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Distribution, abundance and reproduction of *Undaria pinnatifida* (Harvey) Suringar from the Marlborough Sounds, New Zealand

Weiwei CHEN

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of an university or other institution of higher learning.

Signature: Date:

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Abstract

Undaria pinnatifida, known as wakame in Japan, is cultivated in Asia in a multi-million dollar industry. It is primarily farmed for human consumption, with many different products made from it. In the 1980s *U. pinnatifida* was accidently introduced to New Zealand. It has subsequently spread on the East Coast of New Zealand from Stewart Island to Tutukaka.

MAF Biosecurity classed *U. pinnatifida* as an “unwanted species” and so harvest or cultivation in New Zealand was illegal. In 2010 the policy changed and it can now be harvested and cultivated in some areas of New Zealand under certain conditions. It is prolific on existing mussel farms, prompting owners of mussel farms to apply for harvesting permits. Before this seaweed can be widely commercialized in New Zealand, it is important to understand its basic ecology, biology and reproductive phenology. This study will examine these aspects of *Undaria pinnatifida* growing on mussel farms in the Marlborough Sounds.

This study included a total of nine farms and data were gained monthly from 29th of March to 09th of December. Six farms from Pelorus Sound included Farm 23, Farm 122, Farm 233, Farm 280, Farm 314 and Farm 353. Three farms from Port Underwood included Farm 106, Farm 253 and Farm 327. The growth rate, biomass, percentage of mature *U. pinnatifida*, zoospores released, fecundity, and seasonality of sporophyte germination were measured from these farms. In September the biomass on Farm 8052, Farm 8053, Farm 8054 and Farm 8057 was also measured.

The density and biomass of *U. pinnatifida* differed significantly between farms in across this study. In Pelorus Sound, the abundance of *U. pinnatifida* peaked in October, November and December, with the maximum biomass 4.85kg per drop at Farm 122. This pattern was different in Port Underwood, where abundance peaked in September, with Farm 327 having the maximum 3.96kg per drop. In general, most *U. pinnatifida* was found in the top 3-5m.

Concomitant with the increase in abundance, the growth rates of the sporophytes increased markedly in September, October and November.

While the peak abundance was in Spring, there were plants present throughout this study on most farms, with many having mature sporophylls (24% of Port Underwood plants and 16% of Pelorus Sound plants). The spore production from these sporophylls was similar to those of previous studies (up 5.20 X 10^3 spores cm^{-2} of sporophyll tissue at Farm 327 on September), and the sporophylls were competent to produce spores throughout this study. For the first time, this study found conclusive evidence that not only are there spores released throughout the year, but germination of the sporophyte also occurs throughout the year.

Chapter 1: Introduction

1.1 General introduction

In this general introduction, the background of *U. pinnatifida* will be examined. First, a brief discussion of the general characteristics of seaweeds will be provided, followed by the examination of the biology and ecology of *U. pinnatifida*. This then will lead to a discussion of the population distribution of *U. pinnatifida* across the world and within New Zealand. A brief review of New Zealand's previous and current legislation regarding to the management of this kelp will be carried out. From this, a brief prediction will be drawn regarding the aquacultural potential of *U. pinnatifida* in New Zealand.

1.1.1 Seaweeds

Seaweeds are classified as algae, which are photosynthetic organisms with characteristics different to land plants (Thomas, 2002). Most algae are found in marine, freshwater and brackish water (Lee, 2008). Algae range from unicellular to multicellular, from microscopic to macroscopic and are in many forms and in many subclasses. The word seaweed only refers to macroscopic members the divisions of Chlorohyta, which are green algae, Phaeophyceae which are brown algae and Rhodophyta which are red algae (Sumich & Morrissey, 2004).

Brown algae sit within the Phaeophyceae, which is classified under the Phyllum of Heterokontophyta (Lee, 2008). There are approximately 1500 living species of brown algae, with 99.7% being marine species, while the rest inhabit brackish water. Phaeophyceae are entirely multicellular and macroscopic (Sumich & Morrissey, 2004). Therefore, virtually all brown algae can be referred to as brown seaweeds. Brown seaweeds vary in morphological form and in fact vary from olive colour to brown. In comparison to green and red seaweeds, brown seaweeds can be larger and are often referred to as "kelps" (Thomas, 2002)

Seaweeds are not as complex as flowering plants. They lack flowering mechanisms, seeds, true leaves and roots, but do have some comparable structural features to flowering plants. Seaweeds in general may have the following structures, the blade, pneumatocysts, the stipe and the holdfast (Sumich & Morrissey, 2004).

The seaweed blade is the structure which resembles the leaf of a plant. It is flat and can be broad in width (Sumich & Morrissey, 2004). Some seaweed have blades which branch. Large brown seaweeds are likely to be developed from flat, single blades into unique blade arrangements with distinct blade shapes. These include seaweeds in the genus of *Alaria, Nereocystis, Macrocystis, Egregia, Pelagophycus, Chorda* and *Laminaria* (Sumich & Morrissey, 2004).

Pneumatocysts are found in some seaweeds. This is a bladder like structure filled with gases, which include nitrogen, oxygen and carbon dioxide. These floats help to bring the blades upright for the process of photosynthesis. Such buoyancy aids exist in seaweeds such as *Macrocystis pyrfera*, *Pelagophycus porra* and *Ascophyllum nodosum* and many more (Thomas, 2004).

The stipe is the structure which resembles the stem of flowering plants. Stipes allow for attachments of blades and supports photosynthetic tissues to reach for the surface of the water for light absorption (Thomas, 2004). However, some seaweeds lack this structure and their blades are blended into the holdfasts without forming a stipe. *Macrocystis* and a few red and brown algae are found to contain special cells within their stipes for cellular transport of products from photosynthesis (Sumich & Morrissey, 2004).

The holdfasts of seaweeds resemble roots of land plants but are not true roots as they are not responsible for uptake of nutrients. These holdfasts only serve their purpose as acting as anchorage for the seaweed. They hold the seaweed in place resisting motions of wave currents (Sumich & Morrissey, 2004).

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The colourings of seaweeds are due to predomination of their specific

photosynthetic pigments (Thomas, 2002). The grass-green colour of green algae is due to the dominance of chlorophylls compared to other pigments such as carotenes. The expression of brown colour in brown algae is due to the mixture of the golden colour of xanthophylls pigments such as fucoxanthin, and green colour of chlorophylls pigments. However, brown algae are not usually literally brown but a drab olive green in colour (Sumich & Morrissey, 2004). Red algae, however appears in wide range of different colours, from bright green to brown to red or purple in colour. Red algae contain green chlorophyll pigments as well as red phycobilin and blue phycobilin pigment (Sumich & Morrissey, 2004).

U. pinnatifida is a type of brown seaweed commonly called by the Japanese name "*wakame*" (Guiry & Guiry, 2011). *U. pinnatifida* is native to the Japanese, Korean and Chinese seas (Hay & Villuota, 1994). This Asian seaweed has spread to many other parts of the world and was classified as an unwanted species in most countries (Invasive Species Specialist Group, 2007b). *U. pinnatifida* will have many common biological structures when compared to its relatives. Yet it will express unique biological traits that will be explained further in the next part of this thesis.

1.1.2 Biology and ecology of *Undaria pinnatifida*

U. pinnatifida is commonly referred to by its Japanese name – *wakame*. It is known as *Qun Dai Cai* and *Miyok* or *Miyeouk* in Chinese and Korean respectively (Guiry & Guiry, 2011). *U. pinnatifida* is an edible seaweed native to the temperate regions of China, Japan and Korean seas but has spread to 12 countries across four continents (Stuart, 2003).

According to Guiry and Guiry (2011), *U. pinnatifida* has been classified as the following:

Empire: Eukaryota
Kingdom: Chromista
Phylum: Heterokontophyta
Class: Phaeophyceae
Order: Laminariales
Family: Alariaceae
Genus: Undaria
Species: pinnatifida

U. pinnatifida is laminarian kelp which is heteromorphic and has an annual life history. It has two different life stages, the sporophyte stage which is macroscopic and is visible to the naked eye and its gametophyte stage which is microscopic (Stuart, 2003). Although the longevity of its sporophyte stage is only approximately six months, at its gametophyte stage, *U. pinnatifida* is able to remain viable for more than 24 months (Stuart, 2003).

U. pinnatifida appears as a thallus laminate kelp in its sporophyte state. It can vary from yellowish to dark brown in colour. It is usually one to two metres in length in its mature state and can reach up to three metres.

Figure 1: Mature sporophyte with apex eroded.

Mature sporophytes of *U. pinnatifida* have holdfasts which act as anchorage (Stuart, 2003). The holdfast of *Undaria pinnatifida* is slender branches of haptera which resemble the roots of plants (Parson, 1994).

When *U. pinnatifida* reaches its mature state, the sporophylls develop on bilateral sides of the flattened edges of the stipe (Hay & Gibbs, 1996). Sporophyll of the seaweed becomes interleaved when fully matured and appear to have looped around the stipe. It may appear as one piece of sporophyll but are in fact always two discrete pieces (Hay, 1990). The stipe extends away from the holdfast to become the midrib of the frond and the large, translucent blade of the sporophytes grows on the margins of the midrib. The midrib can be as wide as 4cm and the stipe can be as long as 50cm (Hay &Gibbs, 1996). Blades of the sporophytes have tiny hair pits otherwise referred to as cryptostomata scattered across the smooth and glossy surface (Parson, 1994). The apex of the blade is prone to erosion over time; tissues of the blade found in the apex of the sporophytes can sometimes appear rotten due to this process (Hay & Villuota, 1993).

Figure 2: Juvenile sporophytes of *U. pinnatifida*.

Juvenile sporophytes are much simpler in morphology. Young *U. pinnatifida* sporophytes consist of only a holdfast, stipe and undivided blade. The midribs of *U. pinnatifida* become visible only on sporophytes which have a blade longer than 5cm in length (MAF Biosecurity, 2006).

U. pinnatifida are similar in appearance when compared to the native New Zealand kelp, *Ecklonia radiata*. However, *U. pinnatifida* has a distinct midrib and spiral sporophyll when mature. Young *U. pinnatifida* can be easily mistaken as juvenile *E. radiata* due to the absence of these two features (MAF Biosecurity, 2006).

Sporophytes can be up to three metres in length in Japan. In New Zealand, Hay and Villuota (1993) recorded the maximum total sporohyllous plant length to be 56 cm in Wellington Freberg and 135 cm at Timaru (Parson, 1993). However, these measurements were not precise, as the apex of the blade was often eroded, which affected the results of this study (Hay & Villuota, 1993).

U. pinnatifida is an annual seaweed. In late summer and early autumn, mature seaweeds degenerate and new sporophytes become established (Hay & Villuota, 1993). In Japan, sporophytes of *U. pinnatifida* completely die back during autumn. However, in New Zealand, sporophytes are present throughout the year. Hay and Villuota (1993) presumed that this phenomenon could be due to the narrower range of annual sea temperature of the New Zealand water compared to the Japanese and Korean coast water, which have a wider range of annual temperature fluctuation (Hay & Villuota, 1993).

U. pinnatifida favours cold water temperatures, with preference for temperature less than 12℃ (Ministry of Fisheries, 2001). In Asia, the native habitat of U. pinnatifida, sporophytes grow quickly between 5 and 13℃ during winter and spring. Its optimal temperature for growth is approximately 10℃ (Hay & Villuota, 1993). Sporophytes begin to degrade once the temperature reaches above 20℃ and at above 23℃, sporophytes die off (Ministry of Fisheries, 2001).

In Asia, the release of zoospores occurs in spring and summer and mostly at water temperature between 17 and 20℃. Germination of zoospores takes place at a water temperature of approximately 20℃. However, at a water temperature of over 20℃, longevity of zoospores decreases and less germination takes place. No germinating zoospores have been recorded at a water temperature of 27℃ or higher (Hay & Villuota, 1993).

Reproduction of *Undaria pinnatifida* is by the release of asexual zoospores by the mature sporophyll of the seaweed (Hay & Gibbs, 1996). Millions of haploid zoospores drift until they reach a suitable site for attachment. Attached zoospores will then germinate into microscopic female gametophytes and male gametophytes (Ministry of Fisheries, 2001). These gametophytes can remain viable for up to three years in their dormant state before they begin germination (Ministry of Fisheries, 2001). Male gametophytes release mobile sperm into the surrounding water while female gametophytes produce eggs which remain on the gametophyte (Parson, 1994). Mobile sperm fertilise the egg and this fertilised egg will form a germling, which then develop into a sporophyte *in situ* (Parson, 1994).

As described by the Ministry of Fisheries (2001), *U. pinnatifida* is able to grow on any hard surface that it can attach itself to. It inhabits rocky reefs, mudstones, cobbles or even shells of abalone or bivalves. It is also able to grow on sea grasses or epiphytically attaching on other seaweeds. In addition, *U. pinnatifida* can be found in man-made substrates such as buoys, pylons, ropes, bottles, hulls of ships or boats and pontoons (Ministry of Fisheries, 2001).

U. pinnatifida is found in low intertidal zones up to depth of 18m but is mainly found at depth between 1 to 3m. It can often form a thick, dense canopy that shades the sea life beneath it (Invasive species specialist group, 2007b). *U. pinnatifida* has a high tolerance to sunlight and wave exposure. It is able to withstand irradiance of very low intensity to full level of sunlight. It can inhabit and tolerate high level of wave exposures in areas such as open coasts like in Port Underwood and areas such as marinas or harbours (ISSG, 2007b). *U.*

pinnatifida is less likely to be found in areas of high level of fresh water outputs such as estuaries (ISSG, 2007c). This could be due to the preference of the salinity levels of *U. pinnatifida* that can range from 27 to 33% (Department of Fisheries, 2005).

1.1.3 Population distribution and invasion of *Undaria pinnatifida* and possible impacts

Undaria pinnatifida is now distributed into many parts of the world which are beyond its native range (ISSG, 2007b). Its native range includes China, Japan and Korea., but it is now found in Australia (Campbell and Burridge, 1998), Italy (Floc'h, Pajot & Mouret, 1996), England (Fletcher and Manfred, 1996) , the United States (ISSG, 2007a) and mostly importantly for this thesis, New Zealand (Hay and Luckens, 1987). Most of the introductions of *U. pinnatifida* were accidental but there have also been deliberate introduction of the sporophytes by research groups. For example, the introduction of *U. pinnatifida* in the French Atlantic coast of Brittany by the IFREMER research group (Hay, 1990).

Undaria pinnatifida was thought to have been introduced to New Zealand in ballast water. Its further spread within New Zealand was by natural dispersal and through spread on mussel lines being transferred between locations. Each fertile plant is able to release millions of zoospores that can be transported by waves and currents to other parts of the ocean (Ministry of Fisheries, 2001). Depending on the speed and velocity of water currents, spores of *U. pinnatifida* can be carried to new locations that are metres away or even kilometers away from its previous location. Furthermore, this seaweed is able to attach itself on to hulls of ships or boats and other aquaculture equipment and be taken to other parts of the coast (Ministry of Fisheries, 2001).

Undaria pinnatifida is found in many parts of New Zealand (Hay and Villouta 1993). These included the earliest discovery in Wellington in 1987. *U. pinnatifida* was discovered in 1988 in Marlborough Sounds, Oamaru and Timaru and also the Otago Harbour of South Island in 1990. Picton and Lyttelton were invaded in

1991, Porirua in 1992, and Moeraki and Napier in 1995 (Stuart, 2003). In 1997, *U. pinnatifida* was discovered in Port Underwood and Big Glory Bay. Discovery of *U. pinnatifida* in 1998 included the Golden Bay, Nelson and Bluff Harbour. *U. pinnatifida* was then discovered in the Chatham Islands, Akaroa Harbour in 2000 and in Halfmoon Bay in the same year. In 2001, it extended its range into Wainui Bay and in 2002; it found its way into the Firth of Thames and the coast of Kaikoura (Stuart, 2003).

U. pinnatifida was classified as unwanted organism in most parts of New Zealand including the Nelson region and in the Chatham Islands. It was later eradicated from Catham Island (ISSG, 2004). *U. pinnatifida* was reported in the Auckland region in 2004 but its invasiveness status was not specified and the method of introduction was unknown (ISSG, 2005).

The following characteristics were described by the Ministry of Fisheries in 2001 of *U. pinnatifida*, which make it highly successive and invasive:

- *U. pinnatifida* is able to reproduce after 50 days;
- It grows rapidly and competes with many other marine species;
- It is probably able to reproduce all year round;
- Each seaweed sample can release millions of spores microscopic in size and can be easily transported by water currents. These spores are able to lie dormant for up to years before they germinate at a condition suitable for their growth (Ministry of Fisheries, 2001)

The impacts of *U. pinnatifida* are not fully understood and are likely to vary from place to place (ISSG, 2007c). *U. pinnatifida* is a highly invasive species that grows very quickly and has the ability to effectively colonise areas which are not fully occupied by other species, which could lead to changes in the native ecosystem (Ministry of Fisheries, 2001).

Undaria pinnatifida can form a dense canopy which shades the sub-canopy; it competes for sunlight and space with other organisms. Within New Zealand, the impacts of *U. pinnatifida* include the potential in decreasing paua

recruitments by displacing native coralline algae, which greatly impact paua settlement. *U. pinnatifida* invasion could lead to displacement of macro-algal communities which are native to New Zealand. It is reported to have decreased the biodiversity of encrusting and sub-canopy sessile communities (Ministry of Fisheries, 2001).

U. pinnatifida, being a fouling agent, could increase the expenditure of marine farms by increasing labour and harvesting cost (MAF Biosecurity, 2012). The increase in labour could be due to fouling of fish cages, oyster racks, mussel ropes and scallop bags, which restricts water circulation through cages etc (ISSG, 2007c). Fouling of *U. pinnatifida* has the potential to clog machinery of marine farms and heavy fouling on hulls of boats is thought to decrease their efficiency (ISSG, 2007b). The Ministry of Fisheries (2001) determined that more research is needed before the impacts and influences of *U. pinnatifida* in New Zealand can be fully understood.

U. pinnatifida is also a nuisance in other parts of the world. In Jersey, *U. pinnatifida* is reportedly shading underlying species causing physical displacement of the native species (ISSG, 2007c). Irigoyen, Eyras and Parma (2009) suggested that this seaweed reduced the abundance of fish species. As it grows on reefs, covering refuges of fish, that lead to habitat loss of fishes which dwell on the reefs. This was predicted to affect not only the biodiversity of the reef but also recreational or commercial activities which are based on the reefs (Irigoyen *et al*., 2009). Casas, Scrosati and Piriz (2004) carried out a study in the Nuevo Gulf and showed that removal of *Undaria pinnatifida* from invaded sites resulted in an increase in the biodiversity at those sites (Casas *et al*., 2004). Conversely, the spread of *U. pinnatifida* in Brittany has been relatively non-aggressive to native species of that area (Department of Fisheries, 2005).

At this point, it is clear that *U. pinnatifida* has potential impacts to the environments beyond its native boundaries. However the scale of the impacts is difficult to determine. The next section will discuss previous and current legislation of this species in New Zealand along with its management.

1.1.4 *Undaria pinnatifida* management and use in New Zealand

In March 2000, *U. pinnatifida* was classified as an unwanted species according to the Biosecurity Act 1993 under section 164c (Ministry of Fisheries, 2001). This stated that no one should knowingly release or communicate or cause to release or communicate or in any way spread any unwanted organisms, with the following exceptions:

- During pest management programmes;
- For scientific purpose with permission;
- In an emergency situation;
- As permitted by chief technical officers (Parliamentary Counsel Office, 2010)

In 1999, a request was made by the Government to the Ministry of Fisheries to put forward a proposal for a National Pest Management Strategy for *U. pinnatifida* (Ministry of Fisheries, 2001). In 2001, the Ministry of Fisheries published an action plan which stated that the Government decided to not allow the harvest of *Undaria* commercially. Furthermore, the Ministry of Fisheries took action to decrease the rate of spread of *U. pinnatifida*. The action plan included the following:

- Educate marine stakeholders regarding how to avoid spreading *Undaria*;
- Set up vector management programmes
- Investigate ways to treat vectors which spreads *U. pinnatifida*;
- Support regional initiatives on the control of *U. pinnatifida* (Ministry of Fisheries, 2001).

In 2004, the policy was changed and *U. pinnatifida* was allowed to be harvested when this harvest is needed as part of the *Undaria* control programme or as by-catch of other activities such as mussel farming (Ministry of Agriculture and Forestry [MAF], 2010). Harvesting of *U. pinnatifida* from natural or artificial surfaces or harvesting beach cast *Undaria* was prohibited unless the harvest was part of a control programme or by-catch activity (MAF, 2010).

In 2009, the Government reviewed the 2004 policy and a new policy was put forward in April 2010 (MAF, 2010). This new policy permitted the harvest of *U. pinnatifida* to a wider extent. Under the 2010 policy, harvesting from artificial surfaces such as marinas, wharves or mussel farms are permitted because this action is not likely to result in proliferation of *U. pinnatifida*. Harvesting beach cast *U. pinnatifida* was also allowed unless the area is ecologically sensitive or is vulnerable to commercial harvest (MAF, 2010). These areas are identified by the Fisheries (Beach Cast Seaweed Area Prohibition) Amendment Notice 2009. These coastal areas have been clearly stated in the New Zealand Legislation (Parliamentary Counsel Office, 2009). In both the 2004 and 2010 policies, unless it is required as part of a controlled programme, harvesting of *U. pinnatifida* from any natural surface is prohibited. The rationale was that the harvesting from natural surfaces could impact on other species such as lobster or paua. This action could also disturb native canopy species, and the removal of native canopy species could lead to further proliferation of *U. pinnatifida* (MAF Biosecurity, 2010a)

The 2010 policy also permitted the farming of *U. pinnatifida* in New Zealand under certain restrictions. Farming of *U. pinnatifida* would be in selected sites approved by the Ministry of Agriculture and Forestry at infested sites (MAF, 2010)

The Ministry of Agriculture and Forestry (2010) has reviewed the old policy and established a new policy regarding *U. pinnatifida which* does not remove this species from the unwanted organism list. This Asian kelp remained to be an unwanted species in New Zealand under the Biosecurity Act. Any uses of this kelp require permission from the Ministry of Agriculture and Forestry. Any persons or organisations need to go through an application process set by the Ministry of Agriculture and Forestry and must be granted permissions before the harvesting or farming of *U. pinnatifida* (MAF, 2010).The establishment this new policy could hopefully turn this from a pest to a beneficial seaweed that could compensate for the loss of profit of seafood farms. The next section will outline the benefits of aquaculture of *U. pinnatifida* in New Zealand.

1.1.5 Benefits of aquaculture of *Undaria pinnatifida* in New Zealand

As outlined in the previous section the New Zealand government has approved for commercial harvest of *U. pinnatifida* and farming of *U. pinnatifida* under certain conditions (MAF, 2010). This means that the export of *U. pinnatifida* to other countries such as Japan and the rest of Asia could be possible.

