

**The reproductive biology of the surf clams Triangle Shell
(*Spisula aequilatera*), Ringed dosinia (*Dosinia anus*) and
Deep water Tuatua (*Paphies donacina*) from the North-
East of South Island, New Zealand**

Nuha Al jadani



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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 a university or other institution of higher learning”

Signature

A handwritten signature in blue ink, consisting of a long horizontal stroke with a small loop and a vertical stroke intersecting it.

Date: 30/7/2013

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ABSTRACT

Surf clam is an important marine organism which plays vital roles in terms of commercial and ecological roles. Commercial harvesting of them is well established at an international level although still the industry use of them is limited in NZ. To sustainably increase surf clam harvesting at a commercial level, the authorities seek to enhance basic researches, particularly around the reproductive biology of clam species. Thus, throughout this study, the aim is to investigate the gametogenesis and gonadal development of *Paphies donacina*, *Dosinia anus* and *Spisula aequilatera* which the species are economically important in Cloudy Bay environment.

To investigate the reproductive biology of the three species, this study employs two different methodologies which are histological techniques and develop a condition index. The study examines samples extracted from Cloudy Bay from Aug, 2012 to Jul, 2013 for each month to determine the seasonal variations in the reproductive biology of the three species. The study uses statistical techniques extensively to generalize the findings from the samples into the Cloudy Bay populations.

From the above techniques, the current study has found that there are significant seasonal variations in varying extents in the reproductive cycles of the three species. The findings further have concluded that histological techniques are the most appropriate way to investigate the gametogenesis and gonadal development of surf clams over the condition index. It has been clear that temperature is an important exogenous factor that determines reproductive behaviours of *Paphies donacina*, *Dosinia anus* and *Spisula aequilatera* in Cloudy Bay. In addition, this study provides directions for future studies to extend the knowledge discovered from this research. It is hoped that these findings could minimize the research gaps bound around surf clam investigations in New Zealand context.

1 Introduction

1.1 Background/significance of the problem

Surf clams is the collective term for the seven species of clams that live in the soft sediment on sandy, high wave exposure surf type beaches in New Zealand. These species are: Deepwater tuatua (*Paphies donacina*), Fine (silky) dosinia, (*Dosinia subrosea*), Frilled venus shell, (*Bassina yatei*), Large trough shell, (*Mactra murchisoni*), Ringed dosinia, (*Dosinia anus*), Triangle shell, (*Spisula aequilatera*), and Trough shell, (*Mactra discors*) (Ministry for Primary Industries, 2012). The difficulty associated with the commercial harvesting of surf clams has become a major concern for the Ministry of Primary Industries recently. On-going biomass surveys around NZ coastal areas have estimated that the actual biomass is much higher than it was calculated by past studies and hence, reinforce the feasibility of the commercial use of surf clams. The potential is predicted such that surf clams can be among the top most five fishery source to become a source of export.

On an international level, clam fisheries are common and quite large, for example *Spisula solidissima* in the US, *Prothaca thaca* in South America and *Spisula polynyma* in Canada had an annual catch in 2010 of 100,000, 22,000 and 22,000 t respectively. Although there are several evidences that suggest that surf clams harvesting has been done for centuries for recreational and traditional purposes, commercial use of surf clams is less known. There is only one company harvesting these clams on a commercial basis and they harvested just 152.2 t in the 2011-12 fishing year (MPI, 2012).

This is primarily because of the difficulty of harvesting these animals, as they live within the sandy substrate in a very wave impacted environment, necessitating large investments into expensive harvesting equipment such as hydraulic dredges (Beentjes and Baird, 2004). Nonetheless, there are high numbers of these animals on many of New Zealand's surf beaches and while the current industry is small, it appears that significant investment is being made into growing this nascent industry (Cloudy Bay Clams Ltd. pers. comm.). The Ministry of Primary Industries has made it clear to surf clam quota holders, that if they are to continue to increase the Total Allowable Commercial Catch (TACC) in the Fishing Management Areas of New

Zealand, they will require an increase in basic research, particularly around the reproductive biology of the clams species (SWG 2012). These investigations should include investigations on biomass and abundance (H J Cranfield & Michael, 2001b), size and growth rate (Yu, Song, Choi, & Park, 2009), consequences of dredging techniques (Beentjes & Baird, 2004), and gametogenesis and gonadal development (Drummond, Mulcahy, & Culloty, 2006).

While there have been a number of studies on surf clams in New Zealand to date, these have focused only on distribution and abundance (e.g. Cranfield et al., 1994; Haddon et al. 1996; Triantafillos, 2008) or on growth rates and settlement (e.g. Cranfield and Michael, 2001; Conroy et al., 1993), which have been used to inform the setting of quota by the then Ministry of Fisheries (now MPI). Thus, this study believes that the remaining major research focus, gametogenesis and gonadal development, should be investigated in NZ context. Although the development of research is limited in the ambit of gametogenic cycles in NZ, researchers are widely conducted in other parts of the world such as North America, Ireland, Portugal, Japan and Korea, which ultimately influence to set up annual allowable catch limits.

that focus on gametogenesis and gonadal development (Booth, 1983; Hooker and Creese, 1995; Rapson, 1952; Redfearn, 1974 & Gribben, 2005) and many off these have been criticised for having significant number of limitations particularly in relation to the study design. One of the limitations is the lack of strong methodological rigors, where studies used the condition index with no attention at all to use histological techniques which are essential. Other studies, which histological techniques are used, have been conducted specific either to single specie or a particular location. Since the gametogenesis and gonadal development of surf clams vary locally according to exogenous factors (Cranfield & Michael, 2001a; Grant & Creese, 1995; Mid-Atlantic Fishery Management Council, 2010), it is required to investigate the gametogenic development specific to places that surf clam harvesting is done commercial means.

that form the most preferable habitat for surf clam species. According to biomass estimates and growth rates, Diamond shell (*Spisula aequilatera*), Moon shell (*Dosinia anus*) and TuaTua shell (*Paphies donacina*) are the most suited clam species to grow in Cloudy Bay (Cranfield & Michael, 2001b); thus making them the

focus for commercial harvesting. Therefore, the current study focuses on investigating gametogenesis and gonadal development of the three surf clam species in Cloudy Bay. Throughout the current study the ultimate question that will be consistently addressed is:

‘What are the characteristics and dependencies of gametogenesis and gonadal development of Diamond shell (*Spisula aequilatera*), Moon shell (*Dosinia anus*) and TuaTua shell (*Paphies donacina*) in Cloudy Bay, NZ?’

