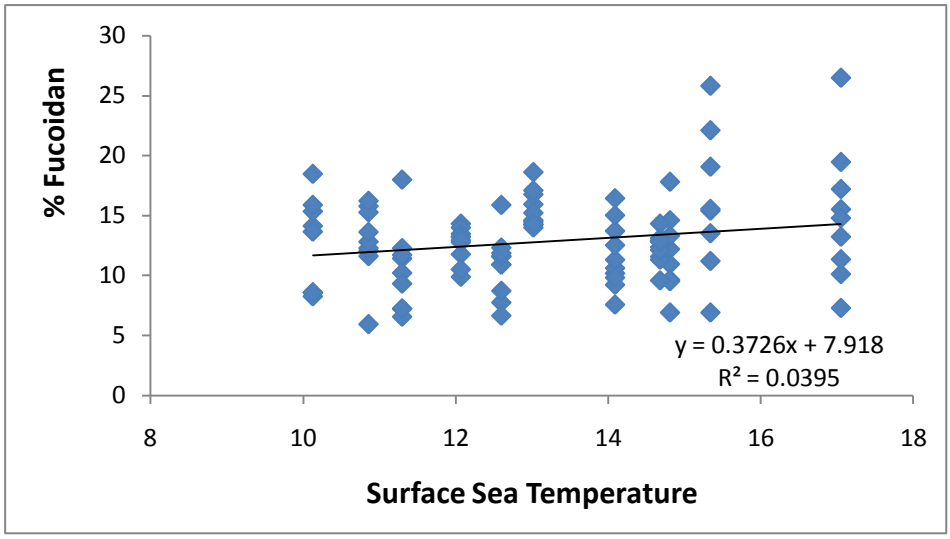

Fig. 15. Scatterplot of the relationship between the average monthly SST and the yield of fucoidan each month, for the period of March 2010 – January 2011.

Personal observations of the blades of *Lessonia variegata* plants at the Wellington study site were made in an endeavour to ascertain the months when the plants were fertile. It was noted that sori were present on the blades from May 2010 until July 2010.

Monthly growth (mm/d^{-1}) within the Wellington Lineage of Lessonia variegata.

The average growth each month over the study period ranged from 0.04 millimetres per day to 10.5 millimetres per day (Appendix 3).

The data collected for the growth of the blades of *Lessonia variegata* displayed non-normal distribution therefore the non-parametric Kruskal-Wallis test of means (equivalent to the parametric one-way ANOVA) was used to test for significant differences in the data. Monthly growth figures were shown to be significantly different to each other ($\alpha = 0.05$), $H = 250.945$, 11 d.f., $P < 0.001$. There is a trend in the data for two distinct growth patterns. The highest amount of growth is from August 2010– February 2011; with each of the means within that period being statistically similar. The least amount of growth occurs from March

2010 – July 2010; with each of the means between March and July being statistically similar, Fig. 16.

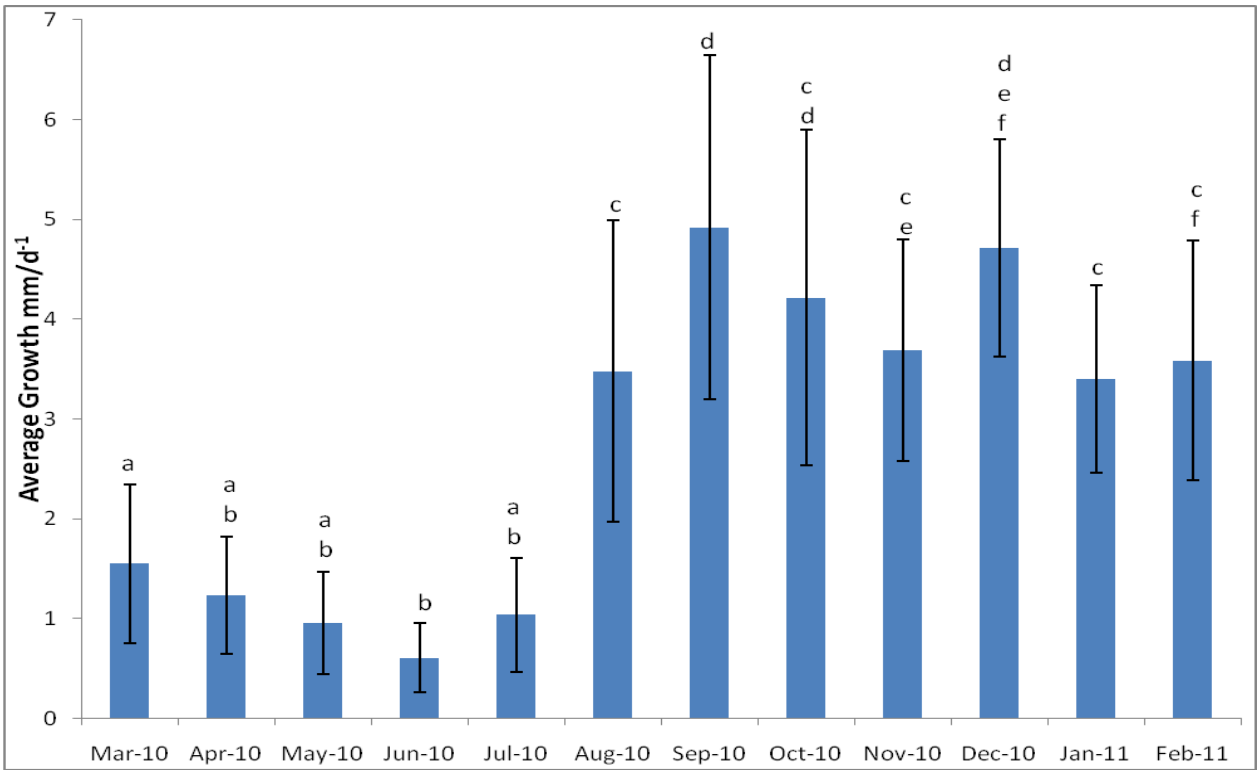

Fig. 16. Histogram showing the average growth per month for blades of *Lessonia variegata* (mm/d^{-1}) (±standard deviation, n = 17 - 38) from the Wellington study site for the period of March 2010 – February 2011. Columns with the same letters are not significantly different at $P < 0.05$.

Correlation analysis was performed to detect any significant relationships ($\alpha = 0.05$) between the factor growth and other factors such as sun hours and SST. There is a positive correlation between the growth of the blades of *Lessonia variegata* at the Wellington study site and monthly sun hours for Wellington for the period from March 2010 until February 2011, ($R^2 = 0.1382$, $P < 0.001$), Fig. 17.

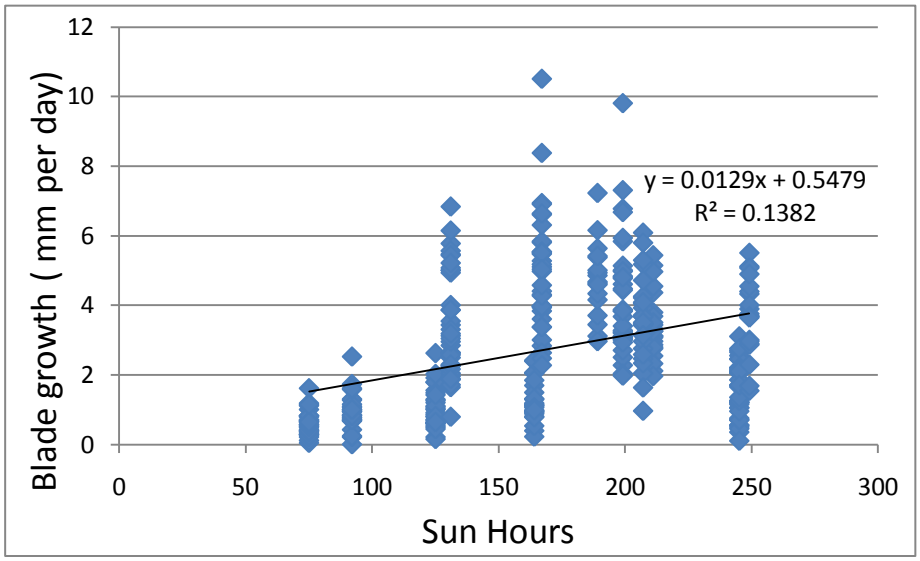

Fig. 17. Scatterplot of the relationship between the amount of sun hours in Wellington each month and the average monthly growth (mm/d^{-1}) for blades of *Lessonia variegata* at the Wellington study site, for the period March 2010 – January 2011.

In order to test whether the location of the *Lessonia variegata* relative to the shore had an effect on the monthly growth rate, the data collected from the 20 plants was grouped according to inshore or offshore locations. Mann-Whitney U tests were conducted to identify differences in the monthly growth between the offshore plants and the inshore plants. The means were only significantly different for the month of January 2011, offshore ($M = 3.08$, $SD = 0.90$) and inshore ($M = 3.82$, $SD = 0.86$); $H = 5.271$, 1 d.f., $P = 0.022$, Fig. 18.

There were no significant differences in the growth between the offshore and the inshore sites for any for the other months.

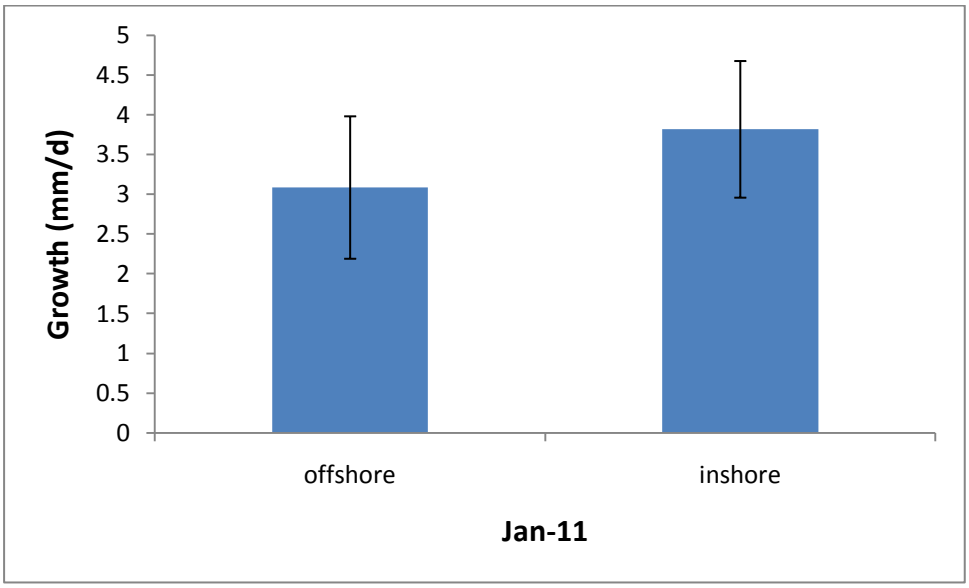

Fig.18. Histogram showing the growth of the blades of 20 *Lessonia variegata* plants at the Wellington study site in January 2011. The ten inshore plants had significantly more growth than the 10 offshore plants ($P = 0.022$).