Wakame is a common daily consumable product in Japan and various other areas of Asia. There are constant needs of wakame imports in Japan as Japan is not able to produce enough kelp to meet the needs of its population. If New Zealand is able to produce high quality wakame, the potential profit and market world-wide could be large. As stated by MAF Biosecurity (2010a) commercial opportunities of this seaweeds are wide ranged including pharmaceutical products, fertilizers and its most commonly use, as food (Carter, 2010). MAF (2009) mentioned that the value of *U. pinnatifida* is based on the quality and end use. It was estimated that every tonne of *Undaria* used for general agricultural use could yield return of approximately five hundred New Zealand dollars and for high quality *U. pinnatifida*, the return could be as high as one thousand New Zealand dollars per tonne. Some have even argued that the commercial value of *U. pinnatifida* has been underestimated when compared to international values (MAF Biosecurity, 2010a).

Given that this seaweed grows prolifically on mussel farms throughout New Zealand, there is considerable interest from mussel farmers in harvesting *U. pinnatifida*. However, before such plans can be undertaken, information on the distribution, density, biomass, growth rate and reproduction rate must be determined. This study was based on a collaboration between researchers at Auckland University of Technology and Wakatu Inc., one of New Zealand's largest mussel processors, to determine the above parameters on mussel farms in Port Underwood and Pelorus Sound in the Marlborough Sounds.

Chapter 2: Methods and Materials

2.1 Study location and collection

The location for this study was the Marlborough Sounds in South Island of New Zealand (41 03’55.34” S 173 58’29.90” E). The license to harvest *U. pinnatifida* was issued by MAF Biosecurity New Zealand, under Biosecurity Act 1993 Section 52, permission was granted to Wakatu Inc. Strict guidelines were followed in the collection and disposal of *U. pinnatifida*.

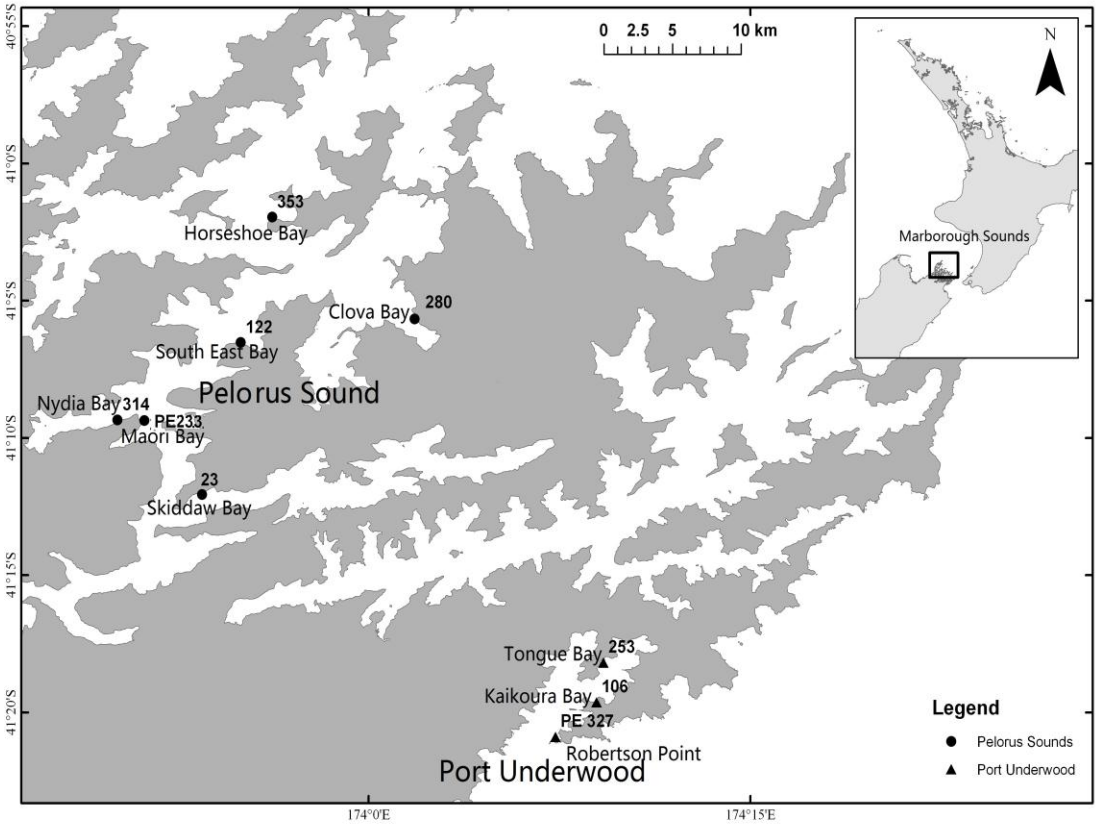

Figure 3: Location of mussel farms where *Undaria pinnatifida* was collected.

U. pinnatifida samples were collected from mussel farms within the Marlborough Sounds from nine sites (Figure 3 and Table 1). The farms were selected to cover a range of locations, but also they had several mussel lines that would not be harvested for the duration of this study.

The farms were visited for sample collection and experimentation on nine occasions in 2011 (Table 1). Due to weather conditions it was not possible to visit all nine farms on each trip. On each occasion the farms were visited on a mussel harvesting or processing vessel, which allowed the mussel lines to be lifted out of the water to collect and measure the seaweed, without disturbing the mussels growing on the lines.

Table 1: Dates that each of the mussel farms were visited during this study. A black square indicate a visit.

Farm	23	122	233	280	314	353	106	253	327
29/03/2011	■	■	■	■	■	■	■	■	■
30/04/2011	■	■	■		■		■	■	■
31/05/2011	■	■	■	■	■	■	■	■	■
06/07/2011	■	■	■	■	■	■	■	■	■
30/07/2011	■	■	■	■	■	■	■	■	■
09/09/2011		■	■	■	■	■	■	■	■
10/10/2011		■	■	■	■	■	■	■	■
11/11/2011		■	■	■		■			
09/12/2011		■	■	■		■	■	■	■

2.2 Biomass, distribution and seasonality

The seasonality and differences between mussel farms in terms of *U. pinnatifida* biomass and growth form of its plants were determined. Each month of collection, four randomly chosen mussel drop lines were selected and pulled out of the water with the crane aboard the mussel harvesting vessel. Each drop line was considered a replicate within each farm and their position was recorded to avoid re-measurement of the same drop in subsequent months. A measuring tape was laid alongside each drop line, and all of the *U. pinnatifida* within each 1m increment was collected, bagged, frozen and transported to the laboratories

at Auckland University of Technology.

At the laboratory, the samples were thawed prior to measurement. Each plant was laid out flat on a bench top and measured with a ruler to the nearest millimeter. The following parameters were measured (see Figure 5): total length of sporophyte (TL), total width of frond (TW), total stipe length (TSL), which is the length of the stipe from the bottom of the blade to the holdfast, and sporophyll length (SL). In addition, the total combined wet weight of *U. pinnatifida* from each 1m increment from each drop line was determined.

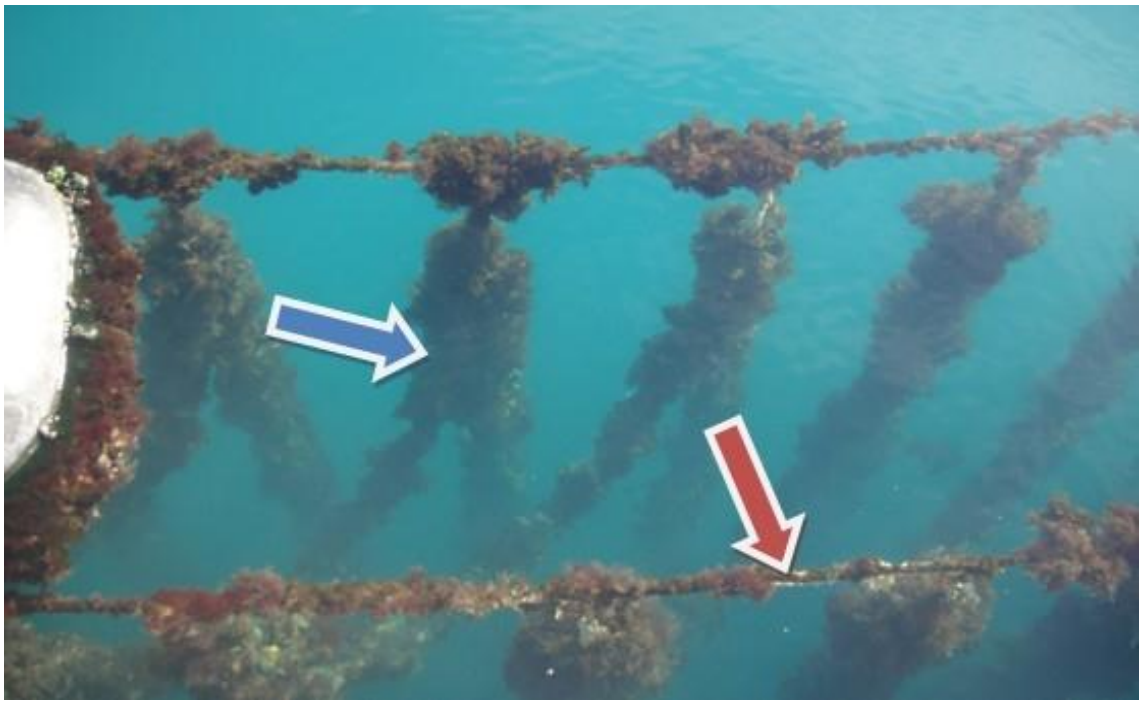

Figure 4: Mussel line backbones (red arrows) and drop lines (blue arrows) which are submerged.

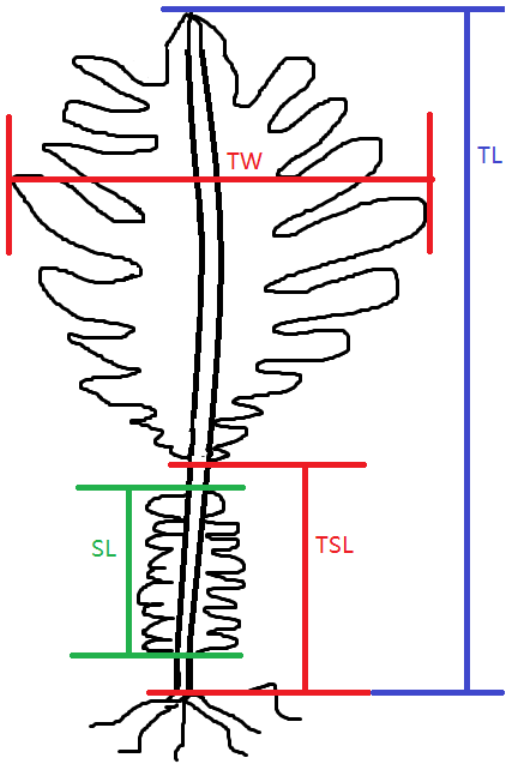

Figure 5: Measurements taken from *Undaria pinnatifida*.

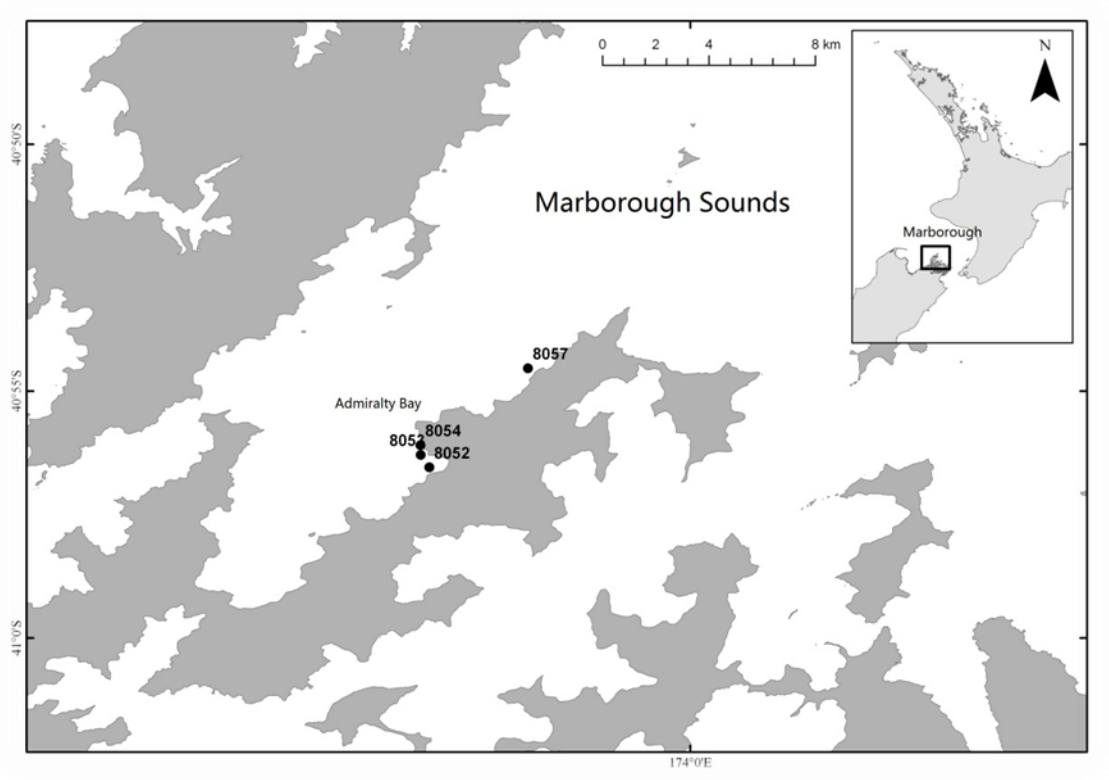

Figure 6: Additional farms visited in November 2011.

In addition to the monthly samples, in November, an additional four farms were visited (Figure 6). As we had determined that the vast majority of *U. pinnatifida* grew in the top 4 m of the water column, replicate drop lines were lifted and counted all *U. pinnatifida* in the top 4 m collected at each farm. The number of plants were counted and the total biomass weighed.

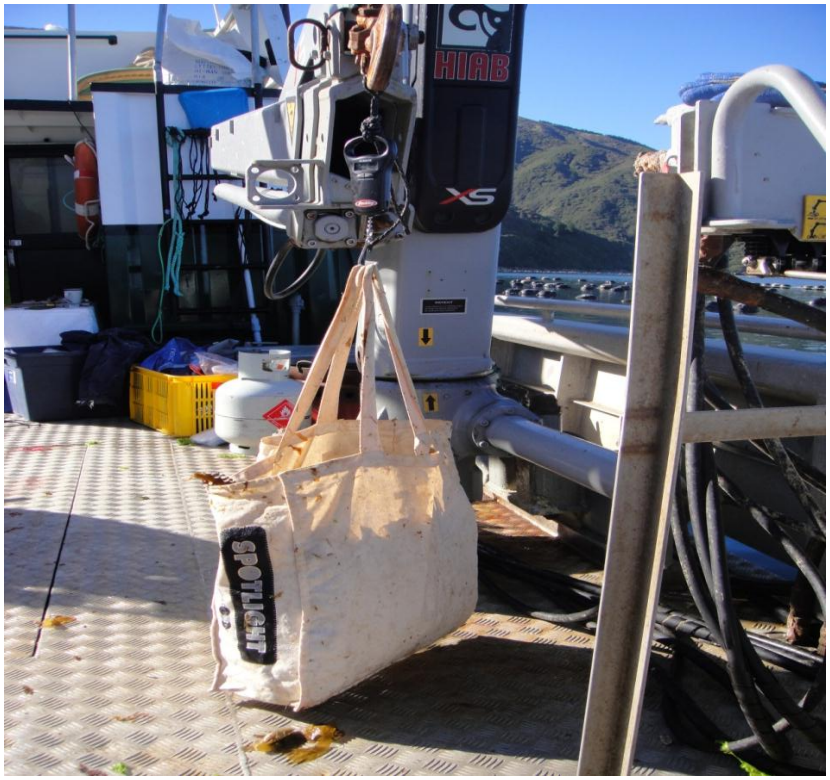

Figure 7: Method of weighing *U. pinnatifida* obtained from the top 4m of droplines.

2.3 Growth rates

From 31st of May 2011, three farms were selected for the measurement of *U. pinnatifida* growth rates. These farms were Farm 122 and Farm 353 in Pelorus Sound and Farm 253 in Port Underwood. Two frames were attached to a mussel line in each of these three farms. The frames measured 75 cm by 75 cm, were made of stainless steel wrapped with biodegradable string that were suspended into sea water at approximately 2 m in depth. These frames were allowed to sit in the water for one month before any plants were tagged. After one month, the frames were removed from the water and all *U. pinnatifida* sporophytes were examined. Up to 18 plants were tagged on each frame, with up to six plants in

each of the following size classes Class I, up to 5 cm, Class II was 5-10 cm and Class III was 10-15 cm. Each of these plants was tagged using coloured plastic cable ties. Six colours were employed, with one tie attached to Class I plants, two ties to Class II plants, and three ties to Class III plants. In this way measurement could be taken in the subsequent months of each individual plant from each frame. When plants were lost, new sporophytes within the lost plant size class were tagged to replace the missing plant where possible.

Figure 8: Metal frame wrapped with biodegradable rope to provide an artificial settlement site.

2.4 Fecundity

Spore release from sporophyll tissue was determined over the course of this study. On each visit, sporophytes were checked for maturity. Sporophytes with mature sporophylls were used to count spores. Three sporophytes were used from each farm in this part of the research.

Three discs of sporophyll tissue 8 mm in diameter were taken from sporophyll tissues of mature sporophytes that were at least 80 cm long with one disc each from the top, middle, and bottom of the sporophyll. Each disc was dried by blotting with tissue paper and was then placed inside a 1.5 ml Eppendorf tube

(Figure 10). These tubes were closed and kept in darkness over night at 4℃.

The next day, 0.96 ml of autoclaved seawater at room temperature was added into each tube to trigger the release of spores. After one hour, 0.4 ml of formalin was added to each tube to fix the spores. These tubes were then transported to the laboratory for spore counting.

Figure 9: A Class I plant tagged with a colour cable-tie.

The spore count was carried out using an Olympus microscope (Olympus CX31). Seawater containing spores was dropped on to Neubauer cell counting chamber and covered with a glass slide. Ten grids with a volume of 0.004 ml were counted for each disc (i.e. 0.04 ml of the 1 ml spore suspension. This number was converted into number of spores per ml of spore suspension, which was then converted into the total number of spores released in one hour in each 8 mm diameter disc of sporophyll and finally to spores released per cm^2 of sporophyll tissue.

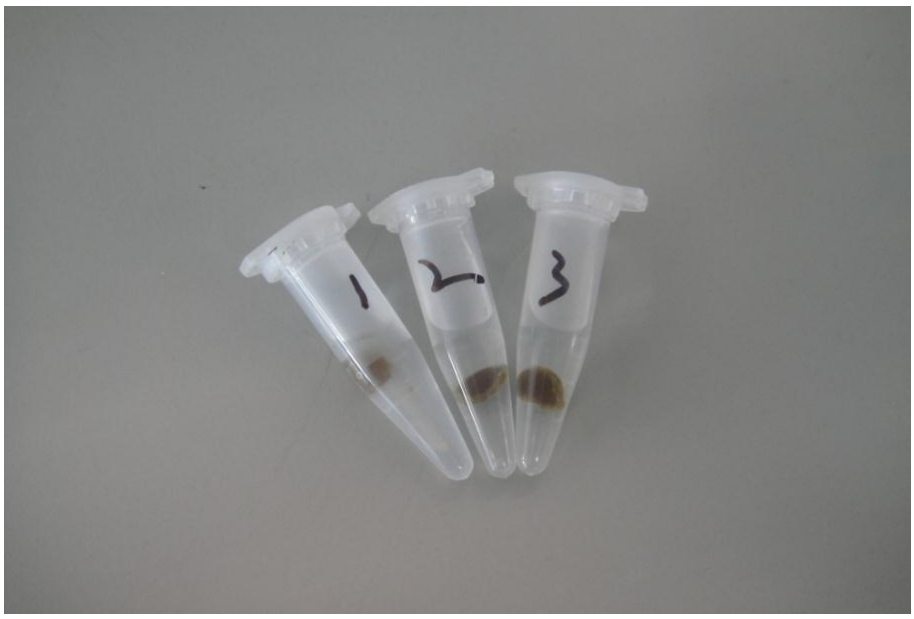

Figure 10: Eppendorf tubes with discs of sporophyll tissue.

Calculations : $\frac{\textit{Sum of spores in 0.04 ml}}{\textit{0.004 x 10}}$

= concentration of spores in 1 ml spore suspension

= number of spores in per 8 mm in diameter disc

In this formula, 10 indicates the number of grids counted, 0.004 indicates volume of the grid, which was 0.04 ml.

2.5 Seasonality of sporophyte germination

Germination of *U. pinnatifida* was measured *in situ* on each farm in both Port Underwood and Pelorus Sound. On each farm, frames as in section 2.3 were attached onto mussel lines. In the subsequent months, frames were taken out of the water and the numbers of plants attached were counted and then placed back for the next visit. At each month new frames were attached to the line. The number of plants on each frame was counted and the number of new plants was recorded. Due to weather constraints, visiting all farms over sampling period were not possible.

This part of the research can potentially show two things. First, if a frame is only in the water for one month and sporophytes were found, this indicated that zoospores have settled and germinated in that month. Therefore seasonality of the reproductive process can be determined. Second, it may show differences in seasonality between different farms and locations.

Chapter 3: Results

3.1 Biomass, distribution and Seasonality

3.1.1 Density and Biomass

Plant density was calculated by the mean number of plants per one metre increment per drop. Biomass was calculated by the wet weight (g) of sporophytes per one metre increment per drop. Blade size was calculated by multiplying the width by the length of each sporophyte. This was used as a proxy for blade size only as sporophytes are in fact multi-pinnate and not rectangular. Clearly, blade size, plant density and biomass are interlinked as biomass is dependent on density and size of the sporophyte.

This section will outline the results from each individual farm in terms of differences in biomass by depth and date, and will then outline the broad differences between farms across the year. First the results from Pelorus Sound will be presented, from the inner-most farm (Farm 23) to the outer-most farm (Farm 353), this will be followed by the results from Port Underwood from the inner-most farm (Farm 253) to the outer-most farm (Farm 327). For the statistical analysis, P values only are reported and the raw statistical analysis can be found in Appendix 1.

Farm 23

This farm was the inner-most farm in Pelorus sound and had the least amount of *U. pinnatifida* of any in this study. Unfortunately, due to time constraints and weather this farm was only sampled from April to August. No sporophytes were found deeper than 5 metres throughout the study period, and only 3 sporophytes were found at 5 metres depth (Figure 11). Significant differences in density were found between both months and depth ($P<0.05$ $P<0.01$ respectively). However no there was significant difference between months for either weight or blade area.