1.2 Aim and objectives

The aim of this study is to investigate the reproductive biology of three species of surf clams: Triangle Shell (*Spisula aequilatera*), Ringed dosinia (*Dosinia anus*) and Deepwater tuatua (*Paphies donacina*) specific to the Cloudy Bay environment.

To achieve the aim of the study, the objectives pursued throughout the study are as follows:

1. To gather histological evidences for the characteristics of the reproductive cycles of the three surf calm species. This objective includes the examinations of gonads to identify their sex by using representative samples of the population. Then, both female and male surf clams are categorized into the distinct stages of their reproductive cycles.
2. To calculate the condition indices. Condition indices are calculated in individual basis for the samples of the three species collected from Cloudy bay throughout the year.
3. To determine correlations between the exogenous factors (considerations will be given only for temperature), reproductive stages and condition index. This is done by using the results which are gathered through the achievement of the 1st and 2nd objective.
4. To compare the reproductive biology of the three surf clam species. The objective helps to ascertain if there are differences in the reproductive cycle between three clams diamond, tuatua and moon

in the same area. The objective is achieved through the field investigations and literature is used in the purpose of comparison.

1.3 Scope and limitation

As described previously, the aim of this study is to investigate the reproductive biology of the most suitable three surf clam species to be grown in Cloudy Bay, which are Triangle Shell (*Spisula aequilatera*), Ringed dosinia (*Dosinia anus*) and Deepwater tuatua (*Paphies donacina*).

The field investigations of the current study are based on surf clams harvested from Cloudy Bay. Since the characteristic of reproductive cycles are influenced by environmental factors (Hashizume, Tatarazako, Kohata, Nakamura, & Morita, 2011), the findings of the current study will be applicable to Cloudy Bay only, which is another limitation of this study.

There are many exogenous factors that the life history of surf clams is influenced such as temperature, salinity, currents and availability of food (Cargnelli, Griesbach, Packer, & Weissberger, 1999). Under the current study, only temperature is considered to investigate the correlation between the reproductive cycles and external factors, which is another limitation. The consideration given to temperature is also limited for the measures of ambient temperatures only and the rate of temperature change, which can be important towards spawning (Cargnelli et al., 1999), is not taken into account.

1.4 Organization of the thesis

Chapter 1 is comprised of an introduction to this research study. In this chapter, background information related to surf clam harvesting and the related research gaps are broadly discussed to describe the significance of the current study. The problem statement includes the establishment of the link between the gametogenesis and gonadal development of surf clams and fishery regulations both in global and NZ contexts. Then, the focus of this research study is described by using aim, objectives and limitations.

Chapter 2 is an extensive review of literature related to surf clam fishery. The chapter is the attempt made to identify the research problem for the current study. The key

areas of the literature review include: identification of the life history and the influence of exogenous factors towards the distinct life cycles of surf clam species, recognizing the abundance of surf clams and their commercial use in global perspectives, identifying the strategies established in other parts of the world to secure the sustainable use of surf clams; reviewing past studies on the abundance and growth rates of different surf clam species in NZ context; evaluating the feasibility of commercial surf clam harvesting in NZ and identifying the research gap related to reproductive biology.

The chapters 3,4,5 describe the field investigations related to the three Cloudy Bay surf clam species which are diamond shells, moon shells and deep water tautau respectively. Only for chapter 3, the research methods are added in detail as long as the same methodology is adapted to investigate the three types of surf clams. The results and interpretations are added for each chapter and followed with a discussion to those interpretations.

Chapter 6 is the conclusion of the current study. The chapter provides a comparison to the findings of the three surf clam species and which are separately discussed under the chapters 3, 4 and 5.

2 Literature review

This chapter provides a literature review of surf clams both in global and local (New Zealand) perspectives. The review of literature include: identifying biological characteristics and stages of their life history with particular emphasis on the reproductive cycle of surf clams. Both strengths and weaknesses of literature in the ambit of NZ surf clams are identified to construct the significance of the current study.

2.1 Biological characteristics of surf clams

Past scholars (Beentjes & Baird, 2004; Cranfield & Michael, 2001; Mid-Atlantic Fishery Management Council, 2010; Ministry for Primary Industries, 2012) have identified Surf clams as one of the most important aquatic organisms which play important commercial and ecological roles. Surf clams are scientifically known as *Spisula Solidissima* and their common names include hen clams, sea clams, bar clams, beach clams and skimmer clams (Fay, Neves, & Pardue, 1983; Ropes, 1980).

Surf clams belong to Phylum Mollusca which is comprised of diverse invertebrate animals; some examples include snails, slugs, mussels, oysters, octopuses, and squid (The Paleontological Society, 2012). In the hierarchy of scientific classification, surf clams belong to the class of Bivalvia which is the second largest class of the phylum Mollusca according to the number of species within the group (The Paleontological Society, 2012). Being in the class of Bivalvia, surf calms have shells consisting of two halves hinged together. Being in the Bivalvia class, they have a soft body completely enclosed with shells (Brink, 2001 as cited in the Paleontological Society (2012). In the taxonomic hierarchy level, surf clams belong to the order of Veneroida and the family of Mactridae (Fay et al, 1983).

Surf clams are aquatic siphon feeders as they burrow beneath the surface of the substrate in which they live (Cargnelli et al., 1999). The siphon is a funnel-like structure in which water and food can flow, as well as being utilized in locomotion and reproduction (Cargnelli et al., 1999). Clams have paired siphons (www.njscuba.net, 2012). Food drawn in through the incurrent siphon is caught by mucus in the gills and then transported to the clam's mouth, while the excurrent siphon carries water away (Fay et al., 1983). The gills also function to draw oxygen

from the water (Cargnelli et al., 1999). Furthermore, after passing the gills, the water passes the genital papillae and carries mature gametes into the water column above during periods of spawning (Ropes, 1986).