To test for differences in growth by season a Kruskal-Wallis test of ranks was performed on samples grouped by season. Growth was shown to differ by season ($H = 179.104$, 3 d.f., $P < 0.001$), Fig. 19 and Table 3.

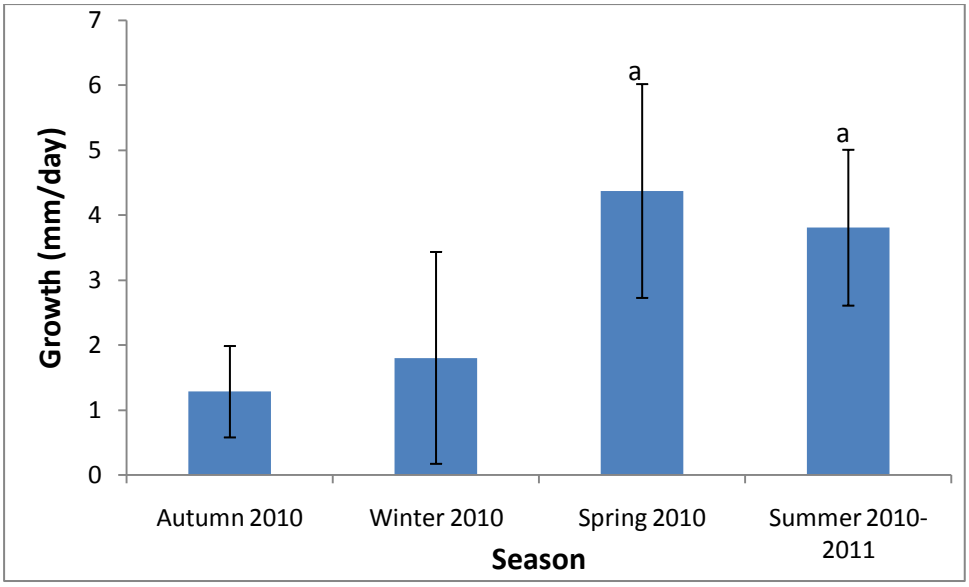

Fig.19. Histogram showing the seasonal growth for the Wellington lineage of *Lessonia variegat*a (±standard deviation, n = 67-100). Columns with the same letter are not significant at $P < 0.05$.

Table 3. Results of Kruskal-Wallis test for seasons and growth, H = 179.104, 3 d.f., $P < 0.001$.

Season	N	Mean Rank
Autumn	89	96.44
Winter	100	119.03
Summer	67	240.90
Spring	89	259.10

Due to the fact that the sun hours showed a correlation with both yield of fucoidan and the growth rate, General Linear Model (GLM) multivariate analysis was performed to test the effect of the amount of sun hours on the factors alginate, fucoidan and growth rate. The amount of sun hours had a significant effect on the factors tested for the Wellington lineage of *Lessonia variegata*, Wilks' Lambda = 0.629, $F_{(3,108)} = 15.485$, $P < 0.001$. In particular, the amount of sun hours was a significant factor in describing changes in the yield of fucoidan ($F_{(1,110)} = 8.057$, $P = 0.005$) and the growth rate ($F_{(1,110)} = 25.100$, $P < 0.001$).

Seasonal means for the yield of alginate and the growth rate were significantly different; therefore the mean sun hours and SST were also tested for significant differences in the means across seasons. One-way ANOVA was performed and both sun hours ($F = 106.185$, 3 d.f., $P < 0.001$) and SST ($F = 758.283$, 3 d.f., $P < 0.001$) were different across the seasons, Table 4.

Table 4. Table of results for One-way ANOVA tests for differences in the means for the factors sun hours and SST across seasons.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Sun hours	Between Groups	493291.904	3	164430.635	106.185	<.001
	Within Groups	528050.896	341	1548.536		
	Total	1021342.800	344			
Surface Sea Temp.	Between Groups	1277.439	3	425.813	758.283	<.001
	Within Groups	176.326	314	.562		
	Total	1453.765	317			

To determine which seasons were significantly different, Tukey HSD comparisons were performed. For the factor sun hours, autumn and winter were significantly different but not spring and summer. For the factor SST, all of the seasons were significantly different, Fig. 20 and Fig. 21.

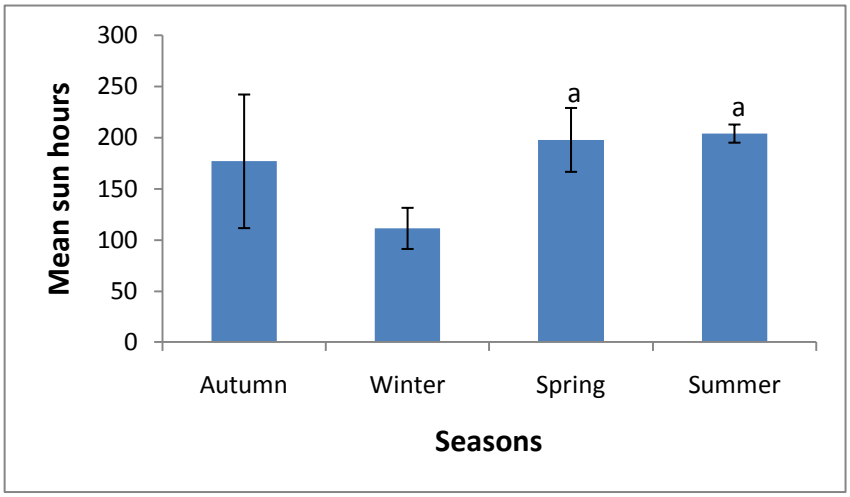

Fig. 20. Histogram showing the mean seasonal sun hours for the Wellington lineage during the study period (± standard deviation, n = 67-100). Columns with the same letter are not significant at $p < 0.05$.

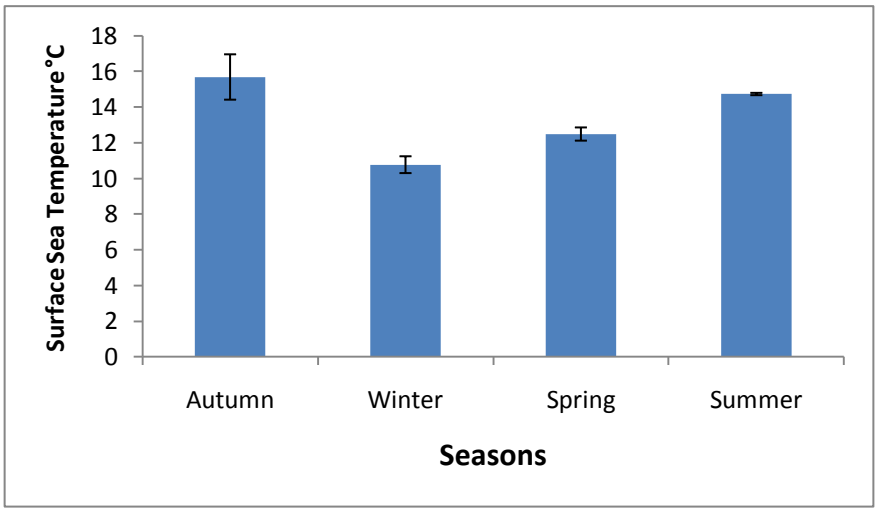

Fig. 21. Histogram showing the mean seasonal SST for the Wellington lineage during the study period (± standard deviation, n = 40-100).

Summary of the results within the Wellington lineage of *Lessonia variegata.*

Variation was detected in the yield of alginate across the months from March 2010 – February 2011. There was a trend for a higher yield of alginate in May, November and December and a lower yield in March.

Some variation was detected for the yield of fucoidan across the months from March 2010 – February 2011 with a trend for a higher yield of fucoidan in November and lower yields of fucoidan in June and September. The amount of sun hours is a significant factor for the yield of fucoidan; GLM multivariate analysis $F_{(1,99)} = 4.965$, $P = 0.028$.

Fertile sori were observed on the blades of *Lessonia variegata* in the field from May 2010 until July 2010.

The monthly growth rates showed significant variation across the months from March 2010 – February 2011. There is a pattern of two distinct growth rates with a higher growth rate evident for the months of August through to February. Sun hours are strongly correlated with growth; multivariate analysis $F_{(1,99)} = 42.157$, $P < 0.001$. Differences in the rate of growth were detected seasonally with the most growth occurring in spring and summer, and the least growth occurring in autumn.

Sun hours is a main effect on both the yield of fucoidan and on the growth rate; GLM multivariate analysis Wilks' Lambda = 0.629, $F_{(3,97)} = 19.090$, $P < 0.001$.

Productivity of the Wellington lineage of Lessonia variegata.

The months with the highest production of both alginate and fucoidan for the Wellington lineage of *Lessonia variegata* were September, October, November and December 2010, Fig. 22. The months with the lowest productivity were May 2010, June and July 2010. The pattern of productivity with low productivity from March to July (autumn – mid-winter) and high productivity from August 2010 to February 2011 (late winter – late summer) is similar to the pattern of growth which has a period of low growth from March 2010 to July 2010 and high growth from August 2010 to February 2011.

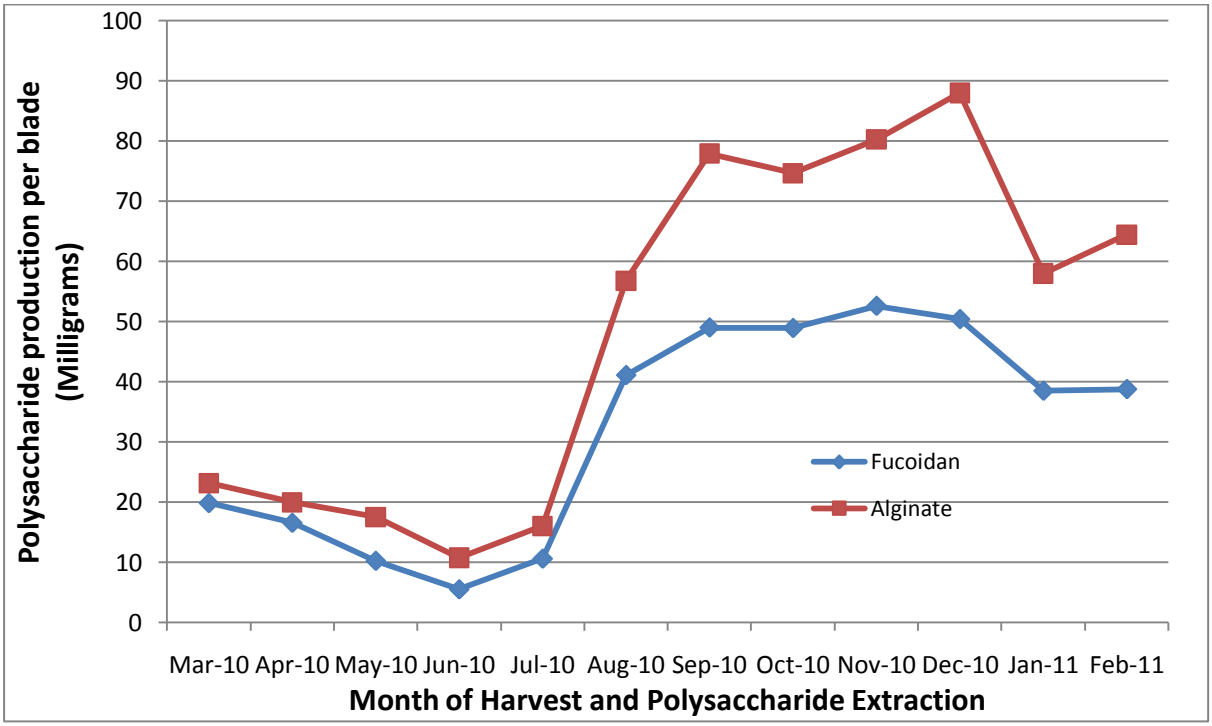

Fig. 22. Line graph displaying productivity figures: the amount of fucoidan (blue diamonds) and the amount of alginate (red squares) produced per blade of *Lessonia variegata* at the Wellington study site for the period from March 2010 – February 2011.