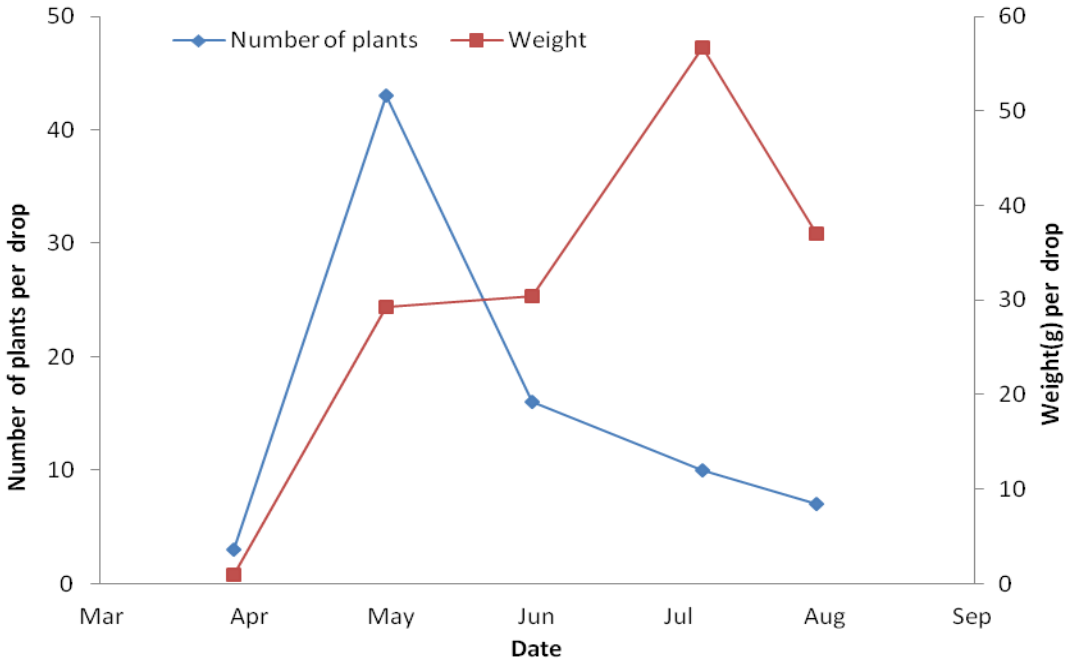

Figure 11: Mean density and biomass of *U. pinnatifida* on Farm 23.

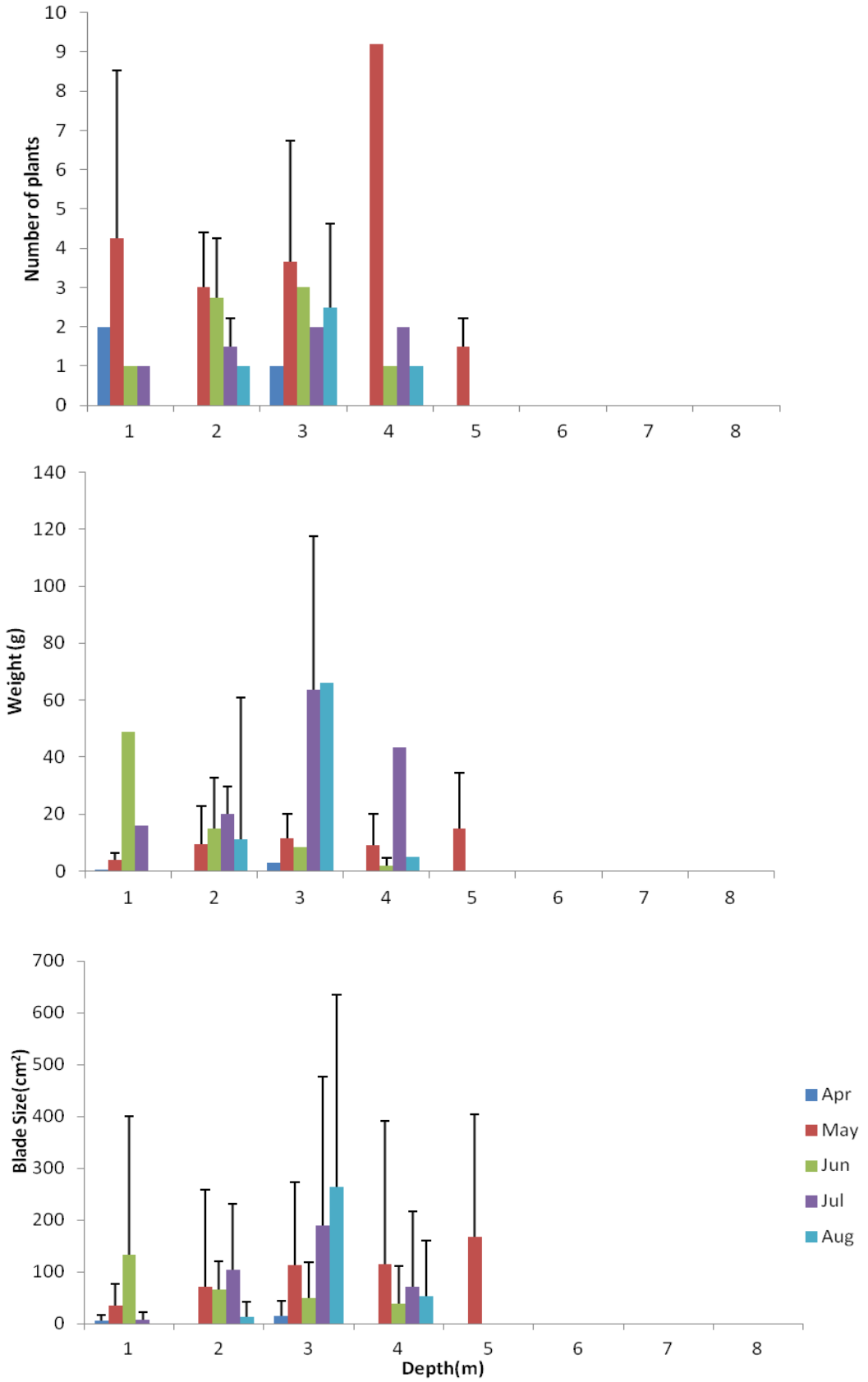

Figure 12: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 23. Error bars = SE.

Farm 233

This farm is located on an exposed point to the North of Maori Bay. There were significant differences (P<0.05) in both density and biomass in terms of both months and depth (Figure 16). The density increased significantly after September and peaked in December. Few plants were found deeper than 5 m, (Figure 14) and were most abundant at depths of 1 to 2 metres in August and October. The maximum mean density was 378 sporophytes per drop while the maximum mean biomass was 4586.2 g per drop.

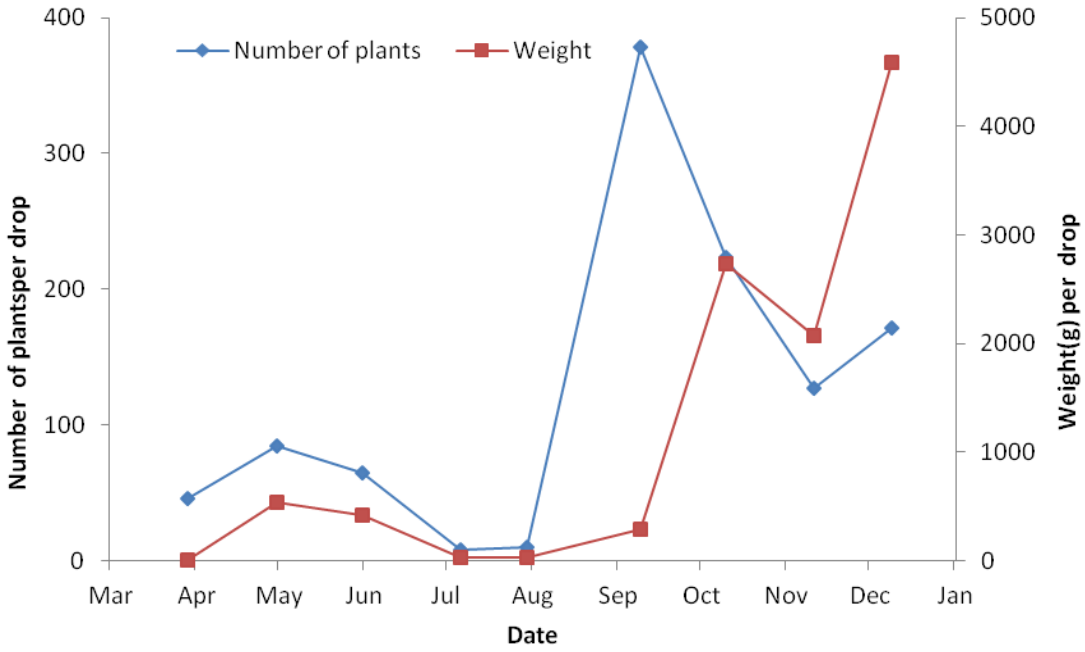

Figure 13: Mean density and biomass of *U. pinnatifida* on Farm 233.

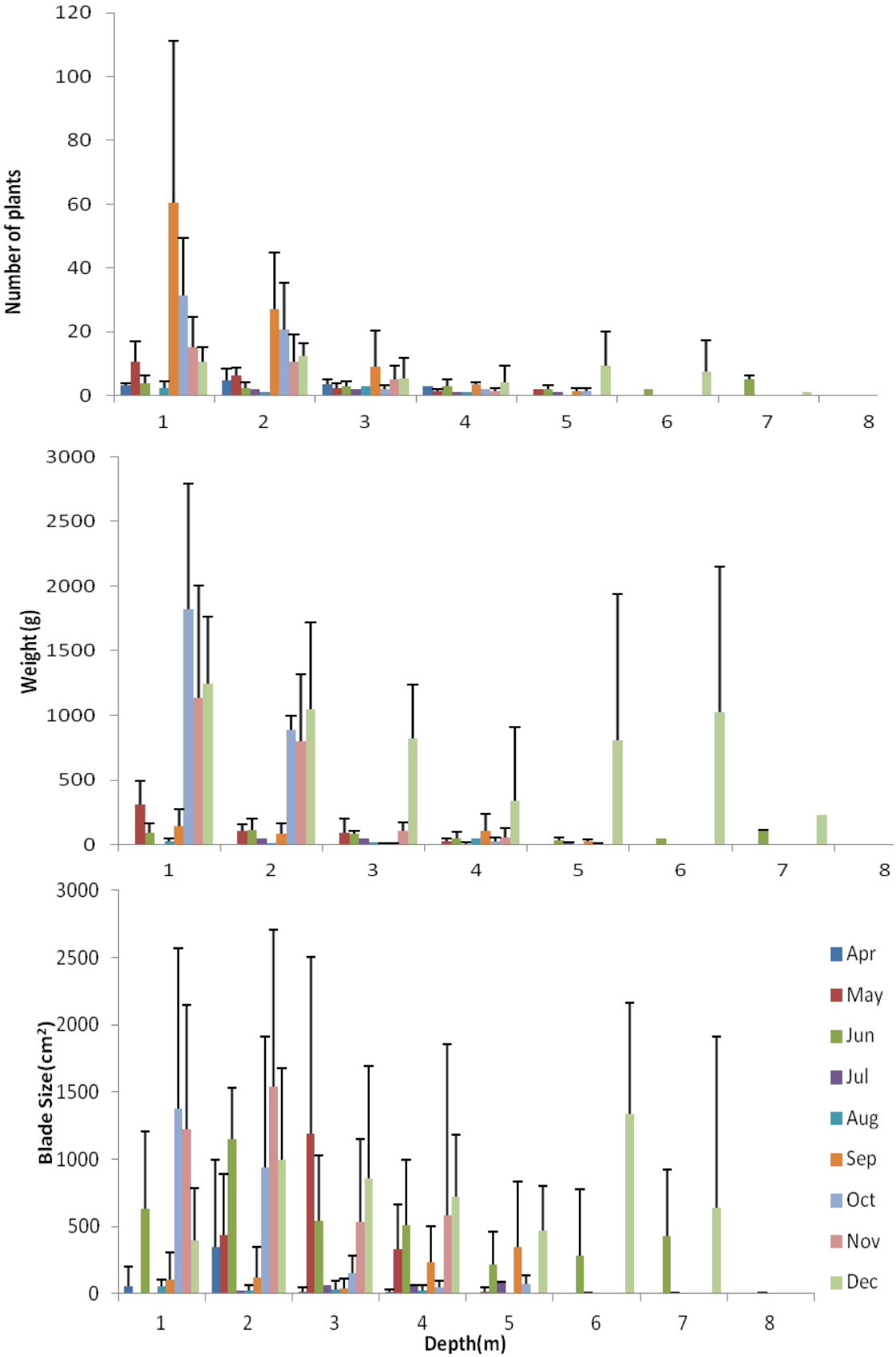

Figure 14: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 233. Error bars = SE.

Farm 314

This farm is located at the mouth of Nydia Bay. As with the previous farms, a statistically significant ($P<0.05$) signal was evident for both season and depth, (Figure 15) with number of plants increasing markedly from August to September and the biomass undergoing the most increase from September to October. Most plants were found at depths from 3 to 5m (Figure 16). The maximum mean density was 64 sporophytes per drop, while the maximum mean biomass was 304.7g per drop..

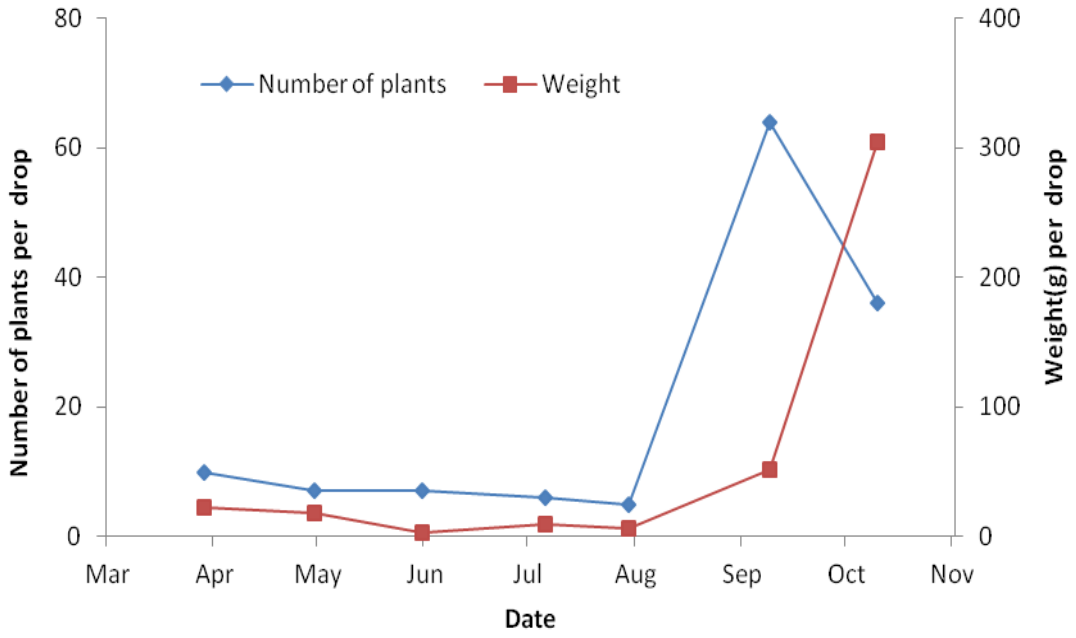

Figure 15: Mean density and biomass of *U. pinnatifida* on Farm314.

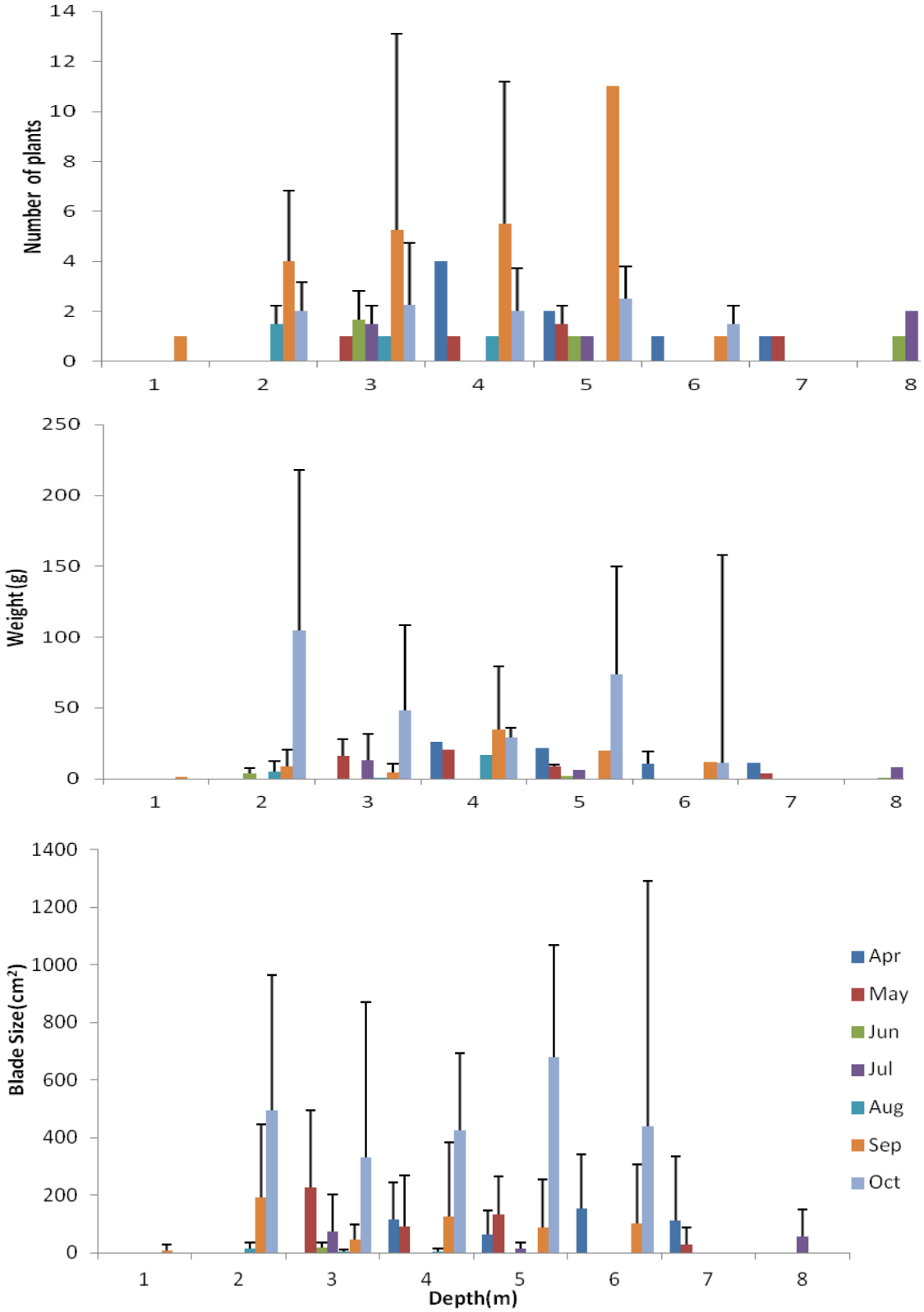

Figure 16: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 314. Error bars = SE.

Farm 122

This farm is located on an exposed point on the South East Bay. There were significant differences (P<0.01) in both density and biomass in terms of both months and depth (Figure 14). The density increased significantly after August and peaked in middle November. Few plants were found deeper than 5 m (Figure 14), and plants were most abundant at depths of 1 to 3 metres in November and December. The maximum mean density was 197 sporophytes per drop. The maximum mean biomass was 4850.5 g per drop in November. There were significant differences between months and depths (P<0.001, P<0.002 respectively) for blade area.

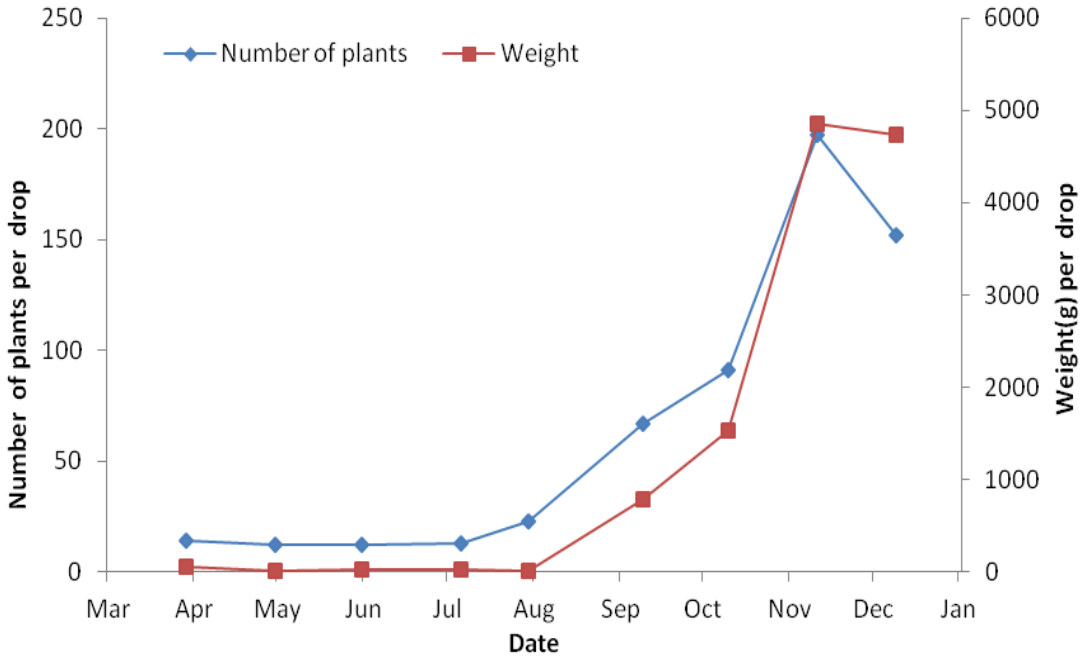

Figure 17: Mean density and biomass of *U. pinnatifida* on Farm 122.