Surf clams are heterosexual organisms with hermaphroditism reported rarely (Ropes & Shepherd, 1980). Histological examination of the gonads is used to identify the sex of surf clams as there are no defining external differences (Ropes, 1980). Sexual maturity is dependent on geographical location (as detailed below in section 2.2.2: Adult surf clams). Additionally, location influences the growth rate and the maximum lengths achieved by surf clams (Sections 2.2.2 and 2.6.2). Surf clams are distributed all over the world except in the Polar Regions, although there are specific locations that their densities are high (Carstensen, Laudien, Leese, Arntz, & Held, 2009). There are several exogenous factors such as suitable temperatures, salinities, dissolved oxygen and availability of food, which determine the presence and densities of surf clam distribution (Yu et al., 2009).

Surf clams positively influence the sustainability of coastal ecosystems in several ways (Brown & McLachlan, 2002). As an example for their ecological effects, Hashizume et al. (2011) stress that surf clams' feeding activities significantly contribute to material cycling between the biogeographic region of sea and the water column. Their ecological importance indicates the requirement of sustainability concerns under the use of surf clams for commercial purposes (Odell, Mather, & Muth, 2005). Their autecology is specifically essential to the sustainable management of shallow coastal areas vulnerable to anthropogenic environmental degradation (Hashizume et al., 2011). Furthermore, they play a vital role in the food chain of coastal ecosystem by being prey for some gastropods, fish, birds and mammals (Ansell, 1969; Carstensen et al., 2009).

Having discussed the taxonomic ranks, other biological facts and the importance of surf clams to marine ecosystems in brief, the next section discusses the life history of surf clam.

2.2 Life history

This section discusses reproductive physiology and the phases of surf clam development: spawning, eggs, larvae, juveniles and adults. The stages of development induce different habitat characteristics and have found to be influenced

by food availability, ambient salinity and more significantly, temperature (Cargnelli et al., 1999). Identification of those stages is mandatory to the current study to understand histological techniques related to studies in reproductive biology.

2.2.1 Reproductive cycle of surf clams

Identification of those stages is mandatory to the current study to

As mentioned in section 2.1, male and female surf clams are identical in external appearance and hence the examination of gonads histologically is used to identify their sex. The gonad is positioned at the top of the muscular foot and extends into the visceral mass as gametogenesis progresses (Drummond, 2006). Ropes (1968) grouped the reproductive cycle of both male and female into five stages based on maturity. These five stages are named from 1-5 and cover the resting, early active, late active, ripe, partially spent and spent phases (Ropes, 1968).

Ropes (1968) finds that oogonia can be seen at the periphery and entrenched in the alveolar walls at the early phase of the reproductive cycle of female surf clams. The nuclei of oogonia are round and oval, and contain basophilic nucleoli at this stage. Ovocytes have almost round and oval nuclei similar to oogonia and contain basophilic nucleoli. As a difference between oogonia and ovocytes, nucleoplasm is thin and irregular shaped in oogonia whereas ovocytes has almost homogeneous nucleoplasm (Fay et al, 1983). At late active phase, ovocytes become larger than the size at the early active phase and nucleoplasm is granular regularly (Ropes, 1968). The next stage is the ripe phase. Ropes (1968) identifies that the vitelline membrane of surf female clams is comprised of cytoplasm and a large nucleus. Ovocytes can be seen free in the lumina of the alveoli (Fay et al, 1983). In partially spawned female clams, A few, but large and ripe ovocytes are present in the lumina of some alveoli while other alveoli do not contain any ripe ovocytes (Ropes, 1968). In the spent phase of female surf clams, the open lumina of alveoli typically become empty from ripe ovocytes (Fay et al, 1983; Ropes, 1968).

At the early active phase of male surf clams, spermatogonia can be seen in alveolar walls as dark stains. Ropes (1968) illustrates that the sizes of spermatocytes vary between 3-4 μ in diameter at the early active stage. These spermatocytes arrange to a

single column and the density of cells increases towards the lumina (Fay et al, 1983). The round and oval nuclei of spermatocytes are surrounded by cytoplasm thin and irregular in their shape. The sizes of spermatocytes are between 2 and 3 μ in diameter at the late active phase and the nuclei are almost filled with vast amount of dark basophilic chromatin threads (Ropes, 1968). At the late active stage, spermatids are transformed and consequently, sperms can be seen in the lumina (Fay et al, 1983; Ropes, 1968). When sperms are matured at the ripe phase, they form into dense masses in the alveoli of male surf clams. Ropes (1968) finds that the alveoli of spent male clams have no or few spermatozoa only. Near spawning, both spermatogonia and primary spermatocytes develop along the basal membrane, which is a similar behaviour to oogonia and early ovocytes (Fay et al, 1983).

In addition to the five stages described above, there is a period where the clam is at rest after stage 5 or has immature gonads, in which both cases the sex cannot be distinguished. This stage is labelled 'Stage 0' as evidenced in other maturity scales (Drummond et al., 2006). Histological characteristics of the reproductive cycles from Stage 0-5 in surf clams are tabulated under the histological techniques which are described in section 3.2.5.

Both exogenous and endogenous factors influence the reproductive activities of surf clams (Hashizume et al., 2011; Mid-Atlantic Fishery Management Council, 2010). Temperature, salinity, light and food are among the environmental factors that has been described in literature (Drummond et al., 2006; Grant & Creese, 1995; Hashizume, Tatarazako, Kohata, Nakamura, & Morita, 2012; Joaquim, Matias, Lopes, Arnold, & Gaspar, 2008a). The Review of literature indicates that there has been in recent years, a focus on finding correlations between the reproductive mechanism and some of the environmental parameters such as temperature and salinity (Drummond et al., 2006; Grant & Creese, 1995; Hashizume et al., 2011; Joaquim et al., 2008a). Although the mechanism between these factors and reproductive activities are not well known, from research investigations done on *Ruditapes philippinarum*, Drummond et al. (2006) emphasise that temperature and salinity influence the seasonal variations of reproductive activities of surf clam species.

2.3 Development of surf clams

Under this section, development of surf clam is discussed with particular emphasis on the influence of exogenous factors. This section discusses about the development of surf clams in terms of global perspectives due to the presence of research gaps in NZ context.