Polysaccharide Yield between Lineages of Lessonia variegata.

The yields of alginate and fucoidan from the Northern, Kaikoura and Southern lineages were compared with the yields for the Wellington lineage from the same calendar month that each sample was harvested. Due to the factor sun hours being a significant factor affecting the fucoidan yield within the Wellington lineage, it was thought that sun hours should be standardized between the Wellington lineage and each of the other 3 lineages. Therefore, the alginate and fucoidan yields from each of the 3 other lineages were compared to yields from months within the Wellington lineage that had similar sun hours to the harvest time of the other lineage. This had no effect on the results; therefore only the results of the samples compared based on calendar month of harvest are reported.

The yield of alginate for the Wellington lineage ranged from 12.3 % to 21.0 % of dry weight and for the Northern lineage from 22.8 % to 28.0 %. The yield of fucoidan for the Wellington lineage ranged from 6.7 % to 15.9 % of dry weight and for the Northern lineage from 7.0 % to 14.1 %.

There was a significant difference in the yield of alginate between the Wellington lineage ($M = 17.24$, $SD = 2.72$) and the Northern lineage ($M = 25.59$, $SD = 1.44$); $Z = -3.780$, $P < 0.001$, Fig. 23.

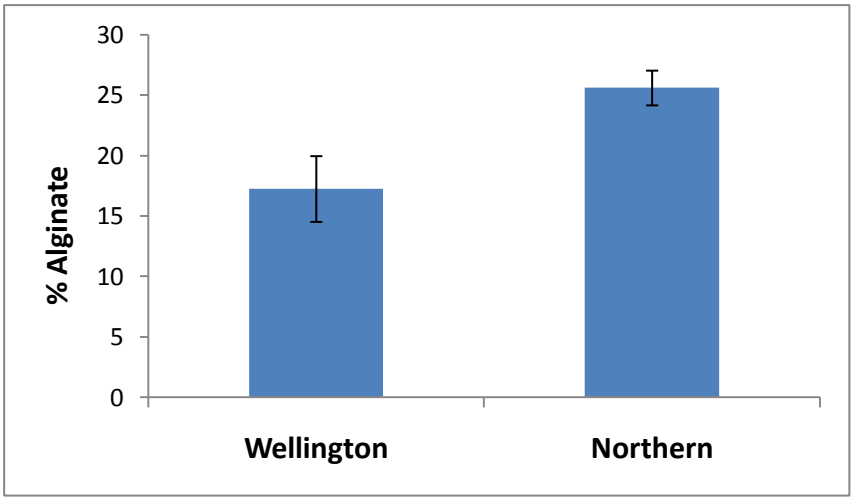

Fig. 23. Histogram showing the average yield of alginate (percent dry weight) for the Wellington lineage and the Northern lineage of *Lessonia variegata* from September, $Z = -3.780$, $P < 0.001$.

There was not a significant difference in the yield of fucoidan between the Wellington lineage and the Northern lineage.

The yield of alginate for the Wellington lineage ranged from 14.2 % to 22.3 % of dry weight and for the Kaikoura lineage from 17.6 % to 26.1 %. The yield of fucoidan for the Wellington lineage ranged from 6.9 % to 25.8 % of dry weight and for the Kaikoura lineage from 5.9 % to 31.6 %.

There was a significant difference in the yield of alginate between the Wellington lineage (M = 17.66, SD = 2.12) and the Kaikoura lineage (M = 22.75, SD = 2.88); Z = -3.175, P < 0.001, Fig. 24.

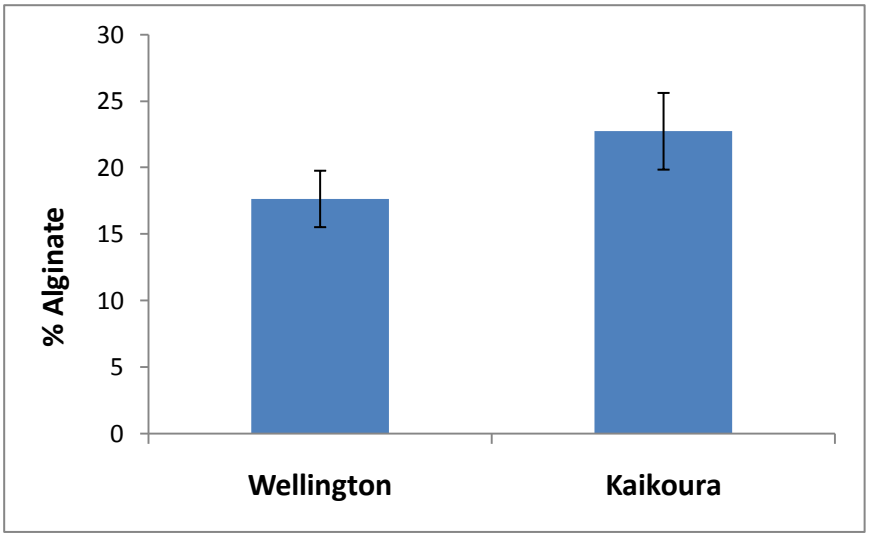

Fig. 24. Histogram showing the average yield of alginate (percent dry weight) for the Wellington lineage and the Kaikoura lineage of *Lessonia variegata* from April, Z =-3.175, P < 0.001.

There was not a significant difference in the yield of fucoidan between the Wellington lineage and the Kaikoura lineage.

The yield of alginate for the Wellington lineage ranged from 12.6 % to 18.5 % dry weight and for the Southern lineage from 17.6 % to 26.1 %. The yield of fucoidan for the Wellington lineage ranged from 7.3 % to 26.5 % dry weight and for the Southern lineage from 5.7 % to 14.2 %.

There was a significant difference in the yield of alginate between the Wellington lineage (M = 16.27, SD= 1.87) and the Southern lineage (M = 22.75, SD = 2.88); Z = -3.553, P < 0.001, Fig. 25.

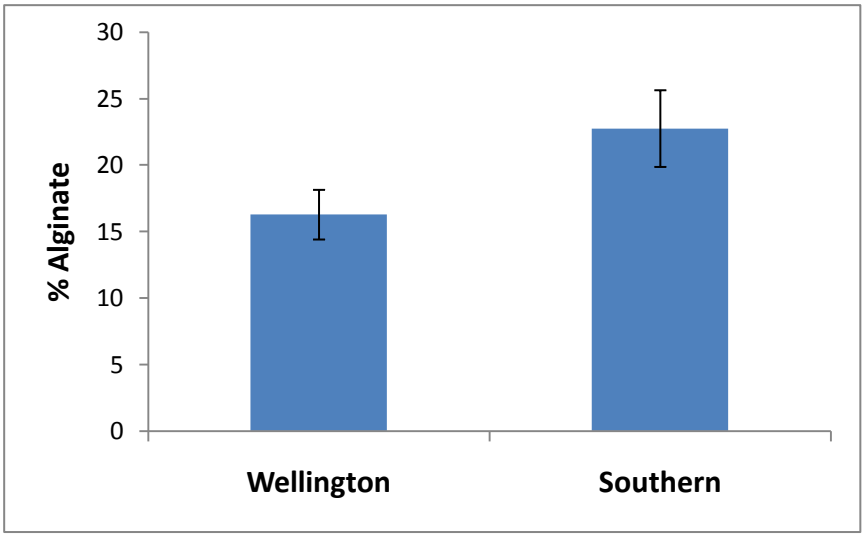

Fig. 25. Histogram showing the average yield of alginate (percent dry weight) for the Wellington lineage and the Southern lineage of *Lessonia variegata* for March, Z = -3.553, P < 0.001.

There was a significant difference in the yield of fucoidan between the Wellington lineage (M = 15.06, SD = 5.66) and the Southern lineage (M = 9.54, SD = 3.02); Z = -2.369, P=0.018, Fig. 26.

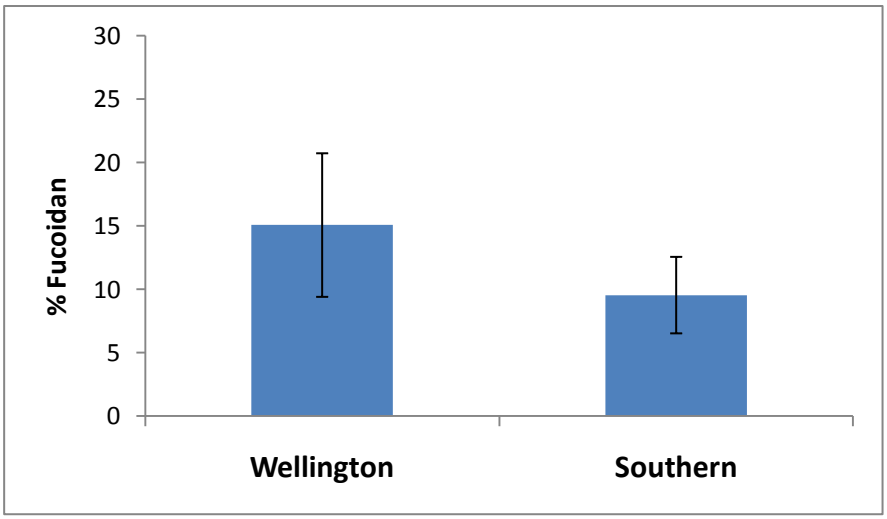

Fig.26. Histogram showing the average yield of fucoidan (percent dry weight) for the Wellington lineage and the Southern lineage of *Lessonia variegata* for March, Z = -2.369, P = 0.018.

Summary of the results for between the Wellington lineage and other lineages of *Lessonia variegata.*

The yield of alginate varied between the Wellington lineage of *Lessonia variegata* and the Northern, the Kaikoura and the Southern lineages. The Wellington lineage had lower yields of alginate than each of the other lineages.