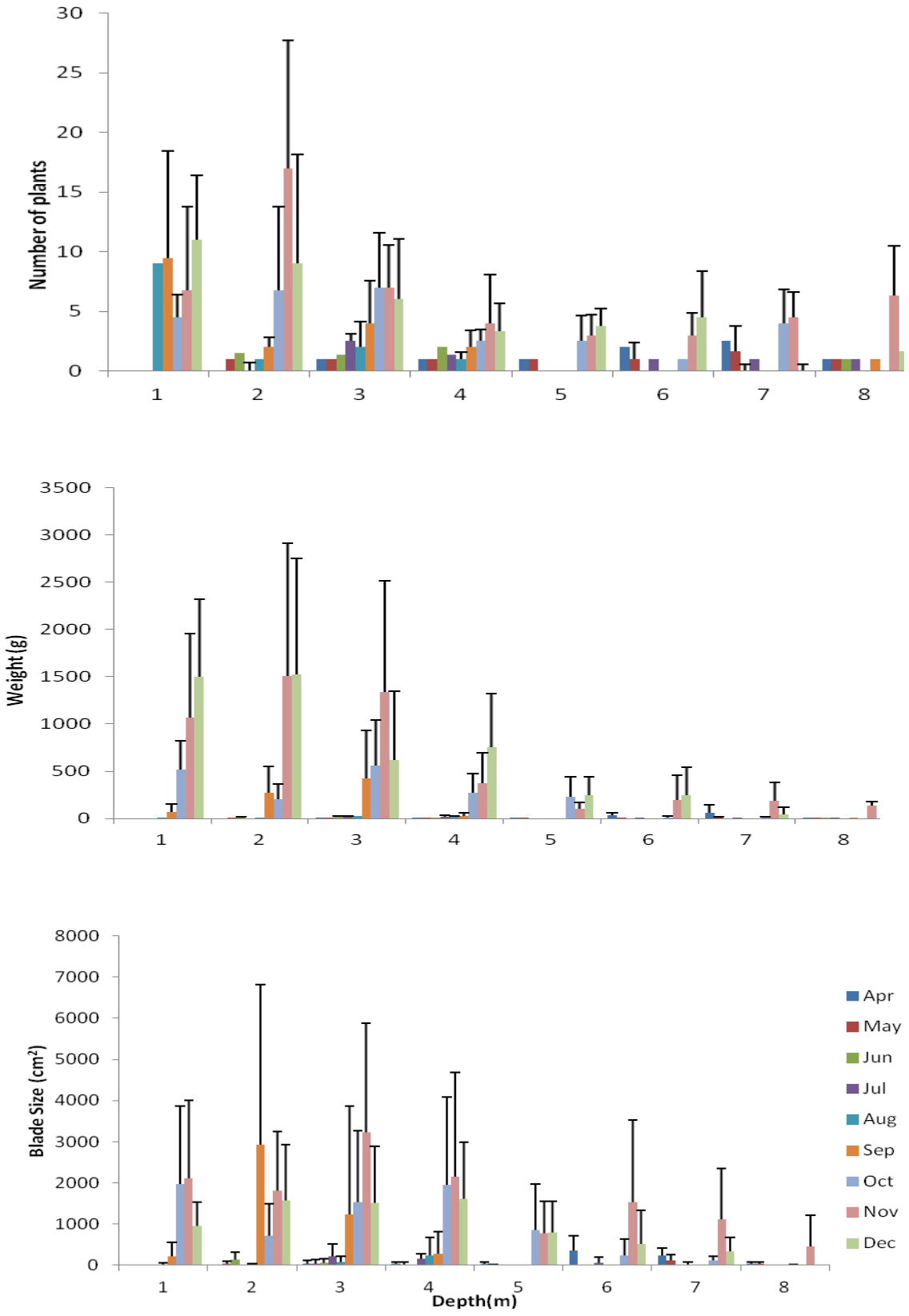

Figure 18: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 122. Error bars = SE.

Farm 280

This farm is located at the mouth of Clova Bay. As with the previous farms, a statistically significant ($P<0.05$) difference was evident for both season and depth (Figure 18) with number of plants increasing markedly from July to August, and then decreasing. In contrast, the biomass underwent a marked increase from August to September and continued to increase right through to December.. Most plants were found at depths from 1 to 4m (Figure 18). The maximum mean density was 136 sporophytes per drop, while the maximum mean biomass was 991 g per drop. There was significant difference between months ($P<0.001$) but no significant difference between depth ($P>0.1$) for blade area.

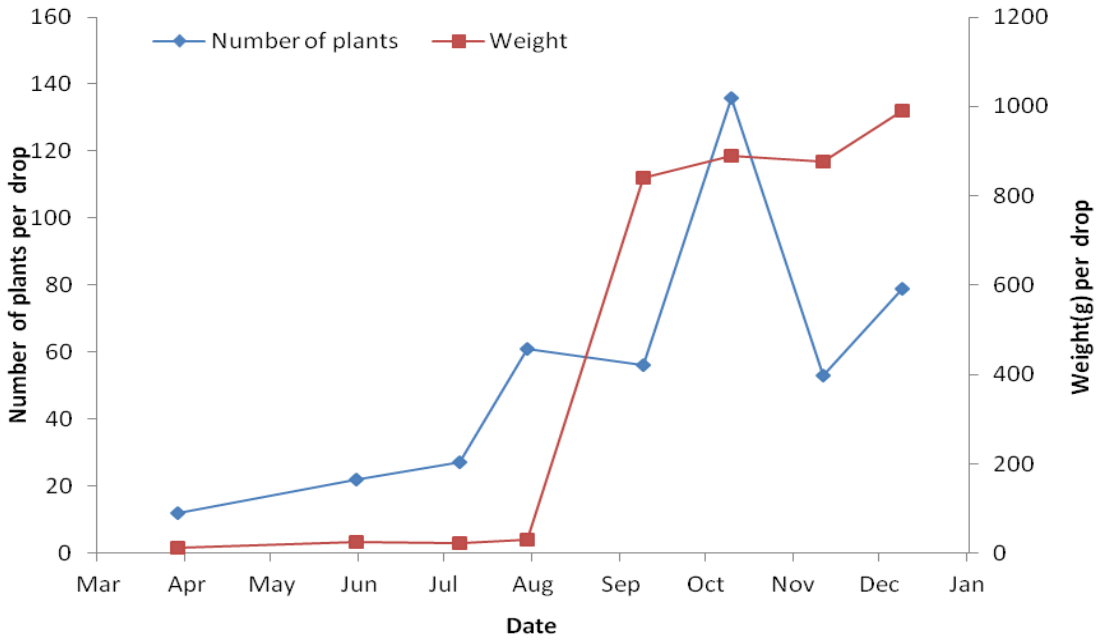

Figure 19: Mean density and biomass of *U. pinnatifida* on Farm 280.

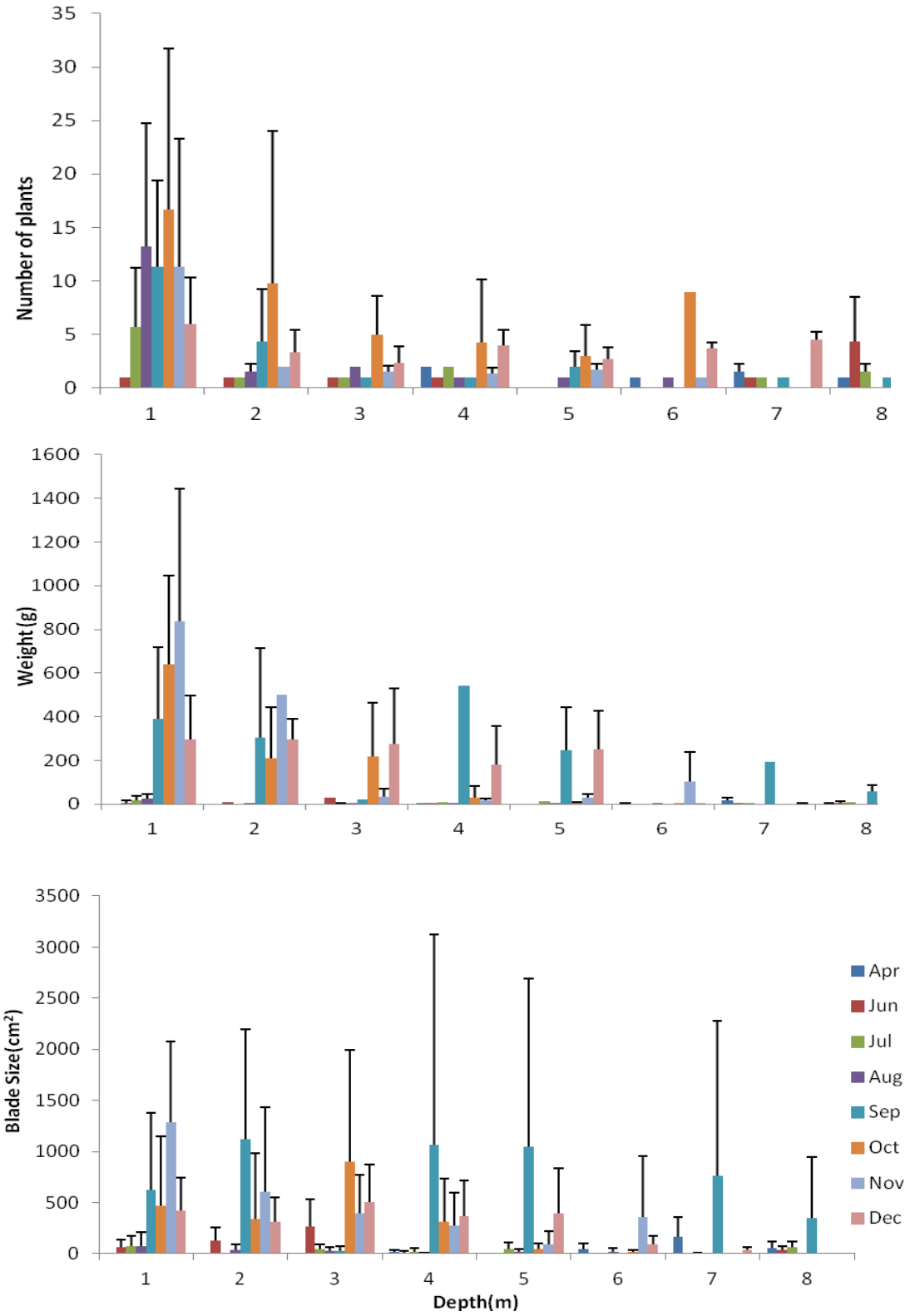

Figure 20: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 280. Error bars = SE.

Farm 353

This farm is located on an exposed point at Horseshoe Bay. There were significant differences (P<0.01) in both density and biomass in terms of month (Figure 21). The density increased significantly after October and peaked in November. While there were no significant differences (P>0.05) in either density or biomass in terms of depth (Figure 22), few plants were found deeper than 5 m, and plants were most abundant at depths of 1 to 3 metres in September, November and December. The maximum mean density was 157 sporophytes per drop. Maximum mean biomass was 3386.7 g per drop. For blade area, there was a significant difference between season (P<0.001) but no significant difference between depths (P>0.5).

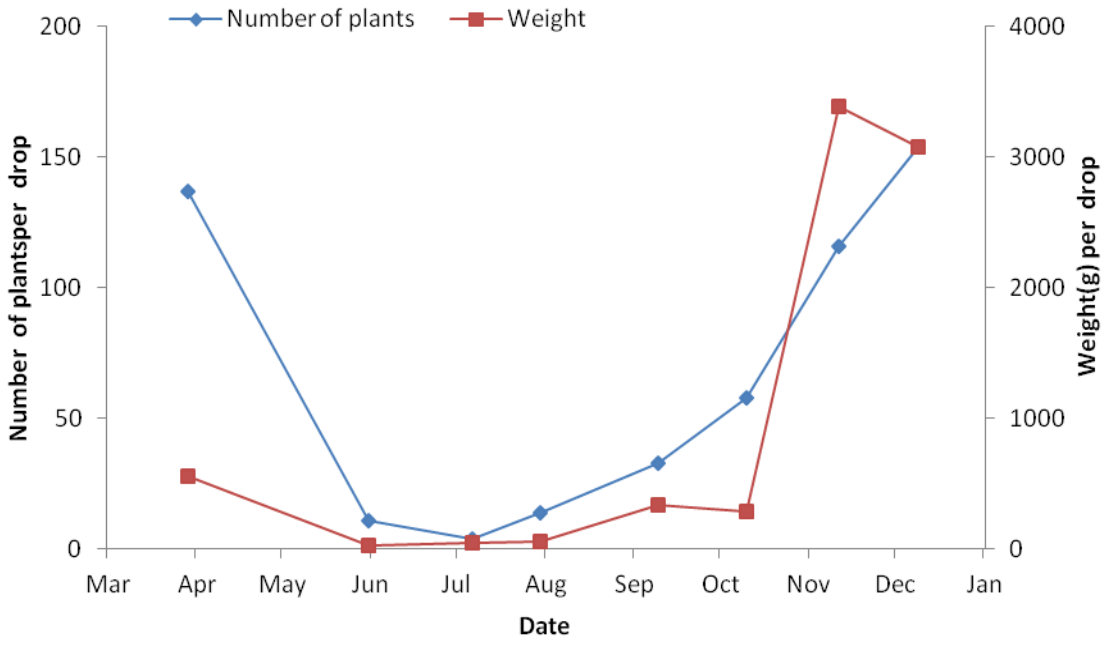

Figure 21: Mean density and biomass of *U. pinnatifida* on Farm 353.

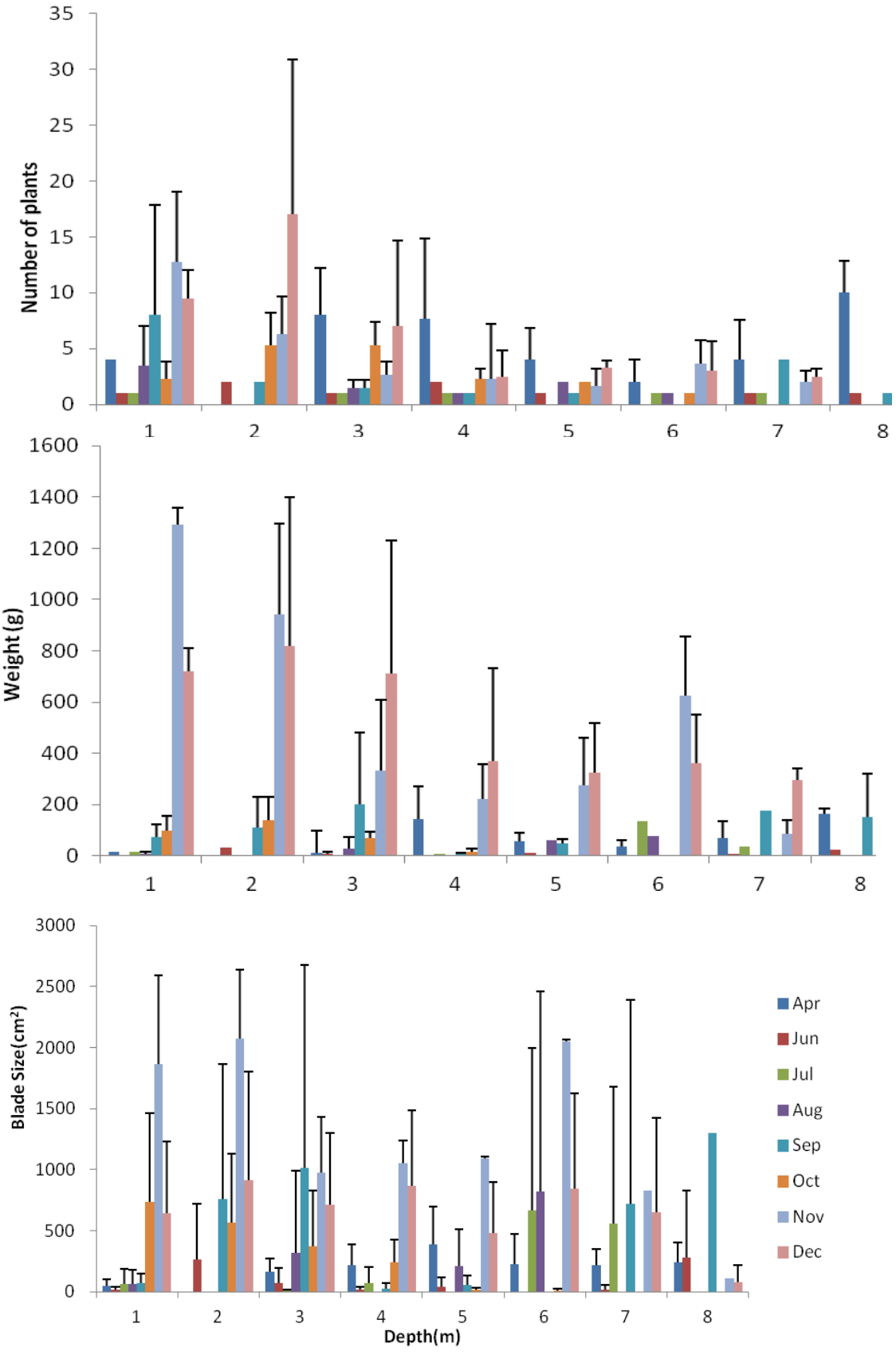

Figure 22: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 353. Error bars = SE.

Farm 253

This farm is located on a corner point of Tongue Bay. There was no significant difference (P>0.05) in density in terms of both months and depth (Figure 25). The density increased after July and peaked in mid-September. Similar numbers of plants was found along the whole drop (Figure 26) with a maximum mean density was 62 sporophytes per drop. There was no significant difference (P>0.05) in biomass between months but there was a significant difference (P<0.005) between depths (Figure 25). Biomass increased significantly after August and peaked in mid-September. The maximum mean biomass was 1166.7g per drop. However for blade area, there was significant differences between both months and depths (P<0.001, P=0.04 respectively)

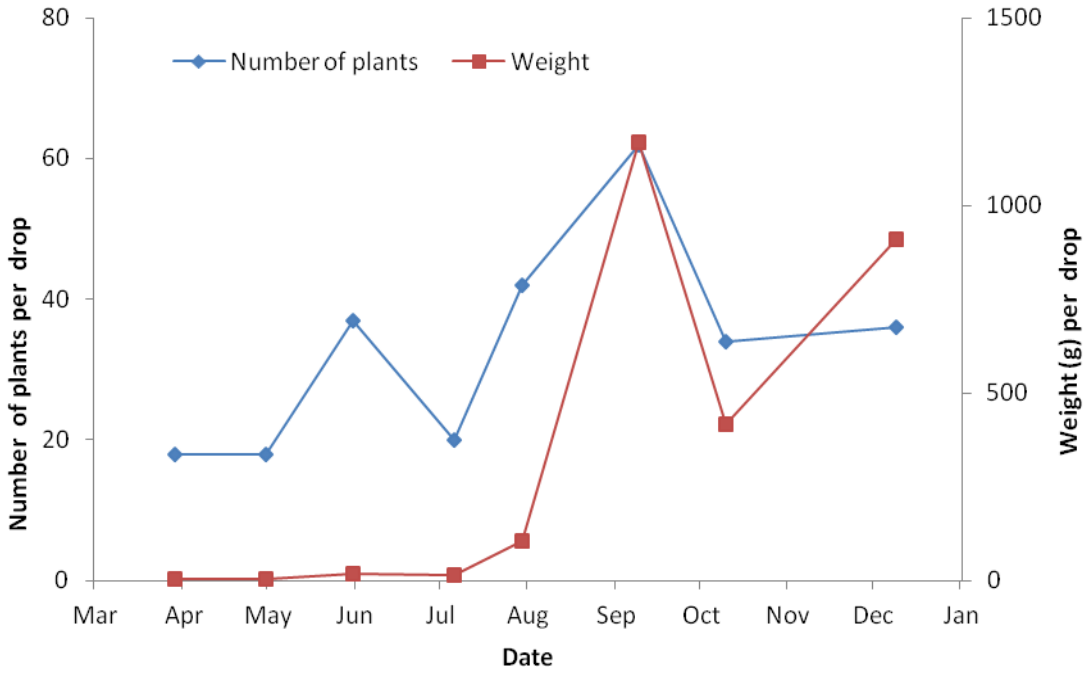

Figure 23: Mean density and biomass of *U. pinnatifida* on Farm 253.

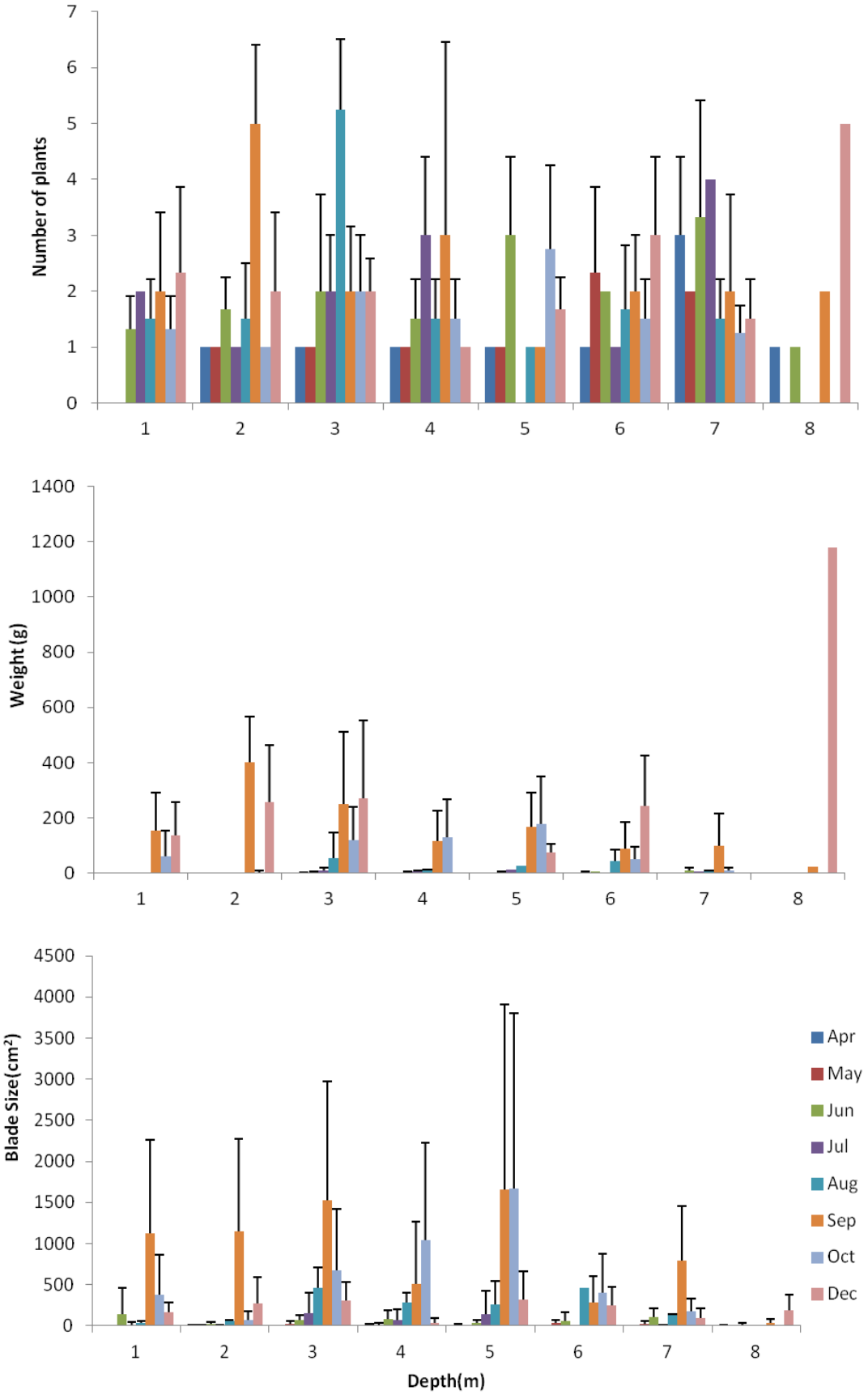

Figure 24: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 53. Error bars = SE.