During the period of spawning, the passage of water for respiration and digestion is the mode in which eggs or sperm are carried into the surrounding waters (Ropes, 1986). Water is drawn through the incurrent siphon and after reaching the gills it passes across the genital papillae, leaving via the excurrent siphon. Rhythmic contractions of the adductor muscle often accompany spawning, and this pulls and releases the two valves together. Spermatozoa are mature and functional before spawning has occurred, as meiosis takes place within the gonads. Conversely, oocytes mature after they are released into the water and post-fertilization; after the gametes unite externally in the water (Ropes, 1980; 1986).

Eggs

Unfertilized eggs are 56µm and the germinal vesicle within is approximately 31µm in diameter (Ropes, 1980). Cargnelli et al. (1999) have identified optimal gamete concentrations of $0.8-4 \times 10^6$ sperm/ml and $5-30 \times 10^3$ eggs/ml for fertilization, under laboratory conditions. Optimal fertilization temperatures depend on the type of species. For example, Atlantic surf clams' optimal temperature ranges from 6-24 °C (Cargnelli et al. (1999). Additional environmental conditions for fertilization included salinities of 20-35 ppt (parts per thousand) and a pH of 7.8-10. Hypo- and hypertonicity was found to cause pathologies within the developing clam such as hermaphroditism. After the sperm penetrates the egg, the polar bodies are extruded within 70 minutes and cleavage of the fertilized egg can begin (Ropes, 1980). Time taken for the developmental process was found dependent on water temperature (Cargnelli et al. (1999). Ropes (1980) observed the first, second and third cleavages after 70, 90 and 110 minutes respectively at an average water temperature of 21.7 °C for Atlantic surf clam. Cell division hereafter occurred very rapidly, with swimming gastrulae found from 5.25 hours post-fertilization.

Larvae

There are three larval stages in the development cycle of surf clams and these are trochophore (planktonic), veliger (bivalve shell present), pediveliger (transitional swimming-crawling stage) (Cargnelli et al., 1999). Planktonic trochophores are pyramid-shaped larvae appearing around nine hours post-fertilization, at 21.7°C (Cargnelli et al., 1999). Lower temperatures cause delayed larval development such that trochophores in 14°C water do not appear until after 40 hours (Loosanoff & Davis, 1963). The next larval stage is 'veliger larvae' which is the first larval stage to have a bivalved shell, becoming an immature free-living form, and occurring after 19-20 hours from fertilization (Cargnelli et al., 1999). At this D-shaped or straight-hinged veliger stage the following can be identified: hinge, retractor muscle, anus, stomach and gut, velum fringed with cilia, and apical flagellum. Larvae use their cilia and flagellum at this stage for locomotion during feeding until they settle and are transformed into juveniles (Ropes, 1980). The final transition of the larvae is called the pediveliger stage which involves the development of a foot for burrowing (Fay *et. al*, 1983). Additionally is the development of the anterior and posterior adductor muscles, mesoderm and the digestive gland. Pediveligers are seen around 18 days after fertilization, at 21.7°C (Cargnelli et al., 1999). Time taken for transition to this stage is also longer at lower temperatures, such that the transformation occurs after 35 days at 14 °C, versus only 19 days at a warmer 22 °C (Cargnelli et al., 1999). Cargnelli (1999) further mention other activities that water temperature influences at the larval stage such as tolerance and mortality.

Juveniles

The movement juvenile clams make is called 'leaping' and is undertaken by forcing their foot against the substrate in which they live (Merrill, 1973 as cited by Fay et al. (1983). However, juveniles do not prefer to move frequently unless they are disturbed by predators or investigators and tend to settle at the same burrow after the disturbances (Fay et al., 1983). Merrill (1973) proposes that these movements are do not influence their overall settlement or distribution patterns (as cited by Fay et al. (1983)). Fay et al. (1983) explain that juveniles are transformed into adults at the age of 1-2 years, but the reviews of literature indicate that their maturity is localized (Cargnelli et al., 1999). According to Cargnelli et al. (1999), Atlantic surf clams in

New Jersey waters reach their sexual maturity as early as about three months old and less than 5mm in length. Ropes (1979) mentions that size is more important than age in determining the degree of sexual maturity in Virginia, U.S.A., where surf clams typically reach their adulthood at 45mm length (as cited by Cargnelli et al. (1999)). In Prince Edward Island, Canada, surf clams spend longer as juveniles and gain their maturity as late as 4-years old and 80-95 mm in length (Sephton & Bryan, 1990 as cited by Cargnelli et al. (1999)). Therefore, the life span of juveniles and adults is not distinctly separated without geographical location being considered, but is rather demarcated with the onset of sexual maturity (Cargnelli et al., 1999).

Adults

Adult surf clams mostly live well below the surface of the substrate (Fay et al., 1983; Ropes, 1980). Adult clams prefer to burrow in sand and gravel substrate, although they can also be found in silty to fine sand (Cargnelli et al., 1999). The habitats of adult surf clams vary from substrates of loose and fairly distributed aggregates to localized or patchy, dense fields (Fay *et al.*, 1983). According to Cargnelli *et al.*, (1999), adult or juveniles surf clams do not live in mud. Low salinity limits the abundance of surf clams in estuarine zones (Ropes, 1980). Despite the differences in substrate of which surf clam can live, Cargnelli et al. (1999) infer that their growth rate is not influenced by substrate types. Cargnelli (1999) also found surf clams to grow similarly offshore and inshore during the first 3-5 years, but the growth rate of inshore clams became less than offshore namely due to inter-tidal differences and changes in food availability. Several studies (Armstrong, 1965; Ropes & Merrill, 1970; and Ropes and Merrill, 1973; Fay et al. 1983) highlight that adult surf clams have no or little voluntary movements, similar to that of juveniles.

As mentioned previously, the length of adult surf clams can differ based on their geographical location. Westman and Bidwell (1946) find surf clams have average specimen length of 163mm at the age of 17; Loesch and Ropes (1977) discover that surf clams are only 147mm in length at age of 20 years while Jones et al. (1978) find a specimen average of 164mm for surf clams of 25-year-old (as cited in Fay et al.(1983)). Cargnelli et al.(1999) summarized the maximum length to be up to 226mm and a maximum longevity of 37 years old. Growth rate and maximum length were also found to be dependent of population densities (Cargnelli et al., 1999).

Higher population densities appear to lower the growth rate and the maximum length. There is a positive correlation between temperatures and growth rate, however an optimal range likely exists for different species (Cargnelli et al., 1999).