The yield of fucoidan was only significantly different between the Wellington lineage of *Lessonia variegata* and the Southern lineage. There was a trend for higher yields of fucoidan in the Wellington lineage than in each of the other three lineages.

Fertile sori were observed on the blades of *Lessonia variegata* in the field from May 2010 until July 2010.

Discussion

The aims of this study were to investigate seasonal changes within the Wellington lineage of *Lessonia variegata* with regards to the yields of alginate and fucoidan, and the growth rate; and to explore differences in the yield of alginate and fucoidan between lineages of *Lessonia variegata.*

Within the Wellington lineage of Lessonia variegata

Alginate

The yield of alginate extracted from blades of *Lessonia variegata* fluctuated over the course of the year from monthly mean amounts of 16.3 to 23.7 percent dry weight. Monthly mean amounts were significantly different to each other. Seasonal differences in the yield of alginate were detected with the highest yield in spring and summer. A similar pattern of increased alginate yield was detected in *Costaria costata* C. Agardh where the alginate yield increased from 15.6 percent dry weight in early spring to 22 percent dry weight in summer (Imbs *et al*. 2009). A previous study on *Lessonia variegata* reported an alginate yield of 18 percent dry weight for algae harvested in October 1994 from within the Wellington lineage (Miller 1996). The yield of alginate extracted by Miller is similar to the October yield of 19.28 percent dry weight (± 1.89 standard deviation) for this study. Other studies on the alginate content of brown seaweeds have also reported spring highs in alginate yields for both *Macrocystis pyrifera* (McKee *et al*. 1992) and *Undaria pinnatifida* (Harvey) Suringar (Skriptsova *et al.* 2004).

Studies on alginates within the Laminariales often focus on detecting differences in the yield of alginate between different species at one point in time (Miller 1996; Usov *et al*.

2001; Rioux *et al*. 2007); or they focus on the changes in the composition of the alginate with reference to its chemical properties and its block structure (Craigie *et al*. 1984; Panikkar and Brasch 1996; Imbs *et al*. 2009; Fenoradosoa *et al*. 2010). The quality of the alginate determines its functional use and is based on its composition, specifically its guluronic acid content (G) and the length of the G blocks (Fenoradosoa *et al*. 2010). Alginates with a high M/G ratio form more elastic gels than those with a low M/G ratio (Fenoradosoa *et al.* 2010).

Alginate composition is also reported to vary between members of the same species at different locations (Craigie *et al*. 1984) and possibly be related to wave action and turbulence regimes (Panikkar and Brasch, 1996). Seasonal variance in the composition of alginate has been reported for *Costaria costata* (Imbs *et al*. 2009), *Macrocystis pyrifera* (McKee *et al.* 1992) and *Undaria pinnatifida* (Skriptsova *et al*. 2004); and is expected to also occur in *Lessonia variegata.* No measurements were made during the course of this study for the velocity of wave action or turbulence. However, due to its habitat on high velocity wave exposed rocky coasts, it is expected that *Lessonia variegata* would have a high M to G ratio which equates to an elastic alginate and perhaps allows for flexibility in such conditions. Miller (1996) reported an M/G ratio of 1.95 for *Lessonia variegata*, which is high and in agreement with the expected ratio based on the environmental conditions; but Miller's work is a one-off analysis and as such cannot address temporal differences.

It would be of value to analyse the alginate extracted from the Wellington lineage of *Lessonia variegata* to determine the seasonal variation in its composition, and to provide knowledge pertaining to the best time to harvest *L. variegata* depending on the proposed

functional use of the alginate. Due to constraints of time and funding, analysis of the alginate extracted during this study was not performed.

Fucoidan

The yield of fucoidan extracted from blades of *Lessonia variegata* fluctuated over the course of the year from monthly means of 10.5 percent dry weight to 16.2 percent dry weight. The effect of sun on the percent of fucoidan was significant. As the sun hours increased the percent fucoidan increased. The strength of this relationship is small though with less than 7% of the variation in fucoidan being explained by sun hours. Therefore it is likely that both fucoidan and the sun hours are correlating with some unmeasured factor.

Increases in fucoidan yield have been reported to be linked with the reproductive status of Laminariales (Zyyagintseva *et al*. 2003; Skriptsova *et al*. 2010). Fucoidan yield in *Laminaria japonica* and *Undaria pinnatifida* are reported to increase five-fold with sorus development from mid-autumn to mid-winter (Skriptsova et al. *2010*) and there is reported to be a strong correlation between the fucoidan yield and the maturity of the blades for *Laminaria cichorioides* Miyabe (Zvyagintseva *et al*. 2003). This study of a population within the Wellington lineage of *Lessonia variegata* did not detect such seasonal changes in the mean monthly fucoidan yield, but the yield of fucoidan per blade did fluctuate within each month with an increase of between 2.2 to 3.7 times within the period from early autumn to early spring (Appendix 1). *Lessonia variegata* within the Wellington lineage is reported to contain some fertile blades across all months except for March (Schwarz *et al*. 2006). Therefore the variation in the yield of fucoidan within each month could be due to fertile blades being present and not detected; natural variance within the population; or an artefact of the extraction process.

Fertile blades of *Lessonia variegata* were noticed in the population studied from May 2010 (late-autumn) until July 2010 (mid-winter). It has been reported that *Lessonia variegata* from a sub-tidal (8-10 m) population of the Wellington lineage had the highest rate of fertility between mid-autumn and early spring in 2003 (Schwarz et al. 2006). This period of highest fertility (mid-autumn-early spring) reported by Schwarz (2006) coincides with the period of the most variance in fucoidan levels between individual plants in this study. Fertile blades of *Lessonia variegata* that were noticed in this study were recognised when the sori had begun to erode and these blades were avoided for the purposes of this study. However, the expected colour variation in blades as they change from vegetative to reproductive was difficult to detect and fertile blades could have been inadvertently utilized in the study.

Variation in levels of fucoidan has also been reported depending on the part of the plant from which it is extracted (Rioux *et al*. 2007). In this study, approximately 10 centimetres of the basal portion of the blades were used in polysaccharide extractions. The sori are located above the basal 10 centimetres utilised in this study therefore the reproductive tissue should have been avoided in fucoidan extractions. However, it is unclear from the literature whether the fucoidan levels of the whole plant increase when the plant is mature or whether it is just the fucoidan levels within the reproductive tissue that increases.

The monosaccharide composition of fucoidan has been reported to change seasonally (Rioux *et al*. 2007; Skriptsova *et al*. 2010). The quality and purpose of use for fucoidan is based on its molecular weight and monosaccharide composition (Skriptsova *et al*. 2010). It would therefore be of value to analyse the fucoidan extracted from the Wellington

lineage of *Lessonia variegata* to determine the seasonal variation in monosaccharide composition; and to provide knowledge pertaining to the best time to harvest *L. variegata* depending on the desired fucoidan. The fucoidan extracted in this study was not analysed to ascertain its molecular weight or monosaccharide composition due to constraints of time and funding.

Growth

The technique of punching a hole in the blade and following the movement of that hole along the blade over time was first described by Parke (1948) as a method of determining the growth rate of the Laminarialean – *Laminaria latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl and G.W. Saunders. This technique measures longitudinal growth of the blade.

Seasonal patterns of growth throughout this study were similar to the results of growth studies for other Laminariales such as *Laminaria latissima* (Parke 1948), *Ecklonia radiata* (Larkum 1986), *Lessonia nigrescens* and *Lessonia trabeculata* (Tala and Edding 2005); which all displayed periods of rapid growth from late winter/early spring through until mid-summer. The growth rate of *Lessonia variegata* in this study ranged from a minimum blade elongation of 0.6 mm/d^{-1} in early winter to a maximum of 4.9 mm/ d^{-1} in early spring. The period of high growth was maintained until mid-summer when the growth rate declined. This observed pattern of growth was similar for other studies of the growth rate of *Lessonia* spp such as *Lessonia nigrescens* 0.8 mm/d^{-1} to 4 mm/d^{-1} and *Lessonia trabeculata* 1.7mm/d^{-1} to 6.5 mm/d^{-1}; with the lowest growth measured for both species in summer and the highest growth measured from late winter to early spring (Tala and Edding 2005). A study on the growth of *Lessonia variegata* from a sub-tidal population (8-10 metres depth) of the

Wellington lineage from 2003 – 2004 also found growth rates similar to this study (0.41 mm/d^{-1} for late autumn – late winter to 1.17 mm/d^{-1} for the period spring – early summer (Schwarz *et al*. 2006)).

Seasonal variation in kelp can be explained by seasonal variation in the amount of light, water temperature or nutrient levels (van Tussenbroek 1993). The growth rate of *Lessonia variegata* in this study showed a positive correlation to the sun hours with the period of highest growth occurring with the onset of spring and therefore the lengthening of daylight hours. The period of the most growth (September - December) had steadily increasing sun hours from its winter lows and also the highest SST of all months studied; and the period of the least growth (March - June) had decreasing sun hours and SST. These results are consistent with van Tussenbroek's (1993) report that seasonal variation in light and water temperature are two environmental factors that can explain seasonal variation in kelp. The month with the least growth was June which also is the month with the shortest days and therefore the least amount of light. However the low growth rate coupled with high sun hours for March 2010 could not be explained using data collected during this study.

The quality of the irradiance was not taken into consideration in this study. Other factors not measured in this study, such as nutrient levels (Craigie *et al*. 1984), water movement (Brown *et al*. 1997; Hurd 2000) and grazing pressure on tagged blades could have had an effect on the growth rate. However, there was no evidence of heavy grazing during this study, therefore strong effects on growth via grazing pressure was not considered significant in this population.

The onset of faster growth in late winter coincided with the sun hours increasing and also the SST increasing from their winter lows of 75 sun hours per month and SST of 10.13°C.

The effect of sun hours on growth was significant but not the effect of SST on growth, therefore in this population of the Wellington lineage of *Lessonia variegata,* seasonal fluctuation in the growth rate seems to be largely due to seasonal fluctuations in irradiance.

Productivity

The basic productivity figures were calculated using the measurements obtained for the monthly yields of alginate and fucoidan, and the monthly growth rates for *Lessonia variegata.* Alginate yield was highest in November and December; fucoidan yield was highest in April and November and there was more growth in September and December than in other months. When the yields of alginate and fucoidan, and the growth rate were analysed together each month; the period of the highest productivity was in November and December (late spring - early summer).

An appropriate time to harvest based on the productivity data would be early to mid-summer immediately following the peak productivity time in November and December. This would allow time for the blades to grow before harvesting in order to utilise the maximum amount of blade. Harvesting in early to mid-summer would also avoid the peak fertility time (mid-autumn to mid-spring); therefore not interfering with the recruitment of *L. variegata.*

Between the lineages of Lessonia variegata

Samples of *Lessonia variegata* blades from each of the three other lineages (Northern, Kaikoura and Southern) were collected and sent to Wellington for the extraction of alginate and fucoidan. As the samples from the Northern, Kaikoura and the Southern lineages were collected in different months to each other, they could only be compared with the Wellington lineage and not with each other. Due to the single sampling of each of the three other lineages, meaningful conclusions pertaining to a lineage with the highest yield of alginate or fucoidan could not be drawn.