Farm 106

This farm is located on a point to the Kaikoura Bay. There was no significant difference (P>0.05) in density for either month or depth (Figure 25). The density decreased in early season and increased after July then peaked in mid-October. Similar number of plants was found along each drop and large amount of plants was found, in depth of 6 and 7m, (Figure 26). The maximum mean density was 66 sporophytes per drop at April. There was significant differences (P<0.001) in biomass in terms of months but there was no significant differences (P>0.7) in terms of depths (Figure 25). The biomass increased significantly after August and peaked in mid-September. The maximum mean biomass was 1420.8g per drop. On the other hand, there were significant differences of blade area between both months and there was no significant differences between depths (P<0.001, P>0.05 respectively).

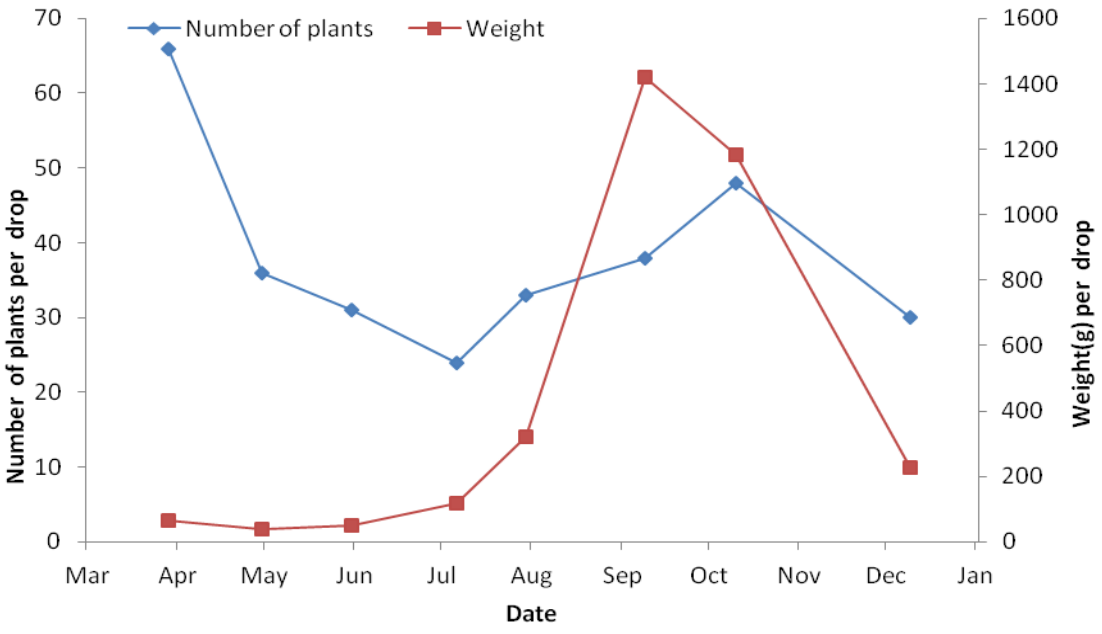

Figure 25: Mean density and biomass of *U. pinnatifida* on Farm 106.

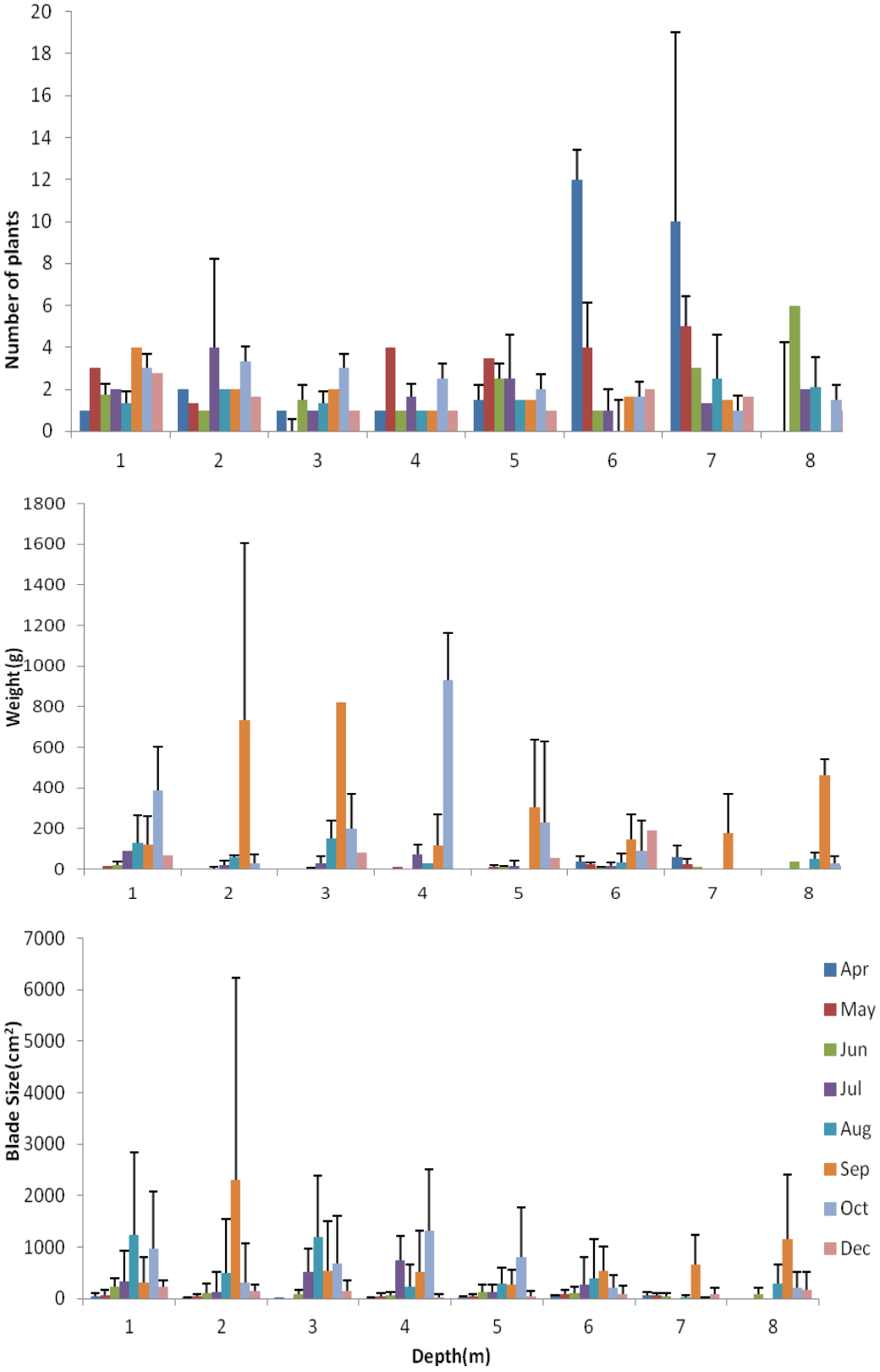

Figure 26: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 106. Error bars = SE.

Farm 327

This farm is located on a point of Robertson Bay. There was significant differences (P<0.01) in density in terms of both months and depths (Figure 27). The density decreased in April and it increased after May and peaked in mid-September. Most plants were found in shallow water with very few plants found at 6m to 8m depth (Figure 28). The maximum mean density was 254 sporophytes per drop in April. Then there was no significant difference (P>0.05) in biomass in terms of months and there was significant difference (P<0.01) in terms of depths (Figure 27). Biomass increased significantly after August and peaked in mid-September. The maximum mean biomass was 3962.2g per drop. However for blade area, there was significant differences between both months and depths (Both P<0.001).

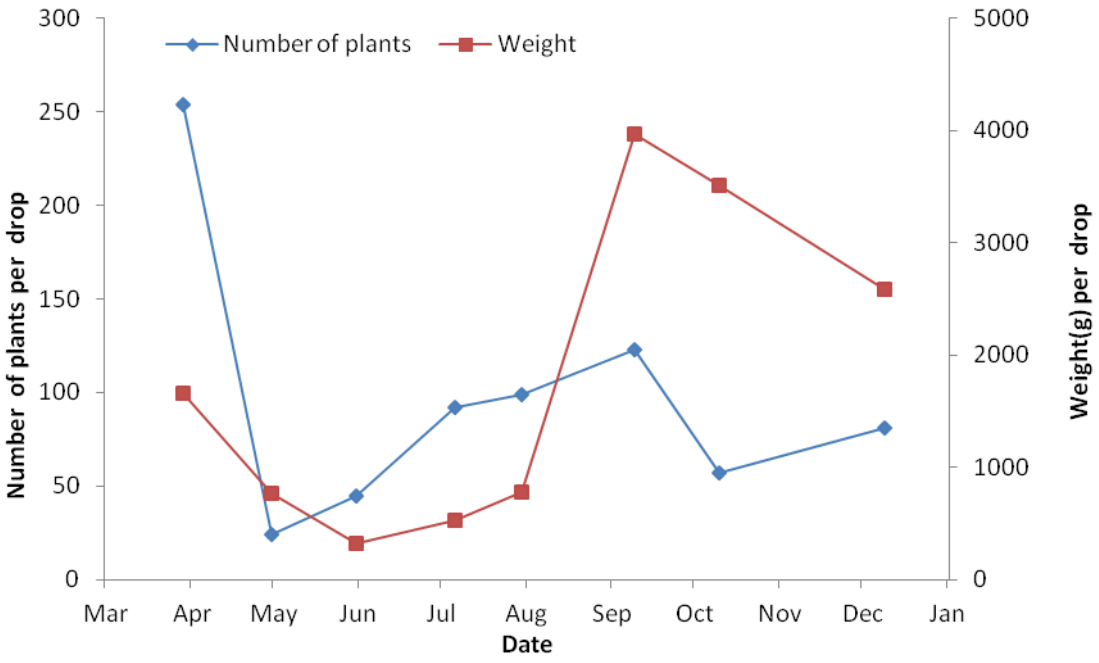

Figure 27: Mean density and biomass of *U. pinnatifida* on Farm 327.

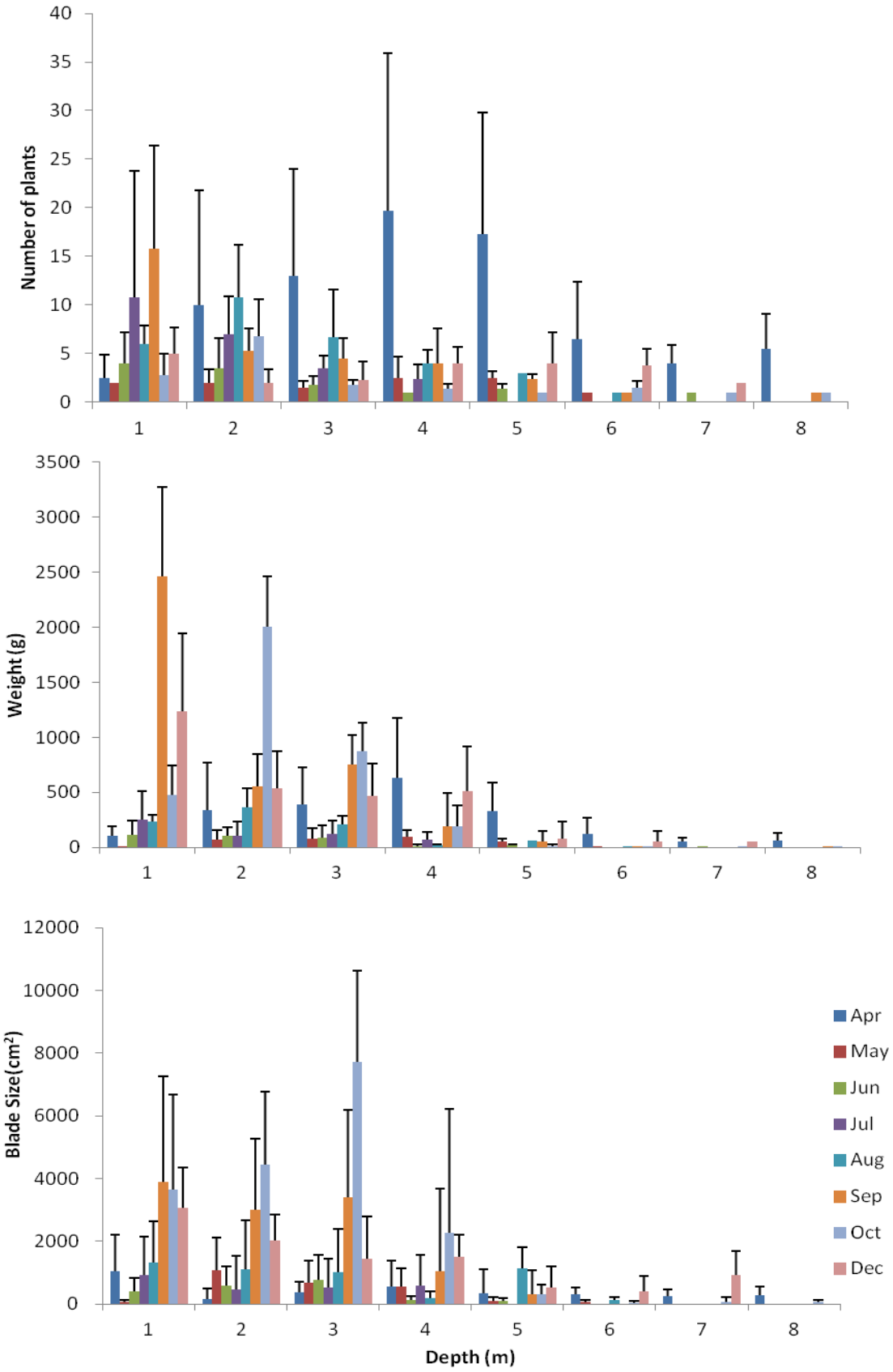

Figure 28: Mean density (per drop), biomass (per drop) and blade area of *U. pinnatifida* on Farm 280. Error bars = SE.

Overall, in all Pelorus Sound farms, plants numbers and total weight were highest at 1m to 3m depth. Maximum mean density was found to be to 60.50 sporophytes per metre per drop, but usually were under 20 sporophytes m^{-1}. Farms 122, 233 and 353 produced the most seaweed biomass (Figure 29) with a large increase after September for Farms 122 and 233 and after October on Farm 353. Farm 122 achieved the highest mean weight in Pelorus Sounds with a mean weight of 4850.5 gm^{-1} per drop in November.

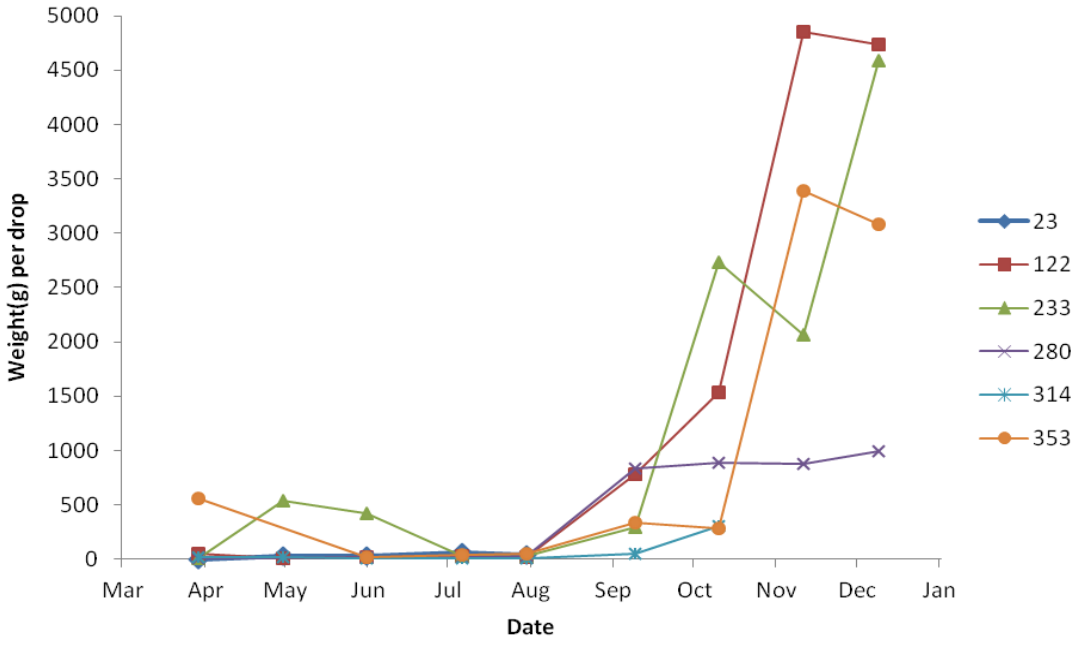

Figure 29: Mean biomass of *U. pinnatifida* of Pelorus Sound farms.

In Port Underwood the most productive farm was No. 327 (Figure 30), located at the most exposed site. While all farms increased in biomass from August, with a peak in September, Farm 327 had a peak weight of 3962g per drop while Farms 106 and 253 had a peak of 1420g and 1166g per drop respectively.

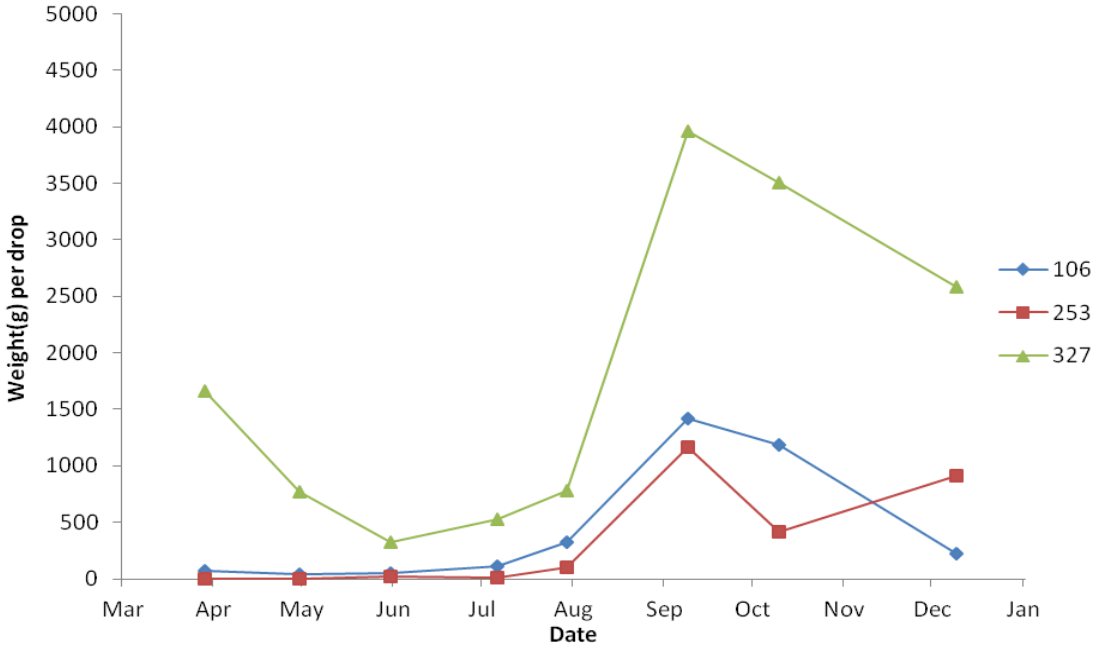

Figure 30: Mean biomass of *U. pinnatifida* of Port Underwood farms.

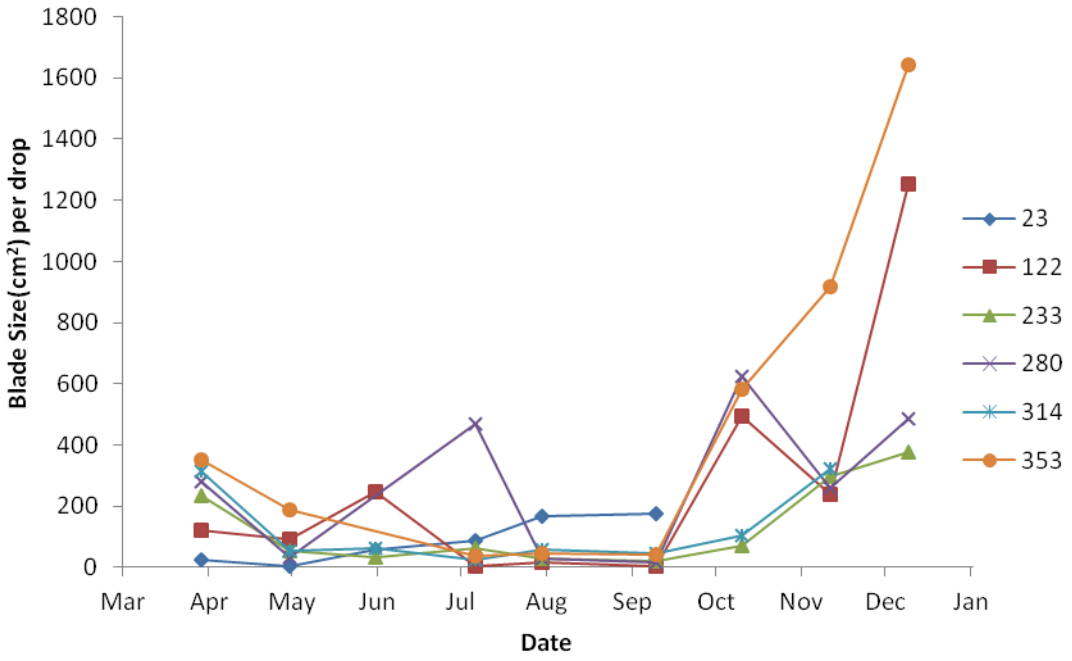

Figure 31: Mean blade size of *U. pinnatifida* from Pelorus Sound farms.