2.4 Distribution of surf clams throughout the world

As mentioned in section 2.1, surf clams are distributed all over the world except in the Polar Regions. Several environmental factors such as temperature, salinity, dissolved oxygen and availability of food as discussed can determine the density of their distribution (Yu et al., 2009).

Hare and Weinberg(2005) mention that Atlantic surf clams are abundant in the western North Atlantic region between the Gulf of St. Lawrence and the Gulf of Mexico where they are better able to burrow in sandy sediments. In this region, there are two kinds of sub-species distributed and these are *Spisula solidissima similis* (the region of the south of Cape Hatters) and *Spisula raveneli* (Hare & Weinberg, 2005). In the US, George Bank, the Delmarva Peninsula, southern New Jersey and off Long Island are the most abundant areas for Atlantic surf clams(Hare & Weinberg, 2005). Furthermore, Cargnelli et al.(1999)find that their distribution over the depth depends on the habitat location. Surf clams of the surf zones along Long Island and New Jersey are densely populated at depths below 18 m, whereas the concentration is at the highest from 18 to 36 m in the Delmarva Peninsula (Ropes, 1978 as cited in Cargnelli et al. (1999)).

In South Africa, the presence of blooms of diatoms on the southeast coast and high coastal phytoplankton production on the west coast increase the abundance of surf clams (Soares, Schlacher, & McLachlan, 1997). This is due to the upwelling process whereby deep and often nutrient-rich, cold water rises to the surface and then this surface water moves offshore. The ecosystems of sandy beaches influence not only the abundance of surf clams, but also the dominant type of surf clams species in South Africa (Brown et al. 1989 as cited by Soares et al. (1997)).

Similarly, surf clams are distributed along the west coast of South American continent from northern Chile to northern Ecuador which is the area associated with Humboldt Current Upwelling System (Carstensen et al., 2009). In addition, Surf clams are inhabitants in coastal areas of the eastern Asia, especially in Japan (Hashizume et al., 2011) and Korea(Yu et al., 2009). Similar to other parts of the

world, surf clams are abundant in New Zealand (Ministry for Primary Industries, 2012). A detailed review of literature on the distribution of surf clams is provided in section 2.6.1.

2.5 Commercial fishery of surf clams

In the east coast of North America, surf clams were used as a source of food from as far back as the 17th century (Parker, 1971 and Yancy, 1970 as cited in Fay et al. (1983)). Before the Second World War, surf clam harvesting was limited for traditional techniques only such as the use of manually operated rakes and tongs, but hydraulic dredging started between 1950's and 60's. Surf clams play a vital role in local economies of the regions where these organisms are abundant. Some of the examples include New York, New Jersey, Delaware, Maryland, and Virginia (Fay et al., 1983), the west coast of Korea (Yu et al., 2009) and the west coast of South America (Carstensen et al., 2009). On the other hand, there are consequences to marine ecosystems due to harvesting. Unsustainable use of these environments causes increased beach erosion, UV radiation and global warming (Brown & McLachlan, 2002). Since many coastal properties are not owned by the government or privately, making regulations is the only option left for authorities to manage surf clam harvesting (Litz, 1994).

Management processes associated with surf clam harvesting seem to be heuristic, and amendments should be made accordingly to former regulations. For example, the Mid-Atlantic Fishery Management Council revises quota specifications for Atlantic surf clam harvesting at three-year intervals to ensure regulations are pragmatic (Mid-Atlantic Fishery Management Council, 2010). According to the report on quota specification for 2011/12/13 by the Mid-Atlantic Fishery Management Council (2010), the characteristics (such as proportion of biomass for sub-species) of surf clams can significantly fluctuate, and hence new regulations that are stricter are considered mandatory.

Similarly, surf clam harvesting is managed in other countries to ensure minimum influences to marine ecosystems. Harvesting of *Ruditapes philippinarum*, *Macra veneriformis* and *Macra chinensis* in Tokyo Bay is regulated under the sustainability concerns of the Japanese government and citizens (Hashizume et al., 2011). The

efforts taken are supposed to minimise the consequences of overfishing towards migrant birds in the area. A quota system is widely used in United States (US), but there are other techniques, such as specified spacing on hydraulic dredges, which are used in countries such as Italy and Japan (H J Cranfield & Michael, 2001b). Aburto and Stotza (2012) stress considering the high variability of surf clam recruitment locally, before management strategies are regulated. This is if there can be negative consequences to the environment from regulations such as in Chile where copy-paste models resulted to misuse of about 60 species of fauna and algae (Aburto & Stotza, 2012). Existing management strategies related to NZ context are discussed in detail in section 2.7.1.

2.6 Surf clams in NZ

NZ fisheries have recognized seven kinds of sub-tidal surf clams (Ministry for Primary Industries, 2012). These are *Paphies donacina* (PDO), *Crassula aequilatera* or *Spisula aequilatera* (SAE), *Mactra discors* (MDI), *Mactra murchisoni* (MMI), *Dosinia anus* (DAN), *Dosinia subrosea* (DSU) and *Bassina yatei* (BYA). From these seven species, the Ministry of Primary Industries has paid more attention recently to four species: DAN (the family of Veneridae), MMI and SAE (both belonging to the family of Mactridae) and PDO (the family of Mesodesmatidae) (Ministry for Primary Industries, 2012). Surf clams in NZ can be found in surf zones of sandy beaches and immediately beyond those beaches (Ministry for Primary Industries, 2012). The Ministry of Primary Industries (2012) found that different species of surf clams are distributed within distinct depth zones as described below.

2.6.1 Distribution of surf clams in NZ

There are two major studies (Cranfield & Michael, 2001b and Haddon, Willis, Wear & Anderlini, 1996) which focus on evaluating the distribution of surf clams in NZ.

Cranfield and Michael (2001b) investigated the depth that each species of surf clam can be caught. According to their findings, surf clams are inhabitants from shallow water to a depth of 10 m. Their investigation showed that the depth range was a variable of location as well as the type of species (Table 2-1). These depths can become important considerations for commercial use of surf clams since depth ranges can influence the method of harvest and hence the cost of fishing.