Alginate

The Wellington lineage had significantly less alginate than any of the other lineages. Each of the three other collection sites had different amounts of sun hours than the Wellington site for the month of algae collection. Therefore in an attempt to keep the factor sun hours as similar as possible for each of the lineages and to try to detect differences which may be based on genetic differences, the Wellington lineage was matched to each of the three other lineages based on sun hours rather than month of collection. The results were the same when the data was analysed by sun hours as they were when the data was analyzed by calendar month of collection. The different alginate yields between lineages were not found to be related to the sun hours. However the alginate yields are likely to be related to genetic differences or to localised environmental conditions such as the nutrient levels, or wave action and turbulence as discussed for the Wellington lineage above.

Fucoidan

The yield of fucoidan from the Wellington lineage was significantly greater than the yield of fucoidan from the Southern lineage. There was a trend in the data for a higher yield of fucoidan in the Wellington lineage than in the Northern or Kaikoura lineages.

There was variation in the yield of fucoidan from individual blades within each lineage of *L. variegata*. For example, within the Kaikoura lineage the yield of fucoidan per blade ranged from 5.9 to 31.6 percent dry weight. It is unlikely that the blades were fertile as they were harvested in March which is a time when *L. variegata* is vegetative in the Wellington lineage. The variation in the yield of fucoidan could therefore be due to natural variation within the population or be an artefact of the extraction process.

As with the alginate, no differences were noted in the results when the Wellington data set was matched before analysis to each of the other 3 lineages based on the amount of sun hours or when the data was analysed based on the month of harvest.

Conclusion

This study adds to the body of knowledge pertaining to the content of alginates and fucoidan for New Zealand Laminariales. Very little is known about the structure of alginates and fucoidans in the brown seaweeds found in New Zealand waters. Recent literature focuses on the chemical and structural composition of alginate (Panikkar and Brasch 1996; Fenoradosoa *et al*. 2010; Storz *et al*. 2009) and fucoidan (Chizhoz *et al*. 1999; Usov *et al*. 2001, Bilan *et al*. 2002). The focus on the composition of alginate and fucoidan rather than the content may be driven by specific requirements for the use of the polysaccharides for industrial and medicinal purposes based on their chemical properties (Fenoradosoa *et al*. 2010; Skriptsova *et al*. 2010).

Differences were detected in the yield of alginate and fucoidan for the Wellington lineage of *Lessonia variegata* over the period from March 2010 to February 2011. It would be valuable to characterize the alginate and fucoidan extracted from the *Lessonia variegata* in this study to determine the molecular weight, M/G ratio and monosaccharide composition as these highlight the expected biological activities of the alginate and fucoidan.

The best harvest time for the Wellington lineage of *Lessonia variegata* is early to mid-summer, which is the period immediately following the time of highest productivity for alginate and fucoidan. Harvesting efforts would be minimal for the most return at this time. If, as stated in the literature, fucoidan increases 5-fold with the onset of reproductive tissue, then if fucoidan is the desired polysaccharide and harvesting occurs during the period of highest fertility, harvesting quota will need to be ascertained at a level that does not have a deleterious effect on future stocks. If a high level of harvesting occurs when the plant is

fertile, then recruitment rates of subsequent plants could be reduced to a level that affects the population size.

It is a high priority to characterise the alginate and fucoidan from *Lessonia variegata* seasonally. This study ascertains the yield of alginate and fucoidan; but the use of the alginate and fucoidan is governed by its molecular weight and M/G ratio or monosaccharide composition. Therefore whilst this study identifies a harvest time based on the quantity of the resource required; the actual use of the resource is most likely to be identified based on the desired characteristics of the alginate or fucoidan. The alginate and fucoidan in this study were not characterised due to a lack of time and funding.

References

Adams, N. M. (1994). *Seaweeds of New Zealand.* New Zealand, Canterbury University Press.

Bilan, M.I., Grachev, A.A., Ustuzhanina, N.E., Shashkov, A.S., Nifantiev, N.E., and Usov, A.I. (2002). Structure of a fucoidan from the brown seaweed *Fucus evanescens* C.Ag. *Carbohydrate Research* **337**: 719-730.

Bold, H.C. and Wynne, M.J. (1978). *Introduction to the Algae*. New Jersey, Prentice-Hall Inc.

Bolton, J.J. (2010). The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. *Helgoland Marine Research* **64**(4): 263-279.

Boo, S.M. and Yoon, H.S. (2000). Molecular relationships of giant kelp (Phaeophyceae). *Algae* **15**: 13-16.

Brown, M.T., Nyman, M.A., Keogh, J.A., Chin, N.K. (1997). Seasonal growth of the giant kelp *Macrocystis pyrifera* in New Zealand. *Marine Biology.* **129:** 417-424.

Bruno, J.F., Stachowicz, J.J., and Bertness M.D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* **18**: 119-125.

Buschmann, A.H., Moreno, C., Vásquez J.A. and Hernández-González, M.C. (2006). Reproduction strategies of *Macrocystis pyrifera* (Phaeophyta) in southern Chile: The importance of population dynamics. *Journal of Applied Phycology* **18**: 575-582.

Buschmann, A.H, Hernandez-Gonzalez, M.D., and Varela, D. (2008). Seaweed future cultivation in Chile: perspectives and challenges. *International Journal of Environment and Pollution* **33**: 432-456.

Camus, P. (1994). Recruitment of the intertidal kelp *Lessonia nigrescens* Bory in northern Chile: successional constraints and opportunities. *Journal of Experimental Marine Biology and Ecology* **184**: 171-181.

Chandía, N. P., and Matsuhiro, B. (2008). Characterization of a fucoidan from Lessonia vadosa (Phaeophyta) and its anticoagulant and elicitor properties. *International Journal of Biological Macromolecules* **42**(3), 235-240.

Chandía, N. P., Matsuhiro, B., Ortiz, J.S., and Mansilla, A. (2005). Carbohydrates from the sequential extraction of *Lessonia vadosa* (Phaeophyta). *Journal of the Chilean Chemical Society* **50**(2), 501-504.

Chandía, N. P., Matsuhiro, B., & Vásquez, A. E. (2001). Alginic acids in Lessonia trabeculata: characterization by formic acid hydrolysis and FT-IR spectroscopy. *Carbohydrate Polymers* **46**(1), 81-87.

Chapman, V.J. (1970). *Seaweeds and their uses.* 2nd Edition. London, Methuen and Co.

Chapman, V.J., and Chapman D.J. (1980). *Seaweeds and their uses*. 3rd Edition. USA, Chapman and Hall.

Chizhov, A.O., Dell, A., Morris, H.R., Haslam, S.M., McDowell, R.A., Shashkov, A.S., Nifant'ev, N.E., Khatuntseva, E.A., and Usov, A.I. (1999). A study of fucoidan from the brown seaweed *Chorda filum. Carbohydrate Research* **320**: 108-119.

Contreras, L., Medina, M.H., Anrade, S., Oppliger, V., and Correa, J.A. (2007). Effects of copper on early development stages of *Lessonia nigrescens* Bory (Phaeophyceae). *Environmental Pollution* **145**: 75-83.

Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Camara, R. B. G., Nobre, L.T.D.B., Costa, M.S.S.P., Almeida-Lima, J., Farias, E.H.C., Leite, E.L., and Rocha, H.A.O. (2010). Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomedicine & Pharmacotherapy* **64**(1): 21-28.

Craigie, J.S., Morris, E.R., Rees, D.A., and Thom, D. (1984). Alginate block structure in Phaeophyceae from Nova Scotia: variation with species, environment and tissue-type. *Carbohydrate Polymers* **4**(4): 237-252.

Dargalkar V.K. And Verlecar X.N. (2009). Southern ocean seaweeds: A resource for exploration in food and drugs. *Aquaculture* **287**: 229-242.

Edding, M.E., and Tala, F.B. (2003). Development of techniques for the cultivation of *Lessonia trabeculata* Villouta et Santelices (Phaeophyceae: Laminariales) in Chile. *Aquaculture Research* **34**: 507-515.

Fenoradosoa, T.A., Delattre, G.A.C., Laroche, C., Petit E., Wadouachi, A., and Michaud, P. (2010). Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow. *Journal of Applied Phycology* **22**(2): 131-137.

Fitton, J.H., Irhimeh, M.R., and Teas, J. (2008). *Marine Algae and Polysaccharides with Therapeutic Application*. In Barrows, C., and Shahidi, F. (Eds.). *Marine Nutraceuticals and Functional Foods* (p345-365). Florida, USA: CRC Press.

Fraser, C.I., Nikula, R., Spencer, H.G., Waters, J.M. (2009). Kelp genes reveal effects of sub-Antarctic sea ice during the Last Glacial Maximum. *Proceedings of the National Academy of Sciences of the United States of America* **106**(9): 3249-3253.

Gaylord, B., Reed, D.C., Washburn, L., and Raimondi, P. T. (2004). Physical-biological coupling in spore dispersal of kelp forest macroalgae. *Journal of Marine Systems* **49**: 19-39.

Gutierrez, A., Correa, T., Muñoz V., Santibañez, A., Marcos, R., Cáceres, C. and Buschmann, A.H. (2006). Farming of the giant kelp *Macrocystis pyrifera* in southern Chile for development of novel food products. *Journal of Applied Phycology* **18**: 259-267.

Hay, C.H. (1987). *Lessonia adamsiae* sp. nov. (Phaeophyta: Laminariales) from the Snares Islands, New Zealand. *New Zealand Journal of Botany* **25**: 295-308.

Hawes, I., Nelson, W. and Mercer, S. (2004). Hang on to your Haptera. *Water and Atmosphere* **12**: 26-27.

Heatley, Hon. P. (2010) Minister of Fisheries and Agriculture's Decision on Sustainable Measures. HO865. Posted to: http://www.option4.co.nz/Fisheries_Mgmt/bladderkelp.htm (accessed 18 April 2011).

Hemmingson, J.A., Falshaw, R., Furneaux, R.H., and Thompson, K. (2006). Structure and antiviral activity of the galactofucan sulfates extracted from *Undaria pinnatifida* (Phaeophyta). *Journal of Applied Phycology* **18**: 185-193.

Honya, M., Mori, H., Anzai, M., Araki, Y., & Nisizawa, K. (1999). Monthly changes in the content of fucans, their constituent sugars and sulphate in cultured Laminaria japonica. *Hydrobiologia* **399**, 411-416.

Hurd, C.L. (2000). Water motion, marine macroalgal physiology, and production. *Journal of Phycology* **36**: 453-472.

http://glycomix.eu (accessed 18 April 2011).

http://www.niwa.co.nz/our-science/climate/publications/all/cs/monthly (accessed from April 2010).

Imbs. T.I., Shevchenko, N.M., Sukhoverkhov, S.V., Semenova, T.L., Skriptsova, A.V., and Zvyagintseva, T.N. (2009). Seasonal variations of the composition and structural characteristics of polysaccharides from the brown alga *Costaria costata. Cemistry of Natural Compounds* **45**(6): 786-791.