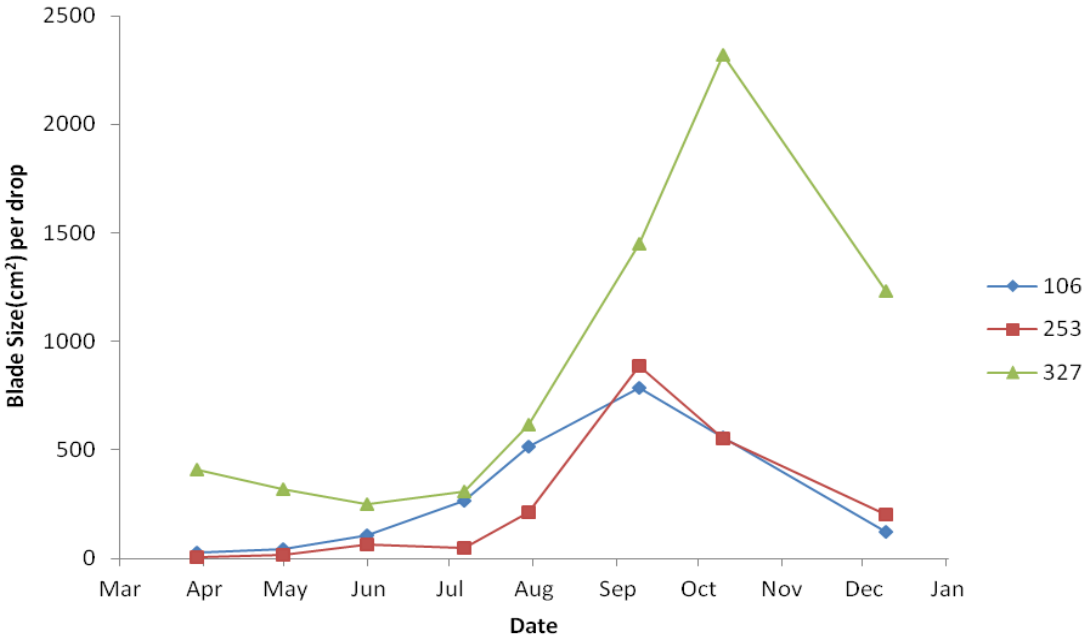

Figure 32: Mean blade size of *U. pinnatifida* from Port Underwood farm

3.1.2 Biomass on additional farms in Admiralty Bay.

In September, four additional farms were visited and the mean density and biomass determined (Figures 33 and 34). There were significant differences in both of these parameters ($P<0.01$, Appendix V).

Farm 8053 had significantly more plants and hence produced a greater total weight than the other three farms, despite all four farms being in the same location (Figure 6). Farm 8053 had an average of 39.8 plants per drop and an average weight of 8.17 kg per drop while the three other farms have less than 10 plants per drop and each drop weighted less than 4 kg on average. Farm 8057 had the least number of plants and minimum weight, which were 3.5 plants and 0.51kg per drop respectively.

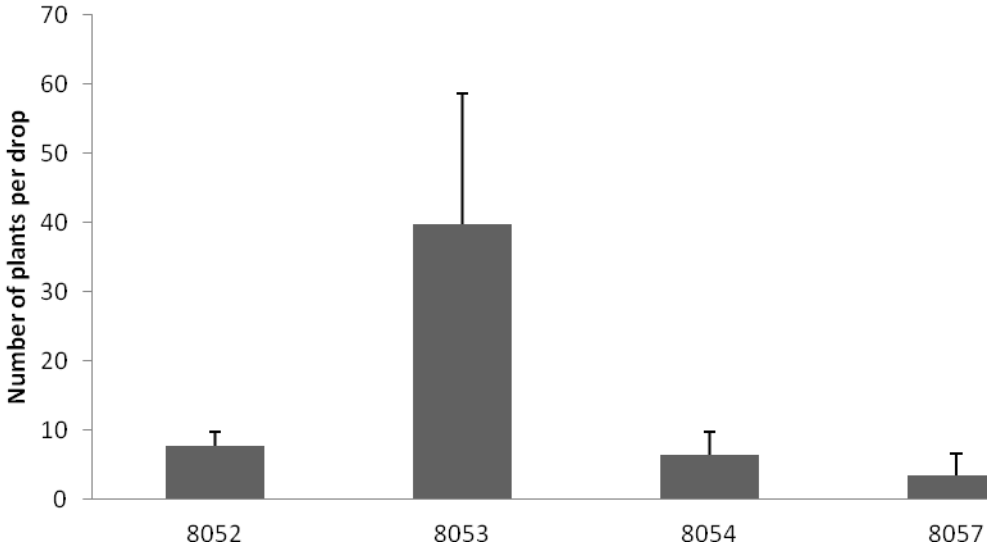

Figure 33: Mean number of *U. pinnatifida* from additional farms. Error bars= Standard Deviation.

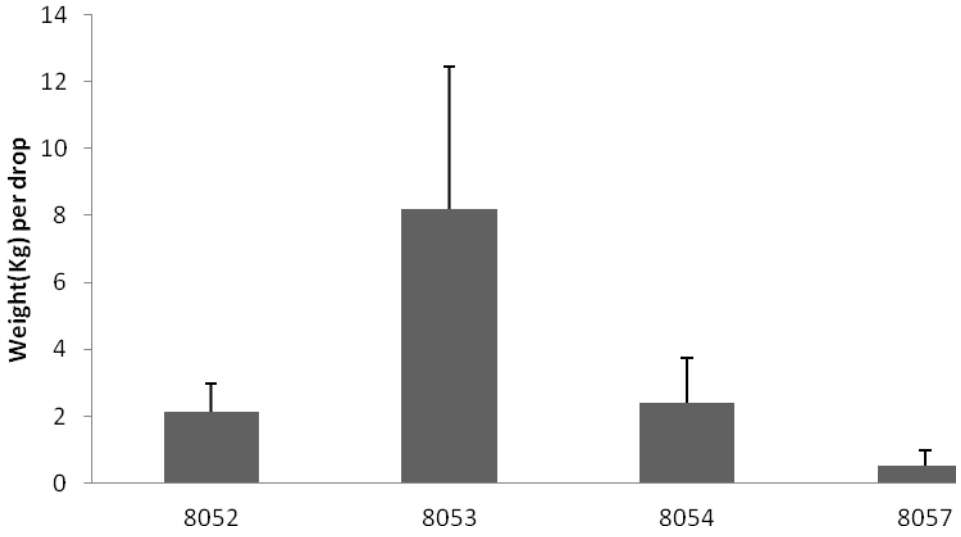

Figure 34: Mean biomass of *U. pinnatifida* from additional farms. Error bars= Standard Deviation.

3.2 Percent of mature sporophyll

The presence of mature sporophylls varied significantly throughout the study for all farms ($P<0.01$, Appendix IV). Farm 327 had the greatest percentage of mature sporophylls followed by Farm 233 and Farm 353. Farm 23 and 314 had the least mean percent of sporophyll. Using the Tukey's comparison test, significant differences were found between Farm 23 and Farm 327 as well as Farm 314 and Farm 327.

On average, Port Underwood farms had 24% mature sporophylls throughout the study period while Pelorus Sound farms had an average of 16%. Port Underwood farms have more mature sporophytes during most visits except in June and December 2011 (Figure 36). Sporophytes in Port Underwood were found to mature in early spring, while sporophytes in Pelorus farms matured in early summer.

For farms in Pelorus Sound (Figure 35) with the exception of Farm 23 and Farm 233, more mature sporophylls were found in the warmer months (April, November and December. The amount of mature sporophyll decreased in winter (June to September) and increased from spring (September).

A similar pattern was found in Port Underwood farms (Figure 36). However, the percent of mature sporophylls in Farm 253 inclined continuously throughout the study period. On the other hand, the percent of sporophylls in Farm 106 and 327 decreased from August (winter).

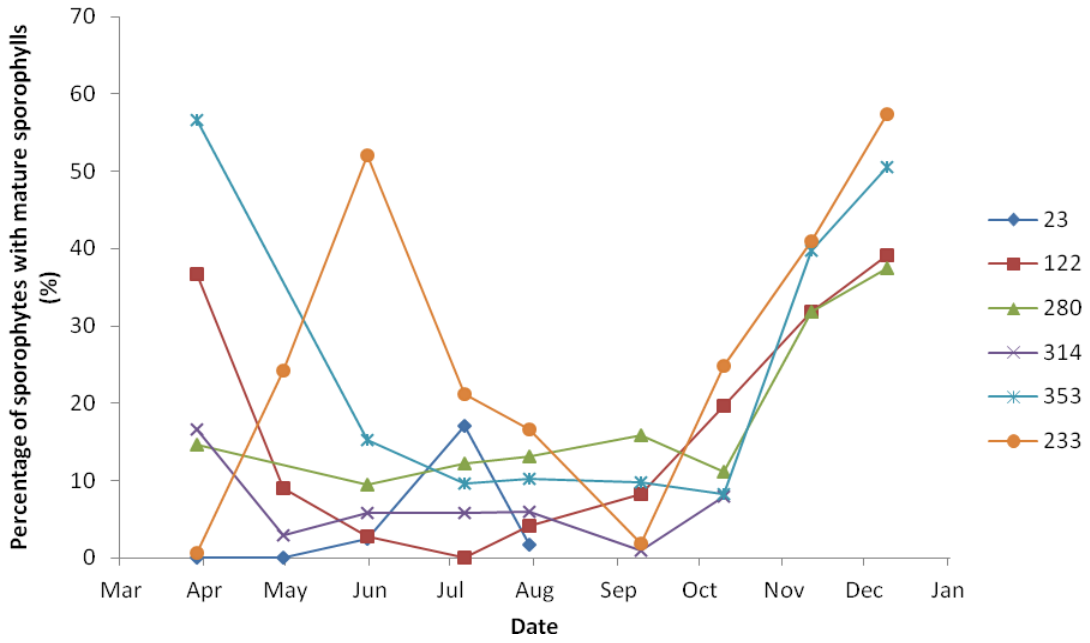

Figure 35: Percentage of sporophytes with mature sporophylls in Pelorus Sound farms.

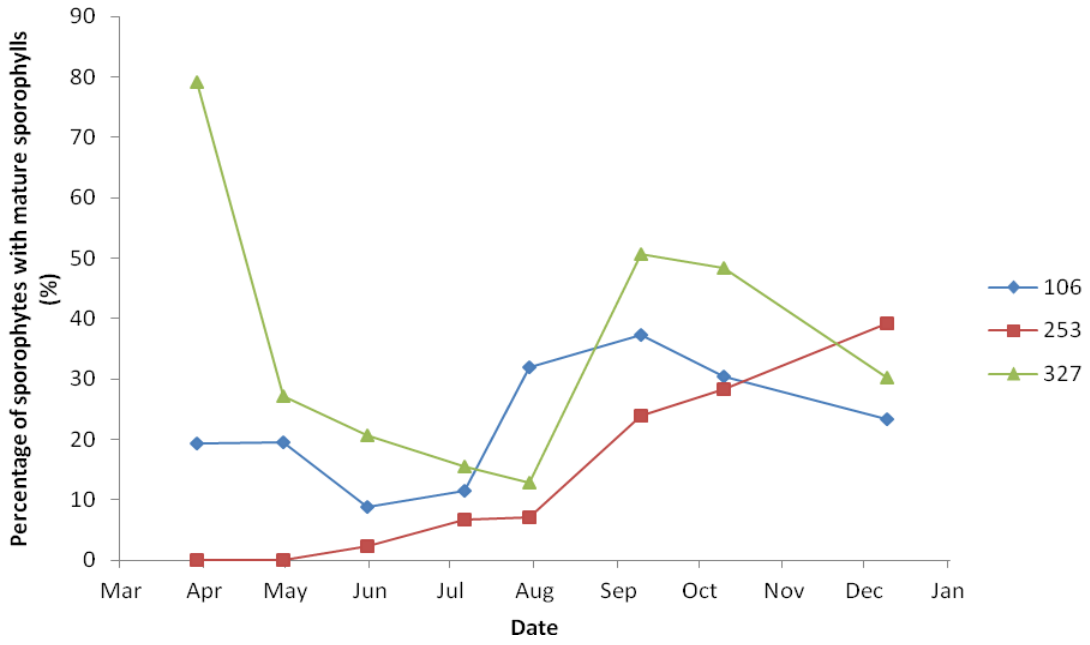

Figure 36: Percentage of sporophytes with mature sporophylls in Port Underwood farms.

3.3 Growth rates

Tagging of sporophytes started from June and ended in November 2011. A total of 174 plants were tagged, but only 102 plants were found in the following month. As the apexes of sporophytes are prone to erosion, the width of sporophytes was used to calculate growth rate. At all farms, significant differences existed in the average monthly growth rate ($P<0.05$, Appendix VI).

Sporophytes were found in Farm 253 and Farm 353 in very few months (Table 2), and the growth rates from these two farms did not differ significantly from Farm 122. Therefore, growth data from all three farms were merged.

A trend of general increase in average growth is evident (Figure 37), with an odd month of a rapid increase in September. Maximum growth was seen in November, when sporophytes grew more than 20cm in one month.

Although, the method of using different classes in the measurement of growth rate in this section was trialed, but only in August, September and October three classes were observed. Significant differences between the three classes was found in August ($P=0.00$), with the larger plants (Class III) growing more rapidly than the other two classes. However in September and October there was no significant difference between the growth rates of the classes. As the months progressed, the rate of growth increased in all three classes.

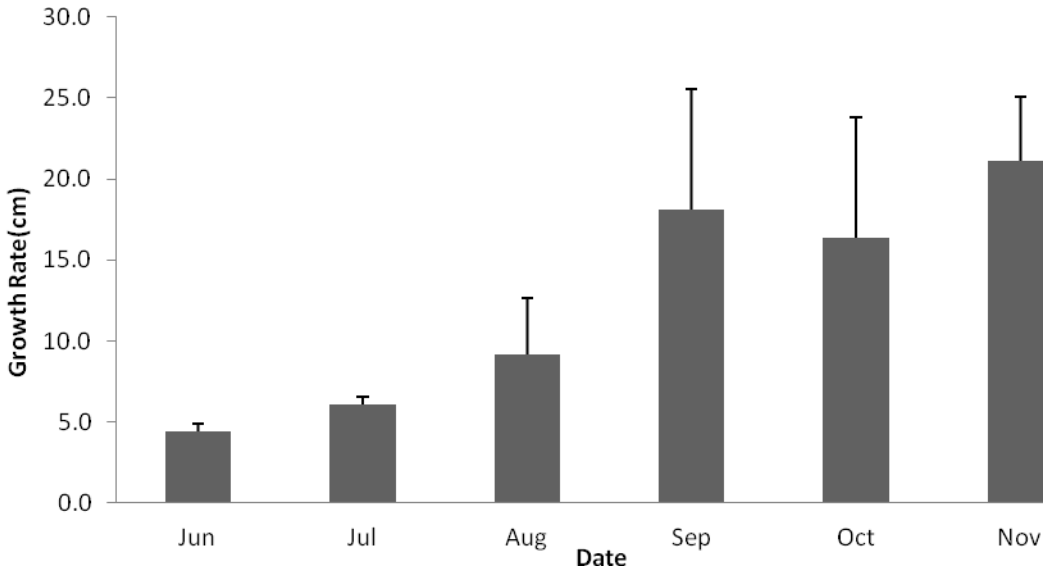

Figure 37: Mean growth rates of *U. pinnatifida* of plants from June to November. Error bars = standard error.

Table 2: Number of plants that were tagged each month in each size class (I, II and III) and the number of these that were still present "found" the following month.

		Farm No.								
		122			353			253		
Date		I	II	III	I	II	III	I	II	III
Jun	Tagged	6								
	Found	4								
Jul	Tagged	6	6							
	Found	4	6							
Aug	Tagged	6	6	6	6	6				
	Found	4	1	3	5	3				
Sep	Tagged	6	6	6	6	6	6	6	6	6
	Found	5	5	4	3	4	2	1	4	0
Oct	Tagged	6	6	6	6	6	6	6	6	6
	Found	4	6	5	5	1	2	4	2	2
Nov	Tagged	6	6	6						
	Found	5	5	3						

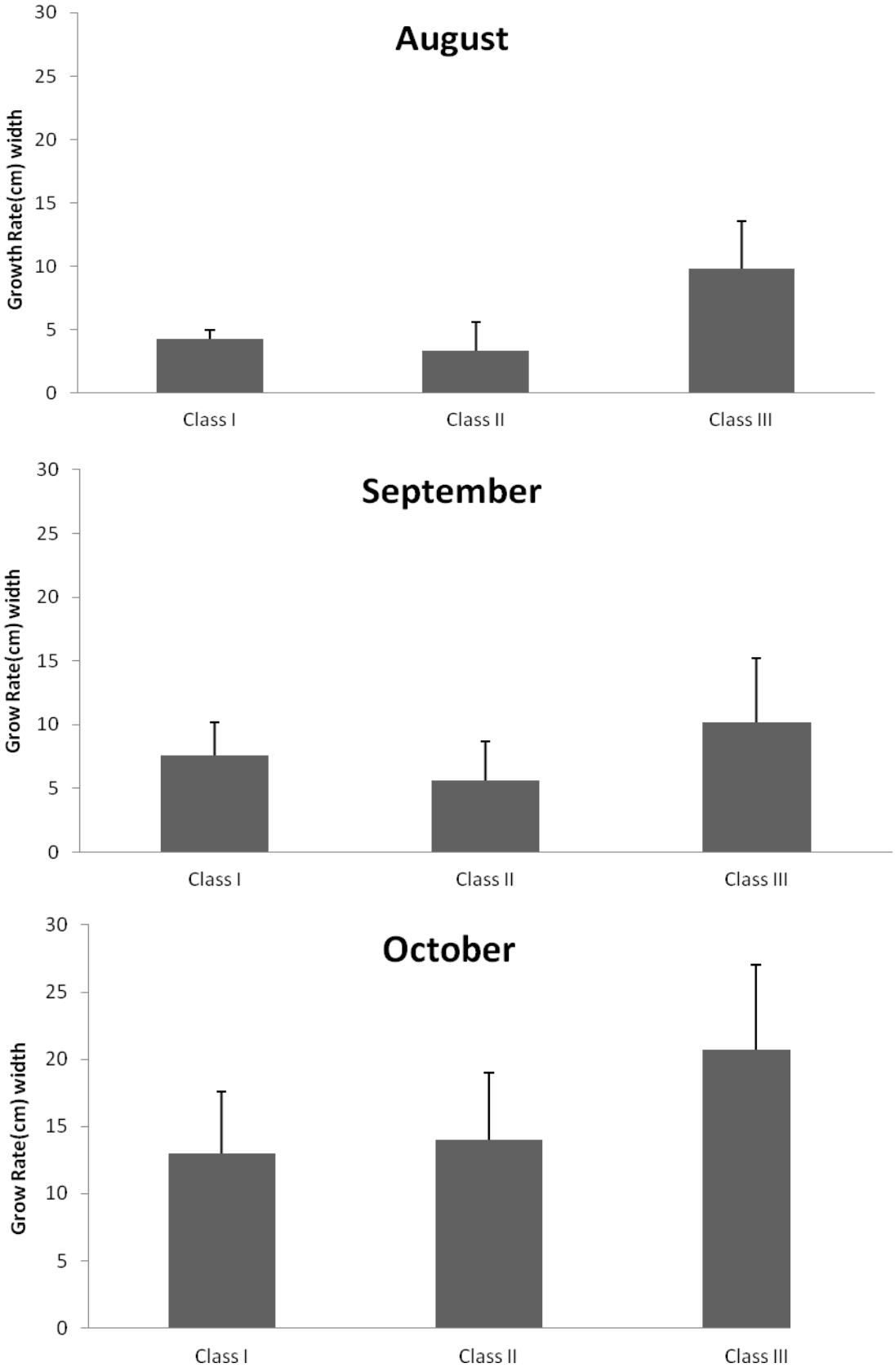

Figure 38: Growth rate of *U. pinnatifida* sporophytes.

3.4 Fecundity

The spore counts only included data when at least one mature plant was found. The spore count originally included 3 different sections of sporophyll which were the top, middle and bottom sections of sporophyll from each plant. No significant differences were found in spore number between these three sections (P>0.05, Appendix VII), so data for these three sections were averaged.

No significant differences in spore number were found between either farms (P>0.05) or months (P>0.05) (Figure 41). Farms 253 and 327 had mature sporophylls in all months. The maximum average spore count occurred in October, with an average of 4.39 X 10^3 spores cm^{-2}. The minimum average spore count occurred in September with only 3.33 X 10^3 spores cm^{-2}. Minimum spore counts were found in Farm 253 on the 31st of May, with a value of 2.47 X 10^3 spores cm^{-2}, while the maximum spore count was at Farm 327 in October with a value of 5.20 X 10^3 spores cm^{-2}.

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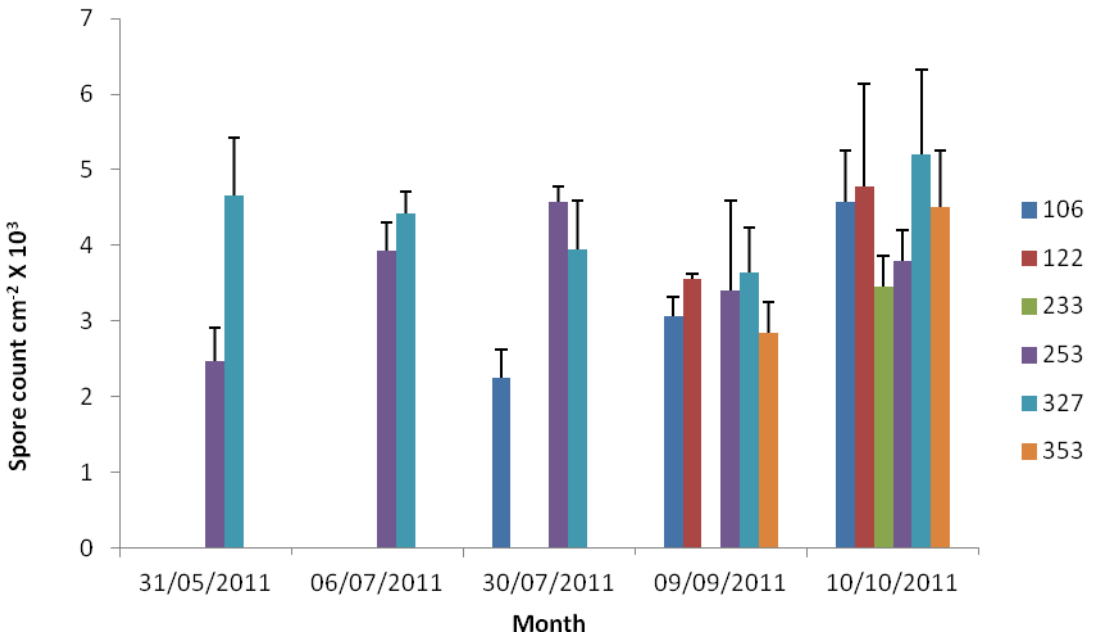

Figure 39: Mean spore release of per cm^2 of *U. pinnatifida* sporophyll.