Table 2-1: Changing depth range for different species of surf clams in the North and South Islands, NZ (Cranfield & Michael, 2001b)

Species	Optimal depth range (m)	North Island depth range (m)	South Island depth range (m)
<i>Paphies donacina</i> (PDO)	2-3	2-4	2-4
<i>Spisula aequilatera</i> (SAE)	3-7	3-5	4-8
<i>Mactra murchisoni</i> (MMI)	4-8	4	5-6
<i>Mactra discors</i> (MDI)	3-7	4-6	3-7
<i>Dosinia anus</i> (DAN)	4-10	5-8	6-10
<i>Dosinia subrosea</i> (DSU)	6-10	6-10	5-8
<i>Bassina yatei</i> (BYA)	6-9	6-9	6-9

From Table 2-1, every type of surf clam in NZ can be caught at depths less than 10 m. Mostly, depth ranges of the North and South Islands are within the optimal depth ranges, except for *Paphies donacina* (PDO) in both islands and *Spisula aequilatera* (SAE) in the South Island. Depth ranges for the species are remarkably different for the North and South Islands for *Spisula aequilatera* (3-5 m versus 4-8 m, correspondingly), *Dosinia anus* (5-8 m versus 6-10 m, correspondingly) and *Dosinia subrosea* (6-10 m versus 5-8 m, correspondingly). Haddon et al. (1996) categorize surf clams into two groups based on their abundant depths: shallow water species (PDO and SAE) and a deeper water group (MMI, MDI and DAN). One of the important findings of their investigation was that the size of the shallow water group becomes smaller towards the shore, whereas the remaining group becomes larger when they are distributed towards the shoreline.

In addition, Cranfield and Michael (2001b) investigated the zonation of surf clams with respect to the depth of water around NZ by investigating a 450 m-wide strip in 16 locations through a stratified random survey. The locations relevant to the survey are depicted in Figure 1-1 in accordance with Cranfield and Michael (2001b). Based on the percentages of biomass at different locations, this study recognized the most abundant area of surf clams in NZ (marked in Figure 6). This area includes Pegasus Bay, Cloudy Bay, Waitarere, Otaki and Peka Peka.

According to the biomass survey, there are three families of surf clams identified as dominant in different regions of NZ. These dominant families are venerid clams in

northern NZ, mactrids in central and southern beaches, and mesodesmatid in central beaches. Cranfield and Michael (2001b) found that *Macraa murchisoni*, *Macraa discors* and *Spisula aequilatera* (the family of mactrids) were most abundant in the South Island, whereas *Dosinia anus* (the family of venerid) were abundant at all locations in the North Island. More specifically, the majority of surf clam biomass in northern locations (Great Exhibition Bay, Te Arai and Matakana) was comprised of *Dosinia anus* (DAN) and *Dosinia subrosea* (DSU). At Ohope, 30% of the biomass was comprised of *Spisula aequilatera* (SAE). An investigation by Cranfield and Michael (2001b) stressed Ohope as an important location for the distribution of surf clams in NZ since the biomass is dominated by mactrids and mesodesmatid to the south of Ohope. Location 5, Nahuka, was a major habitat for *Paphies donacina* (PDO; that belongs to the family of mesodesmatid). Their habitation expands from Nahuka to the East Coast and south to the Kapiti coast in the North Island, and to Cloudy Bay and Pegasus Bay in the South Island. In the rest of the locations, Blueskin Bay, Te Waewae and Oreti (locations in Southland), 80% of the biomass of surf clams included *Macraa discors* (MDI) and *Macraa murchisoni* (MMI) (Cranfield & Michael, 2001b). In this study, there was no indication given to the most abundant region for *Bassina yatei* (BYA).