Kawai, H. and Sasaki, H. (2000). Molecular phylogeny of the brown algal genera *Akkesiphycus* and *Halosiphon* (Laminariales), resulting in the circumscription of the new families Akkesiphycaceae and Halosiphonaceae. *Phycologia* **29**: 416-428.

Kim, K. J., Lee, O. W., & Lee, B. Y. (2010). Fucoidan, a sulfated polysaccharide, inhibits adipogenesis through the mitogen-activated protein kinase pathway in 3T3-L1 preadipocytes. *Life Sciences* **86**(21-22), 791-797.

Lane, C., Maye,s C., Druehl, L. and Saunders, G. (2006). A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *Journal of Phycology* **42**: 493-512.

Larkum, A.W.D. (1986). A study of growth and primary production in *Ecklonia radiata* (C. Ag.) J.Agardh (Laminariales) at a sheltered site in Port Jackson, New South Wales. *J. Exp. Mar. Biol. Ecol.* **96**: 177-190.

Li, B., Lu, F., Wei, X., & Zhao, R. (2008). Fucoidan: Structure and Bioactivity. *Molecules* **13**: 1671-1695.

Macaya, E.C. and Zuccarello, G.C. (2010). DNA Barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales). *Journal of Phycology* **46**: 736-742

Martin, P. (2011). Phylogeny, phylogeography and population connectivity of *Lessonia* (Phaeophyceae). Victoria University of Wellington, PhD thesis.

McKee, J.W.A., Kavalieris, L., Brasch, D.J., Brown, M.T. and Melton, L.D. (1992). Alginate content and composition of *Macrocystis pyrifera* from New Zealand. *Journal of Applied Phycology* **4: 357-369.**

Miller, I. J. (1996). Alginate composition of some New Zealand brown seaweeds. *Phytochemistry* **41**(5): 1315-1317.

Nelson, W.A. (2005). Life history and growth in culture of the endemic New Zealand kelp *Lessonia variegata* J. Agardh in response to differing regimes of temperature, photoperiod and light. *Journal of Applied Phycology* **17**: 23-28.

Nelson, W.A. and Schwarz, A. (2005). Living in the ocean waves: kelp life histories. *Water and Atmosphere* **13**: 22-23.

Neushul, P. (1989). Seaweed for War: California's World War 1 Kelp Industry. *Technology and Culture* **30**(3), 561-583.

Onions, C.T. (Ed.) (1936). *The Shorter Oxford English Dictionary* 2nd Edition. Volume 1. A-M. Oxford, Oxford University Press.

Panikkar, R., and Brasch, D.J. (1996). Composition and block structure of alginates from New Zealand brown seaweeds. *Carbohydrate Research* **293**: 119-132.

Parke, M. (1948). Studies on British Laminariaceae .1. Growth in *Laminaria saccharina* (L) Lamour. *Journal of the Marine Biological Association of the United Kingdom* **27**(3), 651-709.

Raimondi, P.T., Reed, D.C., Gaylord, B. and Washburn, L. (2004). Effects of self-fertilization in the giant kelp *Macrocystis pyrifera*. *Ecology* **85**: 3267-3276.

Rioux, L.E., Turgeon, S.L., and Beaulieu, M. (2007). Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers* **69**: 530-537.

Saunders, G.W. and Druel, L.D. (1992). Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): implications for kelp evolution. *Journal of Phycology* **28**: 544-549.

Saunders, G.W. and Druehl, L.D. (1993). Revision of the kelp family Alariceae and the taxonomic affinities of Lessoniopsis-Reinke (Laminariales, Phaeophyta). *Hydrobiologia* **261**: 689-697.

Schwarz, A. M., Hawes, I., Nelson, W., & Andrew, N. (2006). Growth and reproductive phenology of the kelp Lessonia variegata in central New Zealand. *New Zealand Journal of Marine and Freshwater Research* **40**(2), 273-284.

Shan, T.F., and Pang, S.J. (2009). Assessing genetic identity of sporophytic offspring of the brown alga *Undaria pinnatifida* derived from mono-crossing of gametophyte clones by use of amplified fragment length polymorphism and microsatellite markers. *Phycological Research* **57**: 36-44.

Skriptsova, A., Khomenko, V., and Isakov, V. (2004). Seasonal changes in growth rate, morphology and alginate content in *Undaria pinnatifida* at the northern limit in the Sea of Japan (Russia). *Journal of Applied Phycology* **16**: 17-21.

Skriptsova, A. V., Shevchenko, N. M., Zvyagintseva, T. N., & Imbs, T. I. (2010). Monthly changes in the content and monosaccharide composition of fucoidan from Undaria pinnatifida (Laminariales, Phaeophyta). *Journal of Applied Phycology* **22**(1), 79-86.

South, G.R., and Whittick, A. (1987). *Introduction to Phycology*. Oxford UK, Blackwell Scientific Publications.

Sriamornsak, P. and Sungthongjeen, S. (2007). Modification of theophylline release with alginate gel formed in hard capsules. *AAPS Pharm. Sci. Tech.* **8**(3) Article 51.

Storz, H,. Müller, K.J., Ehrhart, F., Gómez, I., Shirley, S.G., Gessner, P., Zimmermann, H., Weyand, E., Sukhorukov, V.L., Forst, T., Weber, M.M., Zimmermann, H., Kulicke, W.M., and Zimmermann, U. (2009). Physicochemical features of ultra-high viscosity alginates. *Carbohydrate Research* **344**(8): 985-995.

Tala, F. and Edding, M. (2005). Growth and loss of distal tissue in blades of *Lessonia nigrescens* and *Lessonia trabeculata* (Laminariales). *Aquatic Botany* **82**: 39-54.

Tala, F. and Edding, M. (2007). First estimates of productivity in *Lessonia trabeculata* and *Lessonia nigrescens* (Phaeophyceae, Laminariales) from the southest Pacific. *Phycological Research* **55**: 66-79.

Tellier, F., Meynard, A.P., Correa, J. A., Faugeron S. and Valero M. (2009). Phylogeographic analyses of the 30°S south-east Pacific biogeographic transition zone establish the occurrence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*: Vicariance or parapatry? *Molecular Phylogenetics and Evolution* **53**: 679-693.

Tynan C.T. (1998). Ecological importance of the southern boundary of the Antarctic Circumpolar Current. *Nature* **392**: 708 – 710.

Usov, A.I., Smirnova, G.P., and Klochkova, N.G. (2001). Polysaccharides of Algae: 55. Polysaccharide composition of several brown algae from Kamchatka. *Russian Journal of Bioorganic Chemistry* **27**(6): 395-399.

Usov, A.I., Kosheleva, E.A., and Yakovlev, A.P. (1985). Polysaccharides of Algae. XXXV. Polysaccharide composition of some brown algae from the Sea of Japan. *Bioorganicheskaya Khimiya* **11**(6): 830-836

van Alstyne, K.L.(1988). Herbivore grazing increases polyphenolic defences in the intertidal brown alga *Fucus distichus. Ecology* **69**(3): 655-663

van Tussenbroek, B.I. (1993). Plant and frond dynamics of the giant kelp, *Macrocystis pyrifera* forming a fringing zone in the Falkland Islands. *Eur. J. Phycol.* **28**: 161-165.

Villegas M.J., Laudien J., Sielfeld W. and Arntz W.E. (2008). *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales; Phaeophyceae) kelp habitat structures associated macrobenthic community off northern Chile. *Helgoland Marine Research* **62** (Suppl. 1): S33-S43.

Wang, J., Wang, F., Zhang, Q., Shi, X. and Li, P. (2009). Synthesized different derivitives of low molecular fucoidan extracted from *Laminaria japonica* and their potential antioxidant activity in vitro. *International Journal of Biological Macromolecules* **Aa**: 379-384.

Waters, J. M. (2008). Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. *Journal of Biogeography* **35**: 417-427.

Westermeier, R., Patiño, D., Piel, M. I., Maier, I., and Mueller, D.G. (2006). A new approach to kelp mariculture in Chile: production of free-floating sporophyte seedlings from gametophyte cultures of *Lessonia trabeculata* and *Macrocystis pyrifera. Aquaculture Research* **37**: 164-171.

Westermeier, R. , Patiño, D. and Müller, D. (2007). Sexual compatibility and hybrid formation between the giant kelp species *Macrocystis pyrifera* and *M. integrifolia* (Laminariales, Phaeophyceae) in Chile. *J. Appl. Phycol.***19**: 215-221.

Wijesekara, I., Pangestuti, R., and Kim, S. K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers* **84**(1): 14-21.

Witvrouw, M., and De Clercq, E. (1997). Sulfated Polysaccharides Extracted from Sea Algae as Potential Antiviral Drugs. General Pharmacology **29**(4): 497-511.

Yang, C., Chung, D., Shin, I. S., Lee, H., Kim, J., Lee, Y., and You, S. (2008). Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of Undaria pinnatifida. *International Journal of Biological Macromolecules* ***43***(5): 433-437.

Zhuang, C., Itoh, H., Mizuno, T., and Ito, H. (1995). Antitumor active fucoidan from the brown seaweed, Umitoranoo (*Sargassum thunbergii*). *Bioscience Biotechnology and Biochemistry* ***59***(4): 563-567.

Zvyagintseva, T.N., Shevchenko, N.M., Popivnich, I.B., Isakov, V.V., Scobun, A.S., Sundukova, E.V., and Elyakova, L.A. (1999). A new approach for the separation of water-soluble polysaccharides from brown seaweeds. *Carbohydrate Research* **332**: 32-39.

Zvyagintseva T.N., Shevchenko N.M., Chizhov, A.O., Krupnova, T.N., Sundukova, E.V., and Isakov, V. (2003). Water soluble polysaccharides of some far-eastern seaweeds. Distribution, structure, and their dependence on the developmental conditions. *Journal of Experimental Marine Biology and Ecology* **294**(1) 1-13.

Zvyagintseva T.N., Shevchenko N.M., Nazarenko E.L., Gorbach V.I., Urvantseva A.M., Kiseleva M.I., and Isakov V.V. (2005). Water-soluble polysaccharides of some brown algae of the Russian Far-East. Structure and biological action of low-molecular mass polyuronans. *Journal of Experimental Marine Biology* **320**: 123-131.

Appendix 1.

Table 5. Percent of alginate and fucoidan for all samples of *Lessonia variegata* from the Wellington lineage. (NA = Data not available due to spillage during the extraction process, sample number refers to the number assigned to the blades from each plant each month as recorded in my laboratory notebook).