3.5 Seasonality of sporophyte germination

New juvenile sporophytes were found throughout the sampling period from April to November 2011 (Figures 40 and 41). In general, the number of new plants was at its minimum from April to May with little increase in June.

In Pelorus Sound, the most new plants were found on Farm 122 where the maximum number of new plants found attached to a frame (n=126) was found in August. Farm 314 had just one new plant throughout the entire sampling period (Appendix VIII).

The Number of plants found on frames in Port Underwood were similar to plants in Pelorus Sound. Number of plants in Farm 327 increased a little. Interestingly, a decrease in the number of plants in Farm 106 coincided with an increase in number of plants in Farm 253(Figure 41).

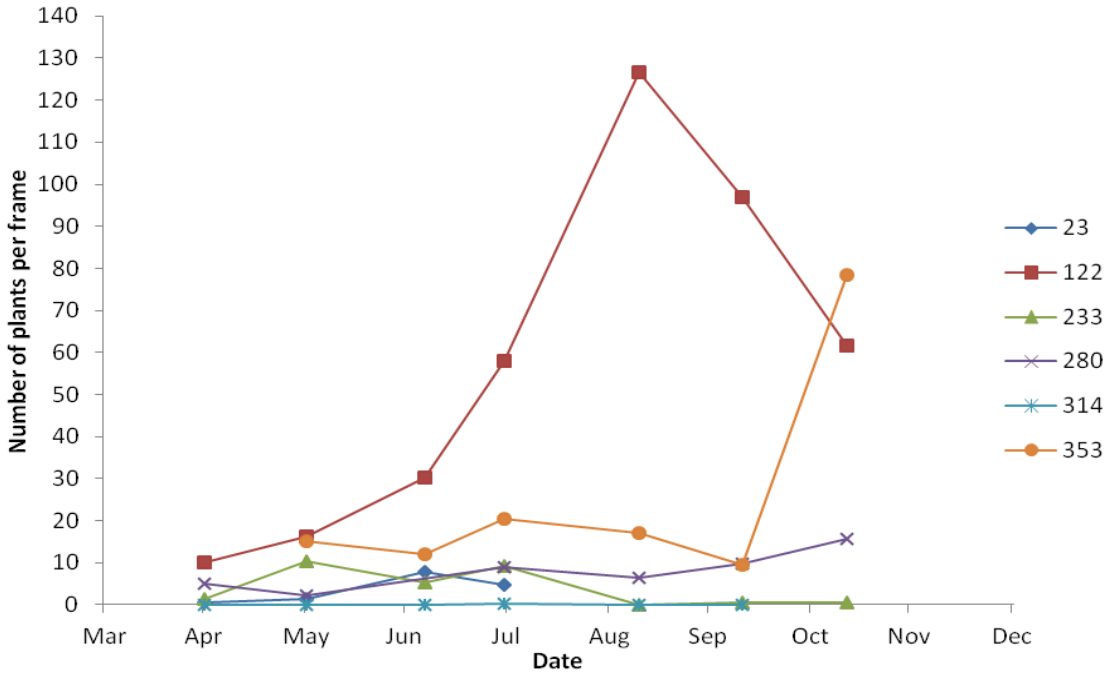

Figure 40: Mean number of new *U. pinnatifida* plants found on frames in Pelorus Sound farms.

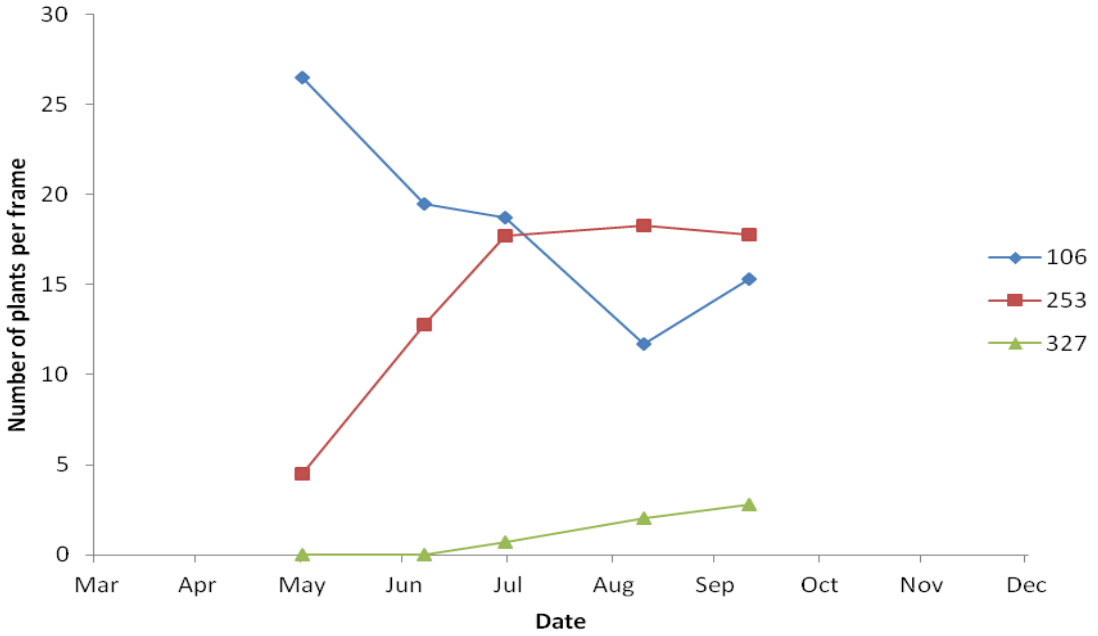

Figure 41: Mean number of new *U. pinnatifida* plants found on frames in Port Underwood farms.

Chapter 4: Discussion

In this study, the most important variable which affected the growth of *U. pinnatifida* was the change of seasons, which was related to the change in water temperature. It was found that monthly, morphological changes were evident. Water depth was also found to affect *U. pinnatifida* density and biomass. Most sporophytes were found between 1 m to 5 m depth, with almost no sporophytes found at 8 m depth.

4.1 Biomass, distribution and seasonality

Within the period of study, sporophytes were much denser in shallow water and grew heavier and denser in later months from spring (September). This may suggest that *U. pinnatifida* matured in late spring (November). However, the density and weight decreased in December accompanied with a decrease in blade size. This suggests that in southern New Zealand, maximal maturation of sporophytes takes place in November and sporophytes die off after they reach maximum maturity.

Results showed that the minimal blade size occurred in mid year around July, suggesting that a new cycle of sporophytes started in winter (July) and produced a large amount of juvenile sporophytes. It was found that in general; mean density rapidly increased around August to September. A sharp increase in biomass was found in October and November along with a sudden increase in mean blade area. This suggests that a large amount of juvenile sporophytes were produced and grew rapidly from September to December.

Piriz, Eyras and Rostagno (2003), reported that in Argentina from 1992 to 1994, the biomass of seaweed wracks consisted mainly of *Ulva spp.* and *Dictyota dichotoma* reached maximum from spring to summer and was at a minimum in winter. This was also supported by Primo, Hewitt and Campbell

(2010), who reported that between the May to September period, *U. pinnatifida* sporophytes were found to be small in size, while from December to January (summer), much larger size plants were found in Blairgowrie and Sandringham, Australia. A very low density of sporophytes was found in January 2009 at only 3 plants m^{-2} in the Blairgowrie location and in February, almost no sporophytes were spotted, with only a few severely damaged plants observed. On the other hand in January, 10 plants m^{-2} was found in Sandringham. Sandringham however, yielded approximately 50 to 65 plants per metre in 2008 to 2009 in most of the months (Primo *et al.*, 2010). In a study in Tasmania, density was highest in November 1998 and lowest in March 1999(Hewitt, Campbell, McEnnulty, Moore, Murfet, Robertson and Schaffelke, 2008). Schaffelke, Campbell and Hewitt (2005) also reported a study in Tasmania in 1998 to 1999. This study reported that in March (autumn), there was only 25 + 2 sporophytes per transect (fixed + floating), while in August (winter) 1999, sporophytes were most dense and reached to a number of 516 +92 sporophytes per transect. In addition, a study by Casas, Piriz & Poradi (2008) based in Argentina also found that maximal density took place in winter, with maximal mean density of 149.1 ± 33 plants m^{-2}. The mean biomass throughout the study in Argentina was at 8391.2 g while the maximal mean biomass was as large as 16501 g ±2359.3 g which was in December 1999 (summer).

The mean biomass in Japan was much different than in the southern hemisphere. In the study by Yoshikawa, Takeuchi and Furuya (2001), it was found that the mean biomass ranged from less than 20 g in January (late winter) to 380 g in April (spring). Shriptsova, Khomeuko and Zsakov (2004) revealed that an increase in temperature may be a crucial growth factor. The weight of plants increased from February (winter) and in March, the mean weight per plant was only 10 g. Blade size of sporophytes also increased approximately 3.5 times from March to June. This led to a large weight gain in sporophytes, which resulted in a mean weight of 468 g in July. Weight decreased after July probably due to erosion (Shriptosova *et al*., 2004). Similarly, in Korea from December 1995 to March 1996, the blade size increased over time and biomass increased

from 93 g cm^{-1} rope in December (winter) to 358 g cm^{-1} rope in March (spring) as the months got warmer. This was negatively associated with the density of plants. Months with lower biomass such as December were associated with higher density of 2.37± 0.2 plants cm^{-1}rope. In months with higher biomass such as March, the density of plants decreased to 0.97 ±0.03 plants cm^{-1} rope.

U. pinnatifida are known to grow rapidly, in Venice, with the total length of *U. pinnatifida* reaching up to 2 m (Curiel, et al, 2001). In 1996, Dean and Hurd (2007) found that in Otago, the average length of sporophytes were 29±0.9 cm then increased to 73.1 ±4.3 cm in July and 79.7±4.4 cm in September (spring). The average length of sporophytes then rapidly decreased to 69.85 ± 5 cm and 34.8 ±6.6 cm in October and November respectively (spring to early summer). Dean and Hurd (2007) also found that the sporophyte growth were maximal in May and June during the autumn to winter period, when growth rate was at 0.80 ±0.12 cm per day. This slowly decreased to -0.27 cm per day in September and October during spring. Casa *et al.* (2008) reported similar findings in Argentina. The average maximal length of sporophytes were found in spring and early summer at around 88.5 ±4.4 cm. In autumn, the mean length rapidly reduced to 12.6 ±1cm due to the increase number of juveniles. These results are similar to the results found in our present study in the Marlborough Sounds. In Pelorus Sound, the smallest mean blade area range was found in August (winter). In August, the mean blade area ranged from 3 cm^2 to 175.2 cm^2 which were found in Farm 122 and Farm 23 respectively. In September (spring), the mean blade area ranged from 70 cm^2 to 624.2 cm^2 which were found in Farm 233 and Farm 280 respectively. On the other hand, in Port Underwood, the mean smallest blade area of 14.2$cm^2$was found in May (late autumn), which was found in Farm 253, while the largest mean blade area was 319.1cm^2 which was found in Farm 327. However, in August(late winter), the blade area of *U. pinnatifida* grew sharply, with smallest mean blade area of 211.6 cm^2 in Farm 253 and largest mean blade area of 614.7 cm^2 in Farm 327.

On the other hand, *U. pinnatifida* was found to be longer in its native region (Yoshikawa *et al.* 2001; Park, Park, Back and Hwang, 2008). In Japan, the mean total length of sporophytes increased linearly from 48 cm in January to 163 cm in April, which was from winter to spring in 1998 (Yoshikawa *et al.*, 2001). Furthermore, in Korea, the length of sporophytes ranged from 27 cm in December 2002 to 160 cm in April 2003. It was found that in December 2002, the size class of 15-29 cm dominated the population of *U. pinnatifida* and in April, the size class of 135-149 cm dominated. In April (spring), some sporophytes were found in the large size class of 195-209 cm (Park *et al.*, 2008). This differed slightly in Venice (Cruiel, Guidetti, Bellemo, Scattolin and Marzocchi, 2001)). In February and March 1998, very small sized sporophytes dominated the population at size classes of 1- 9 cm and 10-19 cm respectively. In May, plants that ranged from 60-69 cm dominated and most plants reached 70cm or longer in June then slowly died off (Cruiel *et al.*, 2001).

The percent of mature sporophytes with sporophylls decreased from maximum of 20.9% to minimum 7.3% in Pelorus Sound and maximum 32.8% to minimum 11.3% in Port Underwood in our present study in Marlborough Sounds in autumn-winter and increased in spring, was similar to results reported in the study by Hay and Villouta (1993). More sporophytes matured from spring onwards. Blade sizes were also maximal in December. However, the study by Hay and Villouta (1993) showed minimal mass in February and maximal blade size in October where thereby after, mass of sporophytes decreased rapidly.

However, our study ended in December 2011 and it was hard to determine whether the smaller density and lighter weight of sporophytes in December was attributed by random error or wether it occurred as a natural process. Hay and Villouta (2003) suggested that mass of sporophytes decreased in spring and summer.

Literatures suggested that the size of sporophyte was largely related to the presence of sporophylls. Larger plants size was associated with an increased amount of sporophyll (Schaffelke *et al.*, 2005). In the study by Stuart *et al.* (1999),

more than 90% of sporophytes in the Otago Harbour were found with sporophylls in May 1994 (autumn). However, in September (spring) the percentage of sporophylls was found to drop to less than 40%. In Tasmania, Australia, the amount of sporophytes with visible sporophyll remained low throughout the study period. The mean number was at 27 plants per month per transect (each transect has area of 50 m X 4 m). In addition, from February to April, no sporophyte was found with sporophylls (Hewitt *et al.*, 2008). In Australia, Schaffelke *et al.* (2005) found that in January, only 23% of small sporophytes carried a visible sporophyll. However, all sporophytes were found to have sporophyll in February (late summer) regardless of size. Casa *et al.* (2008) reported that sporophylls in Argentina could be as large as 12 cm in width in February 2000 (summer), the size of sporophylls dropped dramatically in autumn, with the smallest sporophyll found to be 0.8 cm in width.

The season of sporophyll maturation was slightly different in different regions. In Korea, sporophytes were found to mature in April 2002 which was in spring. In March, only 11% of sporophytes had visible sporophylls but this number increased rapidly to 98% in April (Park *et al.*, 2008). In another study in Korea, between 1995 and 1996, fertility of sporophytes was found to be 38.02% in the first data collection in December. This percentage doubled in the first data collection in January to 72.46%. Consequently, in March 96.55% of sporophytes were found to be fertile (Choi, Kim, Lee and Nam 2007)

Overall, in our study it was found that in Port Underwood farms, depth was not a significant factor affecting density and biomass. Pelorus Sound farms also yielded a slightly higher mean density and biomass than Port Underwood farms. In Pelorus Sound farms, large amount of sporophytes were found in shallower water. It may be true that sunlight was an important variable in sporophyte growth. Blades were able to grow bigger in shallower water in spring. Although *U. pinnatifida* was found to be more dense and heavier in Pelorus Sound farms, mean blade area was higher in Port Underwood farms. This could be due to the difference in water composition in these two different regions but cannot be confirmed in this study as no water quality analysis has been conducted.

In our study, sporophytes were found throughout the study period. This was similar to the study by Hays (1993), which suggests that sporophytes did not die off in New Zealand in the same way as in Asia, as New Zealand weather does not fluctuate as much as in Asia to completely retard the growth of *U. pinnatifida* sporophytes.

For the four farms in Admiralty Bay visited in our study in September 2011, it can be seen that the differences between Farm 8053 and the three others farms were significant, which was an average of 39.8 plants per drop and an average weight of 8.17 kg per drop in Farm 8053 while the three other farms have less than 10 plants per drop and each drop weighted less than 4 kilograms on average. Farm 8053 produced significantly more plants that had heavier weight. This could be due to the location of Farm 8053 compared to Farm 8054 and Farm 8052 as shown in Figure 6. At the position at which mussel lines of Farm 8053 are situated are relatively sheltered, during the visit, it was also observed that Farm 8053 had less wave attacks and weaker water currents, which was more suitable for attachment of *U. pinnatifida* gametophytes. Pang and Shan (2008) suggested that no zoospore attachments occurred in water with flow rates of more than 70cm per second. Hence chances of zoospore attachment will increase in locations with slower water current to a certain extend but is unable to be quantified without further research in the effect of water current in zoospore attachments.

4.2 Growth rate

In our study, growth rate was recorded from June to November, 2011 which was from autumn to late spring. Within this period, water temperature was measured to be approximately between 10 to 14℃. This temperature was found to be suitable for sporophyte growth. Sanderson (1990) suggested that the growth boundaries of *U. pinnatifida* ranged from 3.5 to 20℃. The increasing rate

of overall sporophyte growth within this period was due to the increased in water temperature. \In literature, growth of *U. pinnatifida* sporophytes was found to vary in different times of the year. In a study by Primo *et al.* (2010) in 2008 to 2009 in Victoria, Australia, sporophytes were found to grow rapidly in May (autumn) 2008. Growth rate in June 2008 to January 2009 were also found to be slower when compared to May (Primo *et al.*, 2010).

On the other hand, a study by Schaffelke *et al.* (2005) from 1998 to 1999 in Tasmania, Australia reported that the growth rate in Victoria was slow from May to October, which was from autumn to early spring. Growth rate was at its greatest in November to March (summer). Maximum growth of sporophyte in Tasmania was found in February with a mean growth of 110cm in length in 30 days (Schaffelke *et al.*, 2005). In our present study in the Malborough Sound, the maximal monthly growth rate was found to be approximately 20cm in width. Another study also based in Tasmania in 1997 to 1999 by Hewitt *et al.* (2008) reported that the growth rate of sporophytes ranged from 12 to 41 mm per day and the mean growth rate in length was 24 mm per day.

Growth rate of *U. pinnatifida* in its native region was also reported. A study based in Japan in 1998 by Yoshikawa *et al.* (2001) reported that in colder months in January and February, the growth rate of sporophytes ranged from 1.3 to 1.8 cm per day. However, in the warmer months of early spring in March and April, the growth rate was found to decrease to 1.0 to 1.7 cm per day (Yoshikawa *et al.*, 2001). In Korea, Choi *et al*. (2007) found that there was positive growth from winter to spring. In December 1995, the mean plant length of *U. pinnatifida* was found to be 79.06 cm± 6.09 cm. The mean length in March 1996 of *U. pinnatifida* reached up to 109.12 cm ± 2.79 cm. Mean plant weight in December was found to be 39.98g ± 4.08g and increased to 371.82 g ± 35.32 g in March (Choi *et al.*, 2007).

Overall, in the Southern Hemisphere, studies found varying results in *U. pinnatifida* growth rates and growth seasons that varied from summer to autumn. In Australia, sporophytes grew rapidly from late spring to summer and from

autumn to early spring growth rate slowed down. Growth rate was at its highest in November to March (summer). However, in the Northern Hemisphere, in Japan and Korea growth rate of *U. Pinnatifida* was highest from winter to spring than in summer. This could be due to the difference in water temperature in different regions at which varied in different seasons. However, not enough water temperature data from these studies are available to confirm the best temperature range for *U.pinnatifida* growth.

In our study, it can be seen that in winter especially August 2011, Class III (mean growth of 9.8cm) plants grew faster than the Class I (4.3cm) and Class II (3.3cm) plants, and the differences were significant. This suggests that the growth of juvenile plants was favoured at warmer temperature. However, water temperature had a smaller effect on matured plants. Primo et al, (2010) determined that the smaller sporophytes grew at a faster rate than near mature or mature plants in the early growth season. In our study, difference in growth rates in young sporophyte was insignificant in later months of September and October.

4.3 Fecundity

Studying of the reproduction of *U. pinnatifida* is essential for both the aquacultural or elimination of this species. The release of spores in *U. pinnatifida* has been studied by two groups in Australia. Between 1998 and 1999, Schaffelke *et al.* (2005) studied the zoospore release by measuring size of sporophytes in individual and classes in Tasmania, Australia. Their findings revealed that maximal spore release in *U. pinnatifida* sporophytes could be as high as $4.3X10^{8}$ zoospores per sporophyte, while minimum spore release was at $1X10^{5}$ zoospores per sporophyte. The mean zoospore release in individual sporophytes was $1.3X10^{7}$ zoospores (Schaffelke *et al.*, 2005). In a later study by Primo *et al.* (2010) based in Victoria between 2008 to 2009, spore release in *U. pinnatifida* sporophytes ranged from $0.13X10^{5}$ $cm^{-2}h^{-1}$ to $7.33X10^{5}$ spores $cm^{-2}h^{-1}$, which was 5.7 to 140.9 times more spore release to the results of our

study which ranged from 2.25 to 5.20 X spores 10^3 cm^{-2}.

As stated in Primo *et al.* (2010), *U. pinnatifida* sporophylls matured from the base to the top. Hence by comparing zoospore release in the base, middle and top sections of sporophyll tissue, the life stage of the sporophyll could be determined. Study by Primo *et al.* (2010) showed that from January to February, most sporophytes only releasing spores from the top sections of its sporophyll tissue, indicating that sporophytes reached full maturity. It was also stated that spore release was higher in summer with larger plants having greatest spore release rate in January while smaller plants had greatest spore release rate in February. In addition, Schaffelke *et al.* (2005) found that larger size sporophytes with lengths longer than 110 cm released more zoospores than smaller sporophytes especially from November to January. Similarly, in our study as the plants got more mature, the released zoospores increased from June to August at Farm 253 and from August to October at Farm 106. No zoospores were found in the base, middle and top sections as the plants were not mature in Farm 106, 122, 233, 353 in the early months of this study.

Contradictory to findings by Primo *et al.* (2010), which suggested that spore count in different sections varied according with the stage of maturation, there were no significant differences in spore count found with either months, plant sections or farms in the our study based in the Malborough Sound.

Besides studying zoospore release in individual sporophytes, the release of zoospores was also studied in Tasmania (Schaffelke *et al.*, 2005). Sporophytes which were smaller than 28 cm were excluded in this study as there were very few sporophytes in this class size. In the study, spore release was mainly by Class III, IV and V with length being 55-110 cm, 110-165 cm and > 165 cm respectively. In July and August 2000, sporophytes in Class III to V were less than $30X10^3$ zoospores cm^{-2} h^{-1}. However, the number of zoospore release in Class IV and V significantly increased in October to December, with average spore release ranging from $30X10^3$ zoospores cm^{-2} h^{-1} to $60X10^3$ zoospores cm^{-2} h^{-1}. In contrast, within the same period, Class II sporophytes had a low

zoospore release rate with an average of $1X10^3$ zoospores cm^{-2} h^{-1}. In January 2001, Class II to V were again found to release similar amount of zoospores that ranged from $26X10^3$ zoospores cm^{-2} h^{-1} to 46 $X10^3$ zoospores cm^{-2} h^{-1} (Schaffelke *et al.*, 2005).