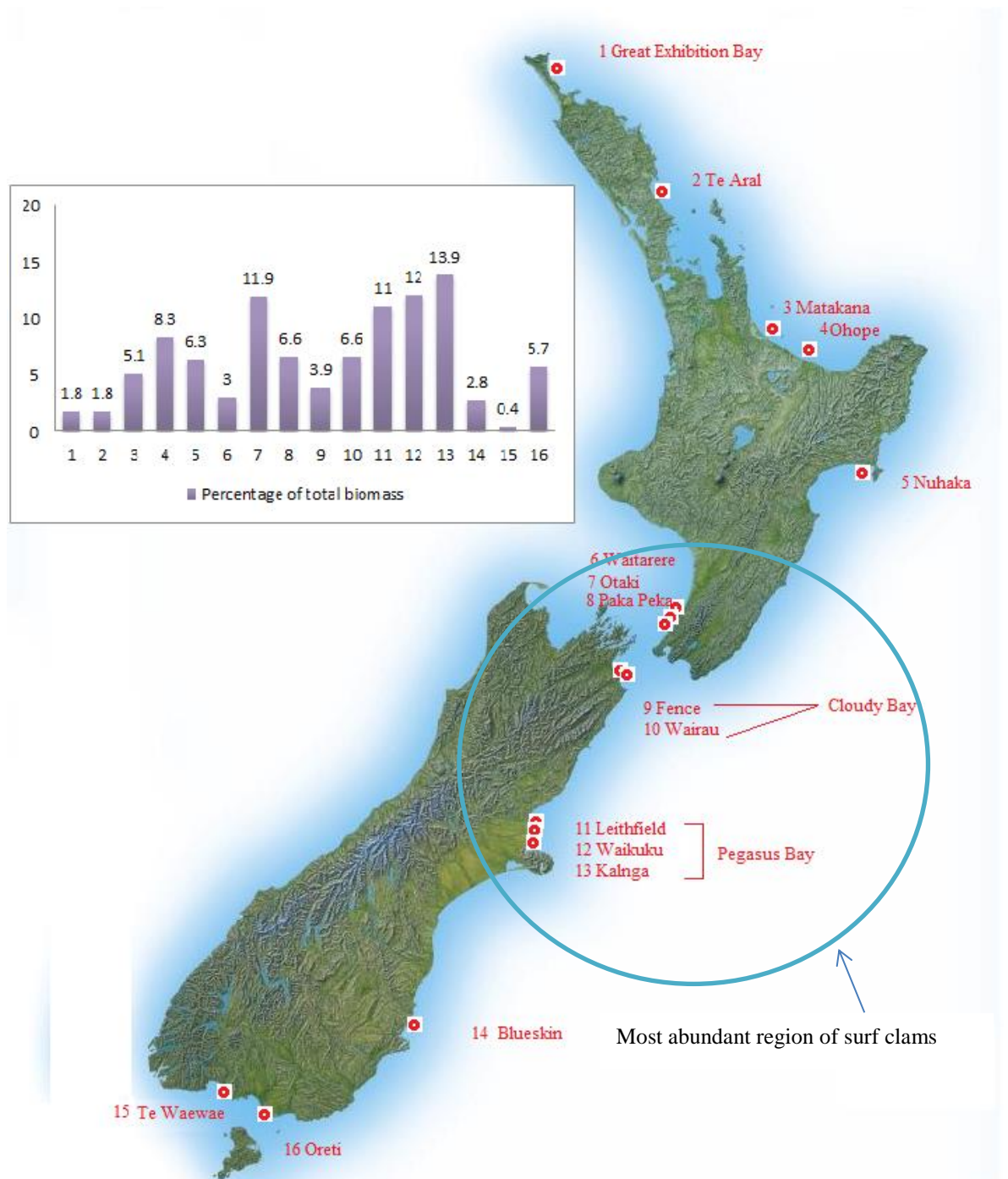


Figure 1-1: 16 sites where biomasses of surf clams are estimated for 450 m wide strips (Cranfield & Michael, 2001b)

2.6.1.1 Surf clams species in Cloudy Bay

Cloudy Bay is the point of interest for many studies (Cranfield & Michael, 2001a; Haddon et al., 1996; Ministry for Primary Industries, 2012) focusing on NZ surf clams. Cloudy Bay consists of mudflats and sand flats, and Diamond shell (*Spisula aequilatera*), Moon shell (*Dosinia anus*) and TuaTua shell (*Paphies donacina*) are

the most suited surf clam species to grow in Cloudy Bay (Cranfield & Michael, 2001b).

Spisula aequilatera is surveyed mainly at two locations in NZ and which are Cloudy Bay and Kapiti Coast (Cranfield & Michael, 2001b). According to the growth rate estimation (von Bertalanffy parameters (k and L_{∞})), Diamond shells are the fastest growing surf clam species in NZ (Cranfield & Michael, 2001a). A systematic and stratified survey done in 2012 that focused on the Manawatu coastal area found that the highest biomass of surf clam species in NZ is *Spisula aequilatera* (Ministry for Primary Industries, 2012). The estimate of biomass of diamond shell is 7992.6 tons. According to the biomass survey done in 2012, the estimates quantity of *Dosinia anus* in NZ is about 3498.1 tonnes (Ministry for Primary Industries, 2012). The remaining species, Tua Tua (*Paphies donacina*), is estimated to weigh up to 3289.5 tonnes in NZ. In detail descriptions for Diamond Shell, Moon Shell and TuaTua are separately added at the beginning of the Chapters 3, 4 and 5 respectively.

2.6.2 Growth rates of surf clams in NZ and influencing factors

In addition to the distribution, the growth rates of surf clam species were investigated in a NZ context. Von Bertalanffy parameters, k and L_{∞} , were used as estimates of growth rates for surf clams in NZ by Cranfield and Michael (2001a). They found that the parameters of growth rates were dependent on the species and habitats. The comparison of five types of surf clams is given in Table 2-2 for two locations (Kapiti Coast in the North Island and Cloudy Bay in the South Island).

Table 2-2: Estimates of von Bertalanffy parameters (k and L_{∞}) for five species of surf clams in Kapiti Coast and Cloudy Bay (Cranfield et.al (1996) as cited in Cranfield and Michael(2001a))

	<i>P.donacina</i>		<i>S.aequilatera</i>		<i>M.murchisoni</i>		<i>M.discors</i>		<i>D. anus</i>	
	k	L_{∞}	k	L_{∞}	K	L_{∞}	k	L_{∞}	k	L_{∞}
Length frequency										
Kapiti Coast	-	-	0.80	52.1	0.60	72.3	0.35	60.1	-	-
Cloudy Bay	0.33	94.1	1.01	60.3	0.57	88.0	0.41	68.0	-	-
Mark-recapture										
Kapiti Coast	-	-	1.44	54.6	1.84	72.4	-	-	0.53	53.0
Cloudy Bay	0.35	84.8	1.74	57.6	0.58	80.6	0.54	61.5	0.36	61.6

In Table 2-2, growth rates are estimated by two methods: length frequencies and mark-recapture. In Cranfield and Michael's (2001a) experiment, length frequencies and incremental growth were calculated by two computer programs: MULTIFAN and GROTAG. Their findings showed that expected increments of growth for surf clams

give different as well as similar observations for the two computer programs, as discussed below.

In GROTAG, there was no dependency towards expected increments of growth from location, but MULTIFAN indicated that expected growth rate for the same species was greater at Cloudy Bay than on the Kapiti Coast. There were different observations compared to the type of surf clams according to Table 2-2. For example, according to the k values of *Spisula aequilatera* and *Macra discors*, both species grow to their asymptotic size faster in Cloudy Bay than on the Kapiti Coast, but k values for *Macra murchisoni* indicated that the growth rate was relatively similar (Cranfield & Michael, 2001a). The k values calculated by GROTAG for *Macra discors* and *Dosinia anus* were different than for the k values for the other three species using MULTIFAN. According to the k values of GROTAG, both species grow to their asymptotic size faster on the Kapiti Coast in than at Cloudy Bay.

A similar observation for both programs was observed for the five species of surf clams where asymptotic sizes (L_{∞} values) were larger in the South Island (Cloudy Bay) than in the North Island (Kapiti Coast).

As one of the concluding remarks, Cranfield and Michael (2001a) stressed that the interpretation from MULTIFAN was more reliable than GROTAG due to the two different sampling procedures the two methods are associated with. In MULTIFAN, both samples were taken over the same period, but in GROTAG sampling was done over different years (Cranfield & Michael, 2001a). Thus they conclude that two (*Spisula aequilatera* and *Macra discors*) out of the five species had faster growth rates in the South Island, while all the species gained greater asymptotic sizes in the South Island. Therefore, the South Island can be considered more suitable than the North Island for the commercial fishing of surf clams.