Month and Year	Sample number	% Alginate	% Fucoidan
Mar-10	78	12.6	15.5
Mar-10	79	16.2	26.5
Mar-10	80	15.5	19.5
Mar-10	81	14.2	13.2
Mar-10	82	18.0	NA
Mar-10	83	17.6	10.1
Mar-10	84	17.3	7.3
Mar-10	85	15.3	11.4
Mar-10	86	17.4	14.8
Mar-10	87	18.5	17.2
Apr-10	98	17.2	11.2
Apr-10	100	22.3	15.4
Apr-10	101	16.2	15.6
Apr-10	102	14.2	NA
Apr-10	103	18.5	13.5
Apr-10	104	16.5	22.1
Apr-10	105	16.8	NA
Apr-10	106	18.4	19.1
Apr-10	107	19.0	25.8
Apr-10	108	17.5	6.9
May-10	119	21.1	10.2
May-10	120	24.8	7.6
May-10	121	24.8	9.2
May-10	122	22.3	10.6
May-10	123	14.1	16.4
May-10	124	16.2	11.3

May-10	125	18.7	9.8
May-10	126	20.6	13.7
May-10	127	15.4	15.0
May-10	128	21.5	12.5
Jun-10	129	21.7	18.0
Jun-10	130	16.9	12.3
Jun-10	131	18.3	NA
Jun-10	132	19.9	11.4
Jun-10	133	17.5	10.2
Jun-10	134	16.4	6.6
Jun-10	135	20.8	7.3
Jun-10	136	21.7	7.2
Jun-10	137	18.0	9.3
Jun-10	138	20.8	11.7
Jul-10	139	15.1	14.1
Jul-10	140	16.6	8.6
Jul-10	141	19.9	NA
Jul-10	142	10.8	15.4
Jul-10	143	19.5	8.3
Jul-10	144	23.1	8.6
Jul-10	145	16.6	15.9
Jul-10	146	17.4	NA
Jul-10	147	13.3	13.7
Jul-10	148	15.8	18.5
Aug-10	149	18.6	11.6
Aug-10	150	15.8	15.8
Aug-10	151	20.5	12.1
Aug-10	152	19.3	12.3
Aug-10	153	16.6	15.3
Aug-10	154	17.5	6.0
Aug-10	155	18.4	12.8
Aug-10	156	15.6	13.6
Aug-10	157	17.5	16.2

Sep-10	159	16.9	10.9
Sep-10	160	17.5	7.8
Sep-10	161	13.0	6.7
Sep-10	162	19.2	10.9
Sep-10	163	21.0	8.7
Sep-10	164	19.3	11.6
Sep-10	165	17.5	12.3
Sep-10	166	18.2	11.6
Sep-10	167	12.3	15.9
Sep-10	168	17.5	11.9
Oct-10	179	21.2	11.8
Oct-10	180	19.7	14.3
Oct-10	181	19.1	9.9
Oct-10	182	18.4	10.5
Oct-10	183	19.9	14.0
Oct-10	184	15.9	13.2
Oct-10	185	17.8	13.5
Oct-10	186	22.9	13.2
Oct-10	187	19.0	12.8
Oct-10	188	18.9	13.0
Nov-10	189	22.9	15.9
Nov-10	190	21.2	14.2
Nov-10	191	24.5	18.6
Nov-10	192	22.4	16.8
Nov-10	193	21.8	14.0
Nov-10	194	21.7	14.5
Nov-10	195	19.6	14.6
Nov-10	196	29.9	17.1
Nov-10	197	27.5	15.2
Nov-10	198	25.6	14.3
Dec-10	199	30.9	14.6
Dec-10	200	22.3	NA
Dec-10	201	17.7	9.7

Dec-10	202	20.8	12.2
Dec-10	203	17.9	11.0
Dec-10	204	19.4	9.5
Dec-10	205	19.8	9.6
Dec-10	206	21.0	13.3
Dec-10	207	20.1	6.9
Dec-10	208	15.2	17.8
Jan-11	209	17.5	11.6
Jan-11	210	18.9	12.9
Jan-11	211	19.9	12.4
Jan-11	212	19.5	9.6
Jan-11	213	18.3	14.3
Jan-11	214	20.3	11.3
Jan-11	215	19.0	13.1
Jan-11	216	17.0	12.1
Jan-11	217	17.1	12.8
Jan-11	218	17.8	12.9
Feb-11	219	19.9	10.6
Feb-11	220	19.9	11.3
Feb-11	221	19.9	11.9
Feb-11	222	20.5	10.9
Feb-11	223	18.8	11.6
Feb-11	224	18.3	12.6
Feb-11	225	16.1	12.2
Feb-11	226	21.6	11.6
Feb-11	227	18.3	11.7
Feb-11	228	22.3	13.3

Table 6. Percent of alginate and fucoidan for all samples of *Lessonia variegata* from the Southern, Kaikoura and Northern lineages. (NA = Data not available due to spillage during the extraction process, sample number refers to number assigned to the blades from each plant each month as recorded in my laboratory notebook).

Lineage	**Month**	**Sample number**	**% Alginate**	**% Fucoidan**
Southern	March	88	17.5	14.2
Southern	March	89	22.6	6.3
Southern	March	90	18.6	5.7
Southern	March	91	24.3	10.5
Southern	March	92	23.6	10.5
Southern	March	93	22.0	6.3
Southern	March	94	25.5	11.1
Southern	March	95	21.8	7.6
Southern	March	96	26.1	13.4
Southern	March	97	25.4	9.9
Kaikoura	April	109	17.6	29.5
Kaikoura	April	110	22.6	6.3
Kaikoura	April	111	18.6	31.6
Kaikoura	April	112	24.3	20.7
Kaikoura	April	113	23.6	19.7
Kaikoura	April	114	22.0	7.2
Kaikoura	April	115	25.5	12.1
Kaikoura	April	116	21.8	9.2
Kaikoura	April	117	26.1	11.4
Kaikoura	April	118	25.4	5.9
Northern	September	169	26.5	NA
Northern	September	170	24. 8	8.9
Northern	September	171	24. 7	8. 7
Northern	September	172	28	8.3
Northern	September	173	26.1	9.2
Northern	September	174	25.3	7.0
Northern	September	175	26.3	7.3
Northern	September	176	24.9	14.1
Northern	September	177	22. 8	7.6
Northern	September	178	26.6	7.5

Appendix 2

Non-significant results of statistical analysis of data

There was no correlation between the yield of alginate extracted and the yield of fucoidan extracted, r^2= .0036, Fig. 27.

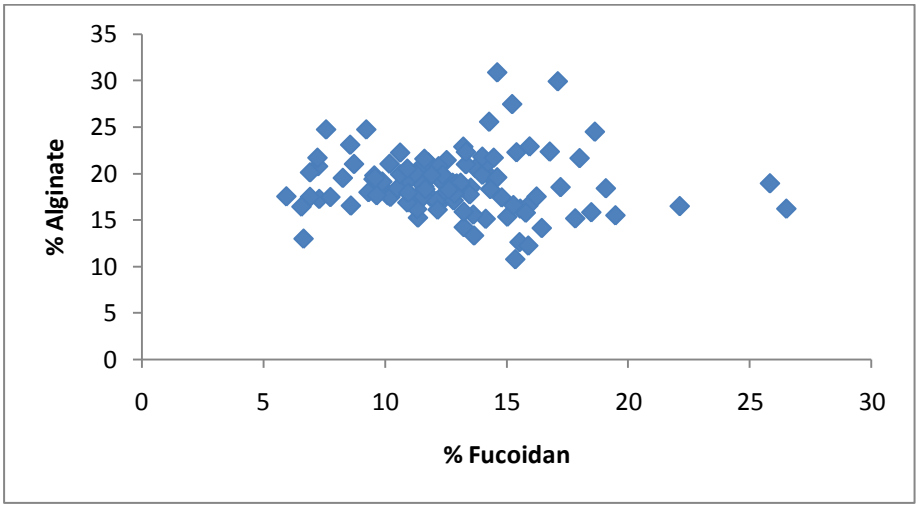

Fig. 27 . Scatter plot of correlation between alginate and fucoidan extracted (calculated as a % of beginning weed weight).

There was no correlation between the yield of alginate and the growth rate, r^2 = 0.0322, Fig.28.

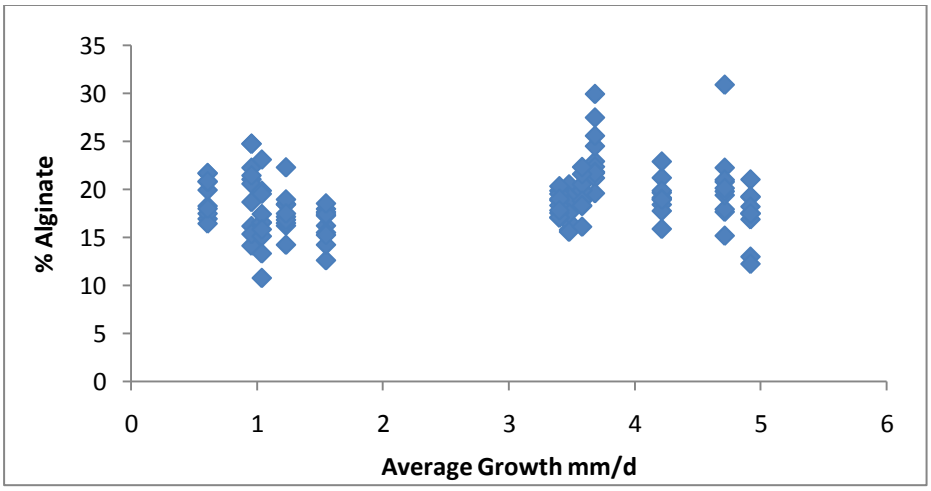

Fig. 28. Scatterplot of correlation between alginate extracted (calculated as a % of beginning weed weight) and the average growth per month (calculated as millimetres per day).

There was no correlation between the yield of alginate and the Sun hours, $r^2 = 0.018$, Fig. 29.

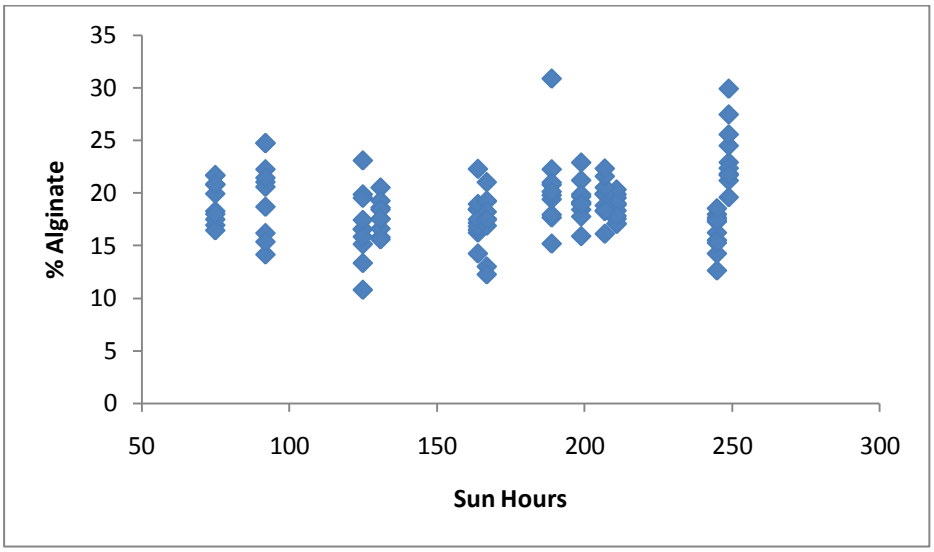

Fig. 29 .Scatter plot of correlation between alginate extracted (calculated as a % of beginning weight) and sun hours for the month of extraction.