Unfortunately, in our study, the number of sporophytes examined for spore count was not sufficient for classification to class sizes. As spore count was only carried out when at least one random mature sporophyte was spotted, spore count was not carried out at all farms in all months. This could have increased the effect of random error due to limited trials. The number of spores released was high and ranged from 2.25 to 5.20 X 10^5 per cm^{-2} h^{-1}. In comparison to the study by Schaffelke *et al.* (2005), maximal spore release was approximately 0.60 X 10^5 cm^{-2} h^{-1} in Tasmania, and Primo *et al.* (2010) reported a spore release of 12 X 10^5 cm^{-2} h^{-1} in Port Phillip Bay. Hence in the Marlborough Sounds, the maximum spore release was almost 10 times higher than in Tasmania but was less than the maximum spore release in the Port Phillip Bay.

4.4 Seasonality of sporophyte germination

Sporophyte germination was one of the important sequences of the life cycle of seaweeds following spore release. Few of the million spores released are able to germinate and grow to maturity as germination of sporophytes are largely affected by temperature, photoperiod and irradiance（Choi, Kim, Lee, Park & Nam, 2005).

Choi *et al.* (2005) studied the effect of daylight and irradiance on sporophyte growth of *U. pinnatifida*. This study revealed that female gametophytes grew faster at daylight of 16 hours per day and at 60 μmol photons m^{-2} s^{-1}. The gametophytes which grew at 30 μmol photons m^{-2} s^{-1} and at only 8 hours light per day had significant delayed growth. However maximal fertility took place at 12 h light per day followed by 8 h and 16 h. Short light length and low light intensity can retard the growth and fertility of gametophytes. This study

concluded that the optimal day length for sporophyte formation was at 12 h of light per day.

The finding by Choi *et al.* (2005) was comparable to the natural seasonal light lengths in Korea. It was known that spore release (growth of gametophytes) of *Undaria pinnatifida* took place from March to June where the day length was between 12 to 15 h. Gametophytes continued to grow at this day length until September, and juvenile sporophytes were seen from October, which had day lengths of 12 h. The study also found that gametophytes require a short day length to mature and produce sporophytes. Similarly in our study, *Undaria pinnatifida* sporophyll formation and growth occurred from September when day length began to increase.

In a study by Pang and Zhan (2008), it was found that the phases of darkness were important to the triggering of fertilization. They suggested that after successful fertilization, the development of sporophytes will begin after one to two days. The mass release of eggs started around two hours after being in darkness and reached a peak rate at midnight, with around 60 percent of gametophytes completing egg release in one night. This study was in agreement with a previous study by Choi *et al.* (2005), which reported that at a temperature of 15℃ coupled with long hours of day light such as 18 h or 16 h of day light were favourable for egg release. However, the phases of fertilization and juvenile sporophyte growth were advantaged by short hours of day light, which in this study were 12 and 8 h of light per day.

The studies by Choi *et al.* (2005) and Pang and Shan (2008) concluded that sporophyll formation of *U. pinnatifida* takes place in summer time, while the day length was long. Reproduction of *U. pinnatifida* was triggered at around autumn when day length reduced to 12 hours per day and a new cycle began.

In our study, juvenile sporophytes were found throughout the study period in the Malborough Sounds. This was supported by Hay and Villouta (1993) who also found sporophytes found throughout the year in New Zealand. In our study,

minimal juvenile sporophyte was found from April to May, while maximal number of juvenile sporophytes was found from June to August in most farms. This further confirmed that young sporophytes developed in the winter period (June to August in New Zealand).

Interestingly in our study, Farm 314 had almost no juvenile plants attachments at all months. It was observed that deforestation on the hill was severe at this farm and coupled with the rainy weather, large amounts of water flowed down the hill bringing large amount of dirt and leaves into the sea. As reported by staff of the mussel farm, water current in Farm 314 are observably stronger compared to other farms. These contributing factors probably made attachment of gametophytes difficult. As mentioned previously by Pang and Shan (2008) with high water velocity, attachment of zoospores and gametophytes became very difficult. Small sporophytes may have been washed away before they could be detected. This could be supported by the negative numbers seen in Appendix VIII. The numbers in the tables indicated the number of plants in the particular month compared to the number of plants counted from the previous month. Hence a positive number is indicative of growth of new plants and negative number is indicative of the number of lost plants. These negative numbers are seen several times, suggesting the loss of plants in frames are usual and are constant. The loss of plants may also be due to consumption of plants by sea animals. The frame itself was made of biodegradable materials and would breakdown slowly in seawater. Although the frames were changed every three months, on some occasions in the third month, the frame was no longer able to support as much sporophyte attachments as a new frame. The results from our study may not be representative of natural or other artificial substrates. Parson (1994) mentioned that *U. pinnatifida* was able to attach to steel, hulls of ships, concrete or wood. However, it attached better to ropes or stones (Brown and Lamare, 1994).

A study in Venice by Cruiel *et al*. (2001) in 1998, examined the length of sporophytes. In February, the population of *U. pinnatifida* was mainly composed of small size sporophytes no longer than 9 cm in length (Cruriel, 2001).

Schaffelke *et al.* (2005) also found a large amount of juvenile sporophytes in July, August and October (winter and spring). This coincided with the reproductive stage in autumn. It was also found that in January and February, a bell shaped pattern was seen in terms of percentage of size classes with the amount of medium size sporophytes predominant and less of small and large sporophytes (Schaffelke, 2005).

In summary from our study, following spore release, sporophyte germination was an important sequence in the life cycle of seaweeds. In spring, when the day length increased, sporophyll formation of *U. pinnatifida* took place. Reproduction of *U. pinnatifida* started at around autumn. Large amounts of juvenile sporophytes were found in winter (July, August) and spring (September).

4.5 General conclusion

This thesis studied the growth patterns of several farms of *U. pinnatifida* in Port Underwood and Pelorus Sound. The effects of depth in terms of the size and abundance of sporophytes was examined. Differences in the size and abundance of sporophytes at the same depth with months of the year were also examined. At the same time, growth rate of *U. pinnatifida*, spore release competency and gametophyte attachment competency were monitored.

The results of this study showed that from March to November 2011, the overall size of sporophyte increased over this period and reached a maximum in November 2011. *U. pinnatifida* was found to grow best in water that ranged from 0 to 3 m depth, while sporophytes can be found even at 8 m of depth. In our study, growth of *U. pinnatifida* was found to be significantly better in Port Underwood farms, particularly in Farm 327 compared to Pelorus Sound farms. The highest amount of spore count was also found in Farm 327. However, more sporophytes were found to have germinated in Pelorus Sound Farm 122. Therefore, spore release competency may not be proportional to sporophyte germination and attachment as these could be affected by various factors.

Results of this study showed that even though the farms were within the

same region of New Zealand, the morphology of *U.pinnatifida* in these farms varied significantly. Hence indicating there is a number of factors which are significant to the growth of this seaweed. Through the completion of this study, various limiting factors have been identified.

The most significant limitation in this study was weather constraint. Another constraint was the limited time frame for the completion of this study. Ideally, this study should involve at least one year of field work and visits to all farms at all months. This complete and comprehensive data is vital for the study of phenology and reproduction of *U. pinnatifida.* Furthermore, there are significant number of factors which have affected in the growth and reproduction of *U.pinnatifida* which are not adjusted in this study. These factors include the water temperature, water quality, water velocity and magnitude. These factors must be addressed in future studies to generate accurate and precise results.

As suggested by this study, the aquaculture of this seaweed is potentially profitable. Even though the density and biomass of *U.pinnatifida* found in New Zealand are not as dense when compared to Asian countries. But when given the right conditions, the growth of this seaweed could be significantly enhanced. However, the right conditions for the growth of *U.pinnatifida* must be identified and put in place before the farming of this seaweed could be successful. A considerable number of future studies are needed to further identify these factors and measure the effects of these factors before any solid planning of commercial farming of *U.pinnatifida* should proceed.

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Appendix I

Two-way ANOVA of plant numbers by depth and month at Farm 23.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	4	27.849	6.96236	3.86	0.013
Depth	7	45.121	6.44584	3.57	0.007
Error	28	50.490	1.80320		
Total	39	123.460			

S = 1.343 R-Sq = 59.10% R-Sq(adj) = 43.04%

Two-way ANOVA of plant numbers by depth and month at Farm 122.

Plant no. av VS Depth, Month

Source	DF	SS	MS	F	P
Depth	7	121.063	17.2947	3.05	0.009
Month	8	268.206	33.5258	5.92	0.000
Error	56	317.387	5.6676		
Total	71	706.656			

S = 2.381 R-Sq = 55.09% R-Sq(adj) = 43.06%

Two-way ANOVA of plant numbers by depth and month at Farm 233.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	8	943.56	117.945	2.26	0.036
Depth	7	1819.61	259.944	4.98	0.000
Error	56	2923.76	52.210		
Total	71	5686.93			

S = 7.226 R-Sq = 48.59% R-Sq(adj) = 34.82%

Two-way ANOVA of plant numbers by depth and month at Farm 280.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	149.642	21.3774	3.43	0.005
Depth	7	306.550	43.7928	7.03	0.000
Error	49	305.084	6.2262		
Total	63	761.276			

S = 2.495 R-Sq = 59.92% R-Sq(adj) = 48.47%

Two-way ANOVA of plant numbers by depth and month at Farm 314.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	6	56.648	9.44136	4.27	0.002
Depth	7	43.306	6.18654	2.80	0.018
Error	42	92.940	2.21286		
Total	55	192.894			

S = 1.488 R-Sq = 51.82% R-Sq(adj) = 36.90%

Two-way ANOVA of plant numbers by depth and month at Farm 353.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	217.152	31.0218	4.24	0.001
Depth	7	100.173	14.3104	1.96	0.081
Error	49	358.637	7.3191		
Total	63	675.962			

S = 2.705 R-Sq = 46.94% R-Sq(adj) = 31.79%

Two-way ANOVA of plant numbers by depth and month at Farm 106.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	27.022	3.86026	0.92	0.498
Depth	7	24.330	3.47574	0.83	0.568
Error	49	205.222	4.18821		
Total	63	256.574			

S = 2.047 R-Sq = 20.01% R-Sq(adj) = 0.00%

Two-way ANOVA of plant numbers by depth and month at Farm 253.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	14.0862	2.01231	1.64	0.148
Depth	7	8.8363	1.26234	1.03	0.425
Error	49	60.2820	1.23024		
Total	63	83.2045			

S = 1.109 R-Sq = 27.55% R-Sq(adj) = 6.85%

Two-way ANOVA of plant numbers by depth and month at Farm 327.

Plant no. av VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	407.84	58.2631	5.83	0.000
Depth	7	245.91	35.1300	3.52	0.004
Error	49	489.69	9.9937		
Total	63	1143.44			

S = 3.161 R-Sq = 57.17% R-Sq(adj) = 44.94%

Appendix II

Two-way ANOVA of plants wet weight by depth and month at Farm 23.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	4	1291.2	322.798	1.49	0.232
Depth	7	3898.6	556.949	2.57	0.035
Error	28	6064.9	216.602		
Total	39	11254.7			

S = 14.72 R-Sq = 46.11% R-Sq(adj) = 24.94%

Two-way ANOVA of plants wet weight by depth and month at Farm 122.

Weight VS Depth, Month

Source	DF	SS	MS	F	P
Depth	7	1575100	225014	3.16	0.007
Month	8	4301721	537715	7.54	0.000
Error	56	3992206	71289		
Total	71	9869027			

S = 267.0 R-Sq = 59.55% R-Sq(adj) = 48.71%

Two-way ANOVA of plants wet weight by depth and month at Farm 233.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	8	3364235	420529	5.83	0.000
Depth	7	2026567	289510	4.01	0.001
Error	56	4038733	72120		
Total	71	9429536			

S = 268.6 R-Sq = 57.17% R-Sq(adj) = 45.70%

Two-way ANOVA of plants wet weight by depth and month at Farm 280.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	504432	72061.7	3.69	0.003
Depth	7	460085	65726.4	3.36	0.005
Error	49	957153	19533.7		
Total	63	1921670			

S = 139.8 R-Sq = 50.19% R-Sq(adj) = 35.96%

Two-way ANOVA of plants wet weight by depth and month at Farm 314.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	6	5972.0	995.327	4.05	0.003
Depth	7	3218.7	459.811	1.87	0.099
Error	42	10312.2	245.529		
Total	55	19502.9			

S = 15.67 R-Sq = 47.12% R-Sq(adj) = 30.76%

Two-way ANOVA of plants wet weight by depth and month at Farm 353.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	2176570	310939	9.36	0.000
Depth	7	386712	55245	1.66	0.140
Error	49	1628040	33225		
Total	63	4191321			

S = 182.3 R-Sq = 61.16% R-Sq(adj) = 50.06%

Two-way ANOVA of plants wet weight by depth and month at Farm 106.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	949949	135707	5.56	0.000
Depth	7	95919	13703	0.56	0.784
Error	49	1196562	24420		
Total	63	2242430			

S = 156.3 R-Sq = 46.64% R-Sq(adj) = 31.39%

Two-way ANOVA of plants wet weight by depth and month at Farm 253.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	559305	79900.7	3.68	0.003
Depth	7	97284	13897.8	0.64	0.720
Error	49	1062467	21683.0		
Total	63	1719056			

S = 147.3 R-Sq = 38.19% R-Sq(adj) = 20.54%

Two-way ANOVA of plants wet weight by depth and month at Farm 327.

Weight VS Month, Depth

Source	DF	SS	MS	F	P
Month	7	2019284	288469	2.04	0.068
Depth	7	3252338	464620	3.29	0.006
Error	49	6918817	141200		
Total	63	12190439			

S = 375.8 R-Sq = 43.24% R-Sq(adj) = 27.03%

Appendix III

Two-way ANOVA of plant blade size by depth and month at Farm 23.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	4	15653	3913.14	1.46	0.241
Depth	7	63281	9040.17	3.37	0.010
Error	28	75106	2682.37		
Total	39	154040			
S = 51.79	R-Sq = 51.24%		R-Sq(adj) = 32.0		

Two-way ANOVA of plant blade size by depth and month at Farm 122.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	8	21379480	2672435	10.30	0.000
Depth	7	5898812	842687	3.25	0.006
Error	56	14525665	259387		
Total	71	41803957			
S = 509.3	R-Sq = 65.25%		R-Sq(adj) = 55.95%		

Two-way ANOVA of plant blade size by depth and month at Farm 233.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	8	3614034	451754	4.63	0.000
Depth	7	2536614	362373	3.71	0.002
Error	56	5469437	97669		
Total	71	11620085			
S = 312.5	R-Sq = 52.93%		R-Sq(adj) = 40.32%		

Two-way ANOVA of plant blade size by depth and month at Farm 280.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	7	2596668	370953	6.01	0.000
Depth	7	766248	109464	1.77	0.114
Error	49	3021980	61673		
Total	63	6384896			

S = 248.3 R-Sq = 52.67% R-Sq(adj) = 39.15%

Two-way ANOVA of plant blade size by depth and month at Farm 314.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	6	505762	84293.6	7.59	0.000
Depth	7	140483	20069.0	1.81	0.111
Error	42	466460	11106.2		
Total	55	1112705			

S = 105.4 R-Sq = 58.08% R-Sq(adj) = 45.10%

Two-way ANOVA of plant blade size by depth and month at Farm 353.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	7	8572336	1224619	8.93	0.000
Depth	7	870081	124297	0.91	0.510
Error	49	6722098	137186		
Total	63	16164515			

S = 370.4 R-Sq = 58.41% R-Sq(adj) = 46.53%

Two-way ANOVA of plant blade size by depth and month at Farm 106.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	7	4526996	646714	5.55	0.000
Depth	7	801282	114469	0.98	0.455
Error	49	5707006	116470		
Total	63	11035285			
S = 341.3	R-Sq = 48.28%	R-Sq(adj) = 33.51%			

Two-way ANOVA of plant blade size by depth and month at Farm 253.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	7	5503030	786147	10.32	0.000
Depth	7	1233910	176273	2.31	0.040
Error	49	3731565	76154		
Total	63	10468505			
S = 276.0	R-Sq = 64.35%	R-Sq(adj) = 54.17%			

Two-way ANOVA of plant blade size by depth and month at Farm 327.

Blade VS Month, depth

Source	DF	SS	MS	F	P
Month	7	12326362	1760909	4.46	0.001
Depth	7	25558910	3651273	9.25	0.000
Error	49	19340822	394711		
Total	63	57226093			
S = 628.3	R-Sq = 66.20%	R-Sq(adj) = 56.55%			

Appendix IV

One-way ANOVA of percentage of sporophylls by all the Farms.

Percent of sporophyll VS Farms

Source	DF	SS	MS	F	P
Farm	8	0.5547	0.0693	2.83	0.010
Error	61	1.4969	0.0245		
Total	69	2.0516			

S = 0.1567 R-Sq = 27.04% R-Sq(adj) = 17.47%

Appendix V

One-way ANOVA of plant wet weight by the 4 new Farms.

Weight versus Farms

Source	DF	SS	MS	F	P
Farm	3	181.88	60.63	9.71	0.001
Error	16	99.87	6.24		
Total	19	281.76			

S = 2.498 R-Sq = 64.55% R-Sq(adj) = 57.91%

One-way ANOVA of plant number by the 4 new Farms.

Plant no. VS Farms

Source	DF	SS	MS	F	P
Farm	3	4793	1598	13.82	0.000
Error	16	1850	116		
Total	19	6642			

S = 10.75 R-Sq = 72.15% R-Sq(adj) = 66.93%

Appendix VI

One-way ANOVA of plant growth by months.

Monthly Growth VS Month

Source	DF	SS	MS	F	P
Month	5	888.1	177.6	4.42	0.004
Error	24	963.6	40.1		
Total	29	1851.6			

S = 6.336 R-Sq = 47.96% R-Sq(adj) = 37.12%

One-way ANOVA of plant width by Class in August.

Width VS Class **August**

Source	DF	SS	MS	F	P
Class	2	117.55	58.77	25.96	0.000
Error	15	33.96	2.26		
Total	17	151.51			

S = 1.505 R-Sq = 77.58% R-Sq(adj) = 74.59%

One-way ANOVA of plant width by Class in September.

Width VS Class **September**

Source	DF	SS	MS	F	P
Class	2	125.1	62.5	1.96	0.156
Error	35	1115.8	31.9		
Total	37	1240.9			

S = 5.646 R-Sq = 10.08% R-Sq(adj) = 4.94%

One-way ANOVA of plant width by Class in October.

Width VS Class **October**

Source	DF	SS	MS	F	P
Class	2	268.6	134.3	2.38	0.115
Error	23	1295.5	56.3		
Total	25	1564.1			

S = 7.505 R-Sq = 17.17% R-Sq(adj) = 9.97%

Appendix VII

One-way ANOVA of plant spores release by sections.

Spore VS Sections

Source	DF	SS	MS	F	P
Section	2	1.18	0.59	0.52	0.599
Error	54	61.81	1.14		
Total	56	62.99			

S = 1.070 R-Sq = 1.88% R-Sq(adj) = 0.00%

One-way ANOVA of plant spores release by Farms.

Spore VS Farms

Source	DF	SS	MS	F	P
Farm	5	3.058	0.612	0.90	0.510
Error	13	8.840	0.680		
Total	18	11.898			

S = 0.8246 R-Sq = 25.70% R-Sq(adj) = 0.00%

One-way ANOVA of plant spores release by Months.

Spore VS Months

Source	DF	SS	MS	F	P
Month	4	3.911	0.978	1.71	0.203
Error	14	7.987	0.571		
Total	18	11.898			

S = 0.7553 R-Sq = 32.87% R-Sq(adj) = 13.69%

Appendix VIII

Number of new plants in Farm 23.

Frame/Month	May	Jun	Jul	Aug
Apr I	0	4	10	
Apr II	1	2	18	
May I		0	3	0
May II		0	1	6
Jun I			9	8
Jun II			5	16
Jul I				7
Jul II				0
Total	1	6	46	37

Number of new plants in Farm 122.

Frame/Month	May	Jun	Jul	Aug	Sep	Oct	Nov
Apr I	9	18	4				
Apr II	11	7	3				
May I		17	45	35			
May II		23	3	9			
Jun I			89	181	208		
Jun II			40	100	385		
Jul I				57	82	118	
Jul II				81	8	97	
Aug I					41	121	100
Aug II					35	52	23
Total	20	65	184	463	759	388	123

Number of new plants in Farm 233.

Frame/Month	May	Jun	Jul	Aug	Sep	Oct	Nov
Apr I	0	23	3				
Apr II	3	9	19				
May I		1	13	29			
May II		8	-2	13			
Jun I			1	3	-4		
Jun II			-2	1	-1		
Jul I				3	2	-1	
Jul II				6	-1	3	
Aug I					0	0	0
Aug II					-2	0	1
Total	3	41	32	55	-6	2	1

Number of new plants in Farm 280.

Frame/Month	Jun	Jul	Aug	Sep	Oct	Nov
Apr I	7	4				
Apr II	3	6				
Jun I		-1	0			
Jun II		0	0			
Jul I			9	10	14	
Jul II			27	-2	22	
Aug I				2	1	28
Aug II				15	2	19
Total	10	9	36	25	39	47

Number of new plants in Farm 314.

Frame/Month	May	Jun	Jul	Aug	Sep	Oct
Apr I	0	0	0			
Apr II	0	0	0			
May I		0	0	0		
May II		0	0	0		
Jun I			0	1	0	
Jun II			0	0	0	
Jul I				0	0	0
Jul II				0	0	0
Total	0	0	0	1	0	0

Number of new plants in Farm 353.

Frame/Month	Jun	Jul	Aug	Sep	Oct	Nov
Apr I	13	9				
Apr II	17	9				
Jun I		13	-1			
Jun II		17	44			
Jul I			14	6	28	
Jul II			25	28	8	
Aug I				17	4	98
Aug II				17	-2	59
Total	30	48	82	68	38	157

Number of new plants found in Farm 106

Frame/Month	Jun	Jul	Aug	Sep	Oct
May I	23	12	39		
May II	30	41	11		
Jun I		9	20	18	
Jun II		16	2	32	
Jul I			34	2	20
Jul II			6	14	22
Aug I				2	14
Aug II				2	5
Total	53	78	112	70	61

Number of new plants found in Farm 253.

Frame/Month	Jun	Jul	Aug	Sep	Oct
May I	9	14	35		
May II	0	0	0		
Jun I		12	16	46	
Jun II		25	26	17	
Jul I			16	8	27
Jul II			13	24	-1
Aug I				15	45
Aug II				0	0
Total	9	51	106	110	71

Number of new plants found in Farm 327.

Frame/Month	Jun	Jul	Aug	Sep	Oct
May I	0	0	0		
May II	0	0	0		
Jun I		0	0	0	
Jun II		0	1	0	
Jul I			1	0	0
Jul II			2	-1	0
Aug I				5	6
Aug II				8	5
Total	0	0	4	12	11