There was no correlation between the yield of alginate and the average SST per month, $r^2 = 0.0006$, Fig. 30.

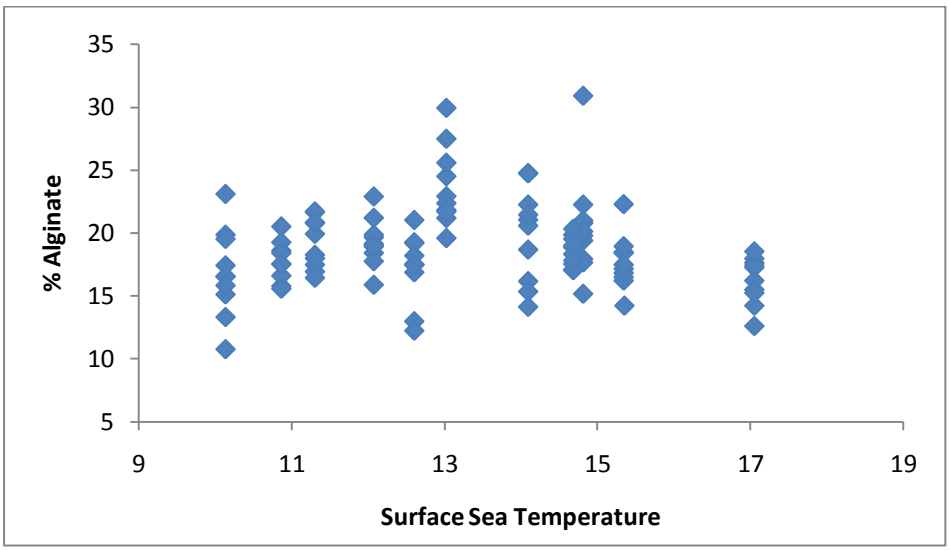

Fig. 30. Scatterplot of correlation between alginate extracted (calculated as a % of beginning weight) and surface sea temperature (SST is calculated as an average for each month).

There was no correlation between the yield of fucoidan and the growth rate per month, $r^2 = 0.077$, Fig. 31.

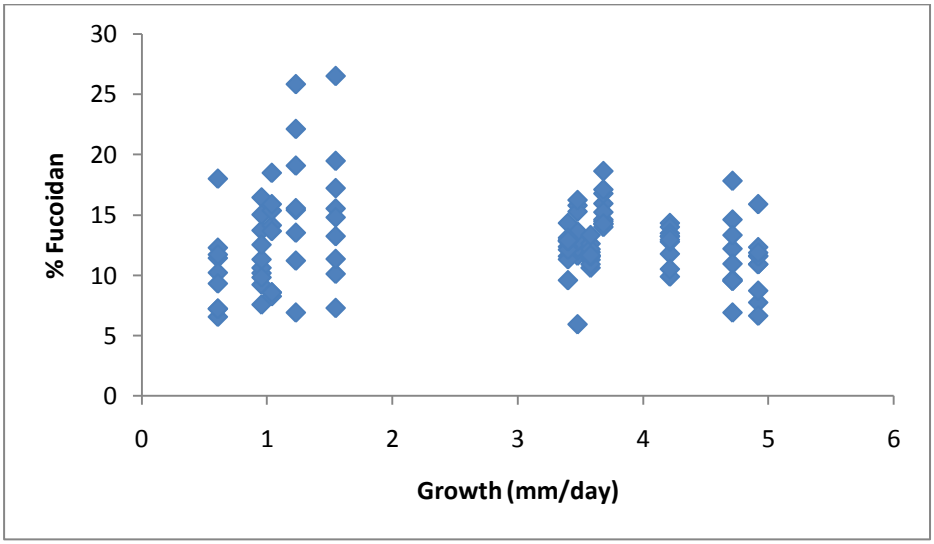

Fig. 31. Scatterplot of the correlation between fucoidan extracted (calculated as a % of beginning weight) and the average growth per month (calculated as millimetres per day).

The season of harvest was not significant for the yield of fucoidan, Fig. 32.

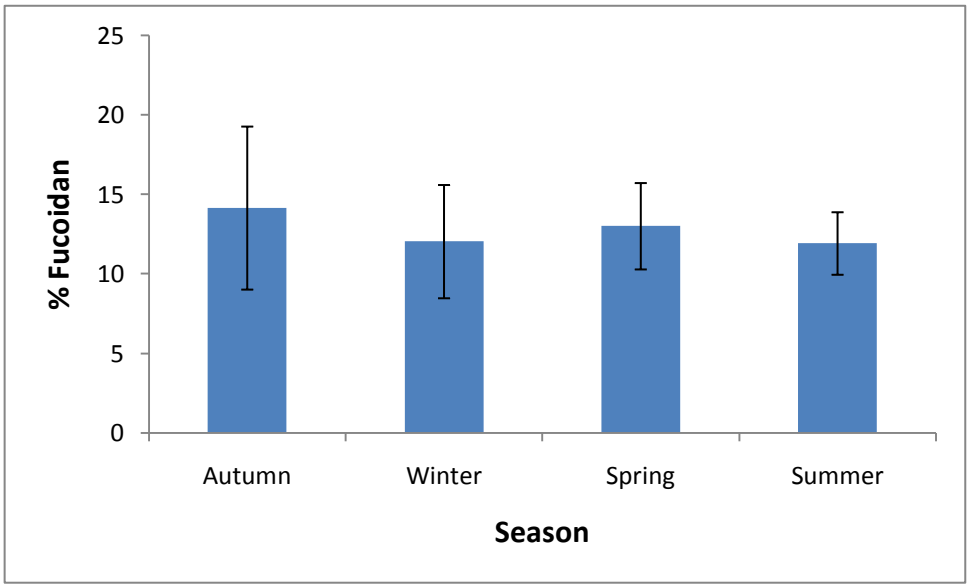

Fig. 32. Histogram showing the seasonal means (± standard deviation, n= 26-30) for fucoidan within the Wellington lineage of *Lessonia variegata* for the period of March 2010 – February 2011, (expressed as a percentage of the beginning weight of the *Lessonia variegata* milled samples).

Appendix 3 Table 7. Growth of *Lessonia variegata* calculated as millimetres per day for each month from March 2010 until February 2011 for two blades of each of twenty plants at the Wellington study site (x = Plant located but blades eroded or lost; * = Plant not located).

Plant ID	Mar-10	Apr-10	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11
1	3.90	1.04	0.42	0.82	0.6	3.43	6.83	x	2.29	*	2.75	5.17
1	3.48	1.61	1.06	1	1.78	4.93	3.83	3.23	x	*	x	x
2	3.95	0.82	0.42	0.82	0.6	3.43	3.97	5	3	3.11	2.11	2.46
2	1.29	0.37	1.06	1	1.78	4.93	5.27	x	1.54	2.96	x	0.96
3	0.52	0.94	0.85	0.29	1.91	1.96	2.83	2.27	5.11	3.44	3.11	2.75
3	2.75	0.84	x	0.04	1.29	1.86	4.3	3.87	4.32	4.67	3.11	x
4	1.25	0.78	0.21	0.79	0.62	2.63	6.6	7.3	*	x	4.36	4.04
4	0.65	0.71	x	0.5	x	2.84	x	5.83	*	x	3.07	3.46
5	2.80	2.39	*	0.38	*	5.46	6.93	3.03	*	x	2.54	2.04
5	0.45	1.45	*	0.7	*	5	5.8	5.93	*	x	2.96	6.08
6	0.60	0.86	x	0.69	0.62	2.04	5.1	3.2	x	4.63	3.79	3.46
6	2.65	0.98	x	0.7	0.71	0.79	x	2.5	x	3.7	2.32	4.21
7	0.65	1.24	0.67	0.54	1.55	5	3.6	3.83	2.89	x	2.86	5.79
7	0.85	1.33	0.24	0.29	x	2.49	x	3.27	x	x	x	3.92
8	1.15	1.10	1.27	0.29	0.21	2.63	3	6.77	5.5	7.22	5.14	*
8	2.20	1.29	0.7	x	x	x	10.5	9.8	5.07	4.85	x	*
9	2.15	1.06	0.76	1.11	0.59	5.07	2.63	x	*	*	x	3.46
9	1.30	0.94	x	0.82	1.79	5.77	3.93	x	*	*	x	x
10	1.80	1.43	1.3	0.43	0.91	3.54	3.37	3.1	*	*	1.96	*
10	1.30	1.27	1.09	0.82	1.2	x	3.37	3.1	*	*	x	*
11	2.55	2.18	0.7	0.11	1.02	2.25	5.47	3.67	3.68	4.93	3.25	3.67
11	0.55	2.53	1.15	0.21	x	1.64	3.97	3.07	3.89	4.15	3.32	3.42
12	1.75	1.84	0.76	0.26	0.15	3.12	4.4	2.03	*	x	2.79	2.96
12	3.20	2.20	0.94	0.35	x	2.16	4.97	1.97	*	x	3.25	3.71
13	2.55	1.63	0.97	0.39	0.86	1.71	3.97	6.67	4.39	5.37	3.43	3.67
13	2.85	2.55	0.7	0.39	x	2.57	8.37	5.13	x	5.63	x	4.25
14	0.80	1.98	0.94	0.65	1.26	2.95	5.83	2.7	3.75	4.33	4.54	2.33
14	2.20	1.45	x	0.65	1	2.26	5.17	4.77	4	x	x	x
15	1.15	1.08	*	*	1.48	5.56	6.9	4.83	3.68	6.15	3.68	4.08
15	1.35	0.53	*	*	x	3.3	2.47	4.43	4.54	x	3.5	x
16	2.30	0.37	*	0.57	0.53	2.29	6.3	3	2.86	*	x	4.71
16	2.30	0.67	*	x	1.07	5.43	6.63	4.6	x	*	x	3.5
17	2.15	1.20	0.82	0.5	0.49	4	2.27	3.4	3.64	*	5.43	1.63
17	0.80	1.02	0.82	x	x	3.04	4.27	x	2.96	*	x	2.58
18	1.95	0.92	1.73	0.57	0.79	3.86	4.57	2.93	4.89	5	4.96	3.13
18	2.25	1.12	1.58	x	x	x	5.53	4.47	x	4.59	x	5.29
19	1.30	*	1.61	1.18	0.69	1.88	5.53	4.97	1.68	5.41	x	x
19	1.05	*	1	0.64	1.1	x	5.5	4.8	x	x	x	x
20	0.20	*	2.52	1.61	2.62	5.21	5.03	3.83	*	*	x	*
20	1.35	*	x	1.14	1.42	6.14	x	x	*	*	x	*