Apart from the above findings about growth rates, the mark-recapture experiments done by Cranfield and Michael (2001a) indicated the importance of considering the effects of water depth and variations of individual growth as such consideration will make the results more comprehensive. Cranfield and Michael (2001a) hypothesized that these differences between the locations and species could have been environmental or genetic.

Typically, the coastal waters of the South Island have temperatures 2-3 °C cooler than the North Island (Uddstrom & Oien, 1999). Therefore, the sexual maturity of surf clams can be delayed in the South Island, so that ultimately the growth rate of juveniles remains more typical for a longer time. As the second hypothesis, Cranfield and Michael (2001a) postulated that lower temperatures in the South Island could have influenced the dominant surf species in each surf zone. However, they stressed that the lack of data on sexual maturity and composition of surf diatoms did not allow for testing these hypothetical constructs. Therefore, the current study believes that its findings could minimize these research gaps in the ambit of surf clams.

The second potential reason for the differences between k and L_{∞} values is related to genetics. It is known that gene flow at the larval phase for surf clams, like most marine organisms is restricted between the North and South Islands, as well as within central New Zealand, due to two current systems: Tasman Front and the Subtropical Front (Cranfield & Michael, 2001a).

Having recognized the distribution patterns and the influencing factors towards the growth rates of NZ surf clam species, the next section describes surf clam abundance in Cloudy Bay, NZ.

2.7 Harvesting of surf clams in NZ

Surf clam harvesting is done in NZ for recreational, traditional and commercial purposes (Beentjes & Baird, 2004). The history of recreational and traditional fisheries can be traced back to many years ago (Cranfield & Michael, 2001b). Both traditional and recreational surf clam harvestings were done by hand held tools, which made the species *Paphies ventricosa* (Toheroa) and *Paphies subtriangulata* (intertidal Tua Tua) the most known species under traditional and recreational purposes (Haddon et al., 1996). As described in section 1.1, the use of surf clams under commercial purposes is limited in NZ. The viability of developing surf clam harvesting has been recognized recently in economic perspectives (www.3news.co.nz, 2013; www.fish.govt.nz, 2010). The former acting Minister of Fisheries, Hon David Carter, says:

“Surf clam fishing is relatively new in New Zealand. The new catch limits will allow the potentially valuable surf clam fishery to develop, within stringent environmental limits” (www.fish.govt.nz, 2010).

In 1995, Foxton Bay was surveyed for the potential yield of surf clams and found that there was a biomass of 21 g/m² of surf clams (www.3news.co.nz, 2013). Recently, based on research studies done by NZ scientists, the media (www.3news.co.nz, 2013) reveals that the biomass of Foxton Bay is much higher than the estimate done in 1995. These significantly distinct estimates are possible due to the various assumptions associated with scholarly studies such as dredge efficiency and the type of sampling (Haddon et al., 1996). Having the newest estimate of the biomass of 400 g/m² in Foxton Bay, NZ researchers identified the potential of surf clam harvesting to be among the top five NZ fisheries contributing to total exports up to \$ 200-300 M/year, up from the current earnings of \$ 10 M/year(www.3news.co.nz, 2013).

2.7.1 Management of surf clams in NZ

The seven species of surf clams are managed through a quota management system introduced in 2004 in NZ(Ministry of Fisheries, 2011). In addition to the authorities of NZ fisheries, there are other authorities such as New Zealand Food safety Authority (NZFSA) and the District Health Board Public Health Units. These organisations investigate catchment areas comprehensively and regularly for pollution and the microbiology of surf clams(Ministry of Fisheries, 2011).

With the identification of the high potential to develop surf clam harvesting as a trade source of export(www.3news.co.nz, 2013; www.fish.govt.nz, 2010), the current quota allocations of surf clam harvesting is under consideration to be increased(Ministry for Primary Industries, 2012). Table 2-3 shows the current allocations and the limits to be increased in future only for FMA8.

Table 2-3: The current and future allowances for surf clam harvesting in FMA8(Ministry for Primary Industries, 2012)

Stock	Total Allowable Catch (TAC) (t)	Customary allowances (t)	Recreational allowances (t)	Total Allowable Commercial Catch (TACC) (t)	Other Sources of fishing related mortality (t)
<i>Paphies donacina</i>					
Current	19	9	9	1	0
Suggested	296	9	9	263	15
<i>Spisula aequilatera</i>					
Current	8	-	-	8	0
Suggested	1821	-	-	1730	91
<i>Macra murchisoni</i>					
Current	25	-	-	25	0
Suggested	631	-	-	599	32
<i>Dosinia anus</i>					
Current	33	-	-	33	0
Suggested	236	-	-	224	12

The Ministry of Fisheries is most concerned about harvesting diamond shells (*Spisula aequilatera*), with the Total Allowable Catch (TAC) and Total Allowable Commercial Catch (TACC) supposedly increased to 1821 t and 1730 t respectively from the current limitation of 8 tonnes. The second highest increment is for *Macra murchisoni*, but this is outside of the scope of the current study. *Paphies donacina* and *Dosinia anus* are to be increased up to TACC of 263 t and 224 t respectively from the correspondent current allowable commercial harvesting limits of one and 33 t. Thus, for the four species of surf clams, the Ministry of Fisheries provides a total of 2984 tonnes additional TAC under the revised regulations for FMA 8 only which may be implemented in future(Ministry for Primary Industries, 2012). The above suggestions on the TAC and TACC limits are based on a biomass survey done in October-November, 2012, in Manawatu coast. The resulting estimates are done by using suitable values of coefficient of variations (Ministry for Primary Industries, 2012). The Ministry estimates a total biomass of 18384 tonnes of surf clams for the four species.

The barrier to increase the annual yield of surf clams is the government regulations on Total Allowable Commercial Catch (Ministry for Primary Industries, 2012; Ministry of Fisheries, 2011; www.3news.co.nz, 2013). These limitations are conservative (Ministry of Fisheries, 2011). NZ researchers as well as the authorities stress that there is a crucial need to increase funding on researches of surf clam

harvesting before future amendments are done (Ministry for Primary Industries, 2012; www.3news.co.nz, 2013). Therefore, the significance of the current study to investigate the reproductive biology of NZ surf clams is reinforced in the review of literature.

2.7.2 Significance of gametogenesis and gonadal development researches

The emphases of past studies on NZ surf clams are limited to a few areas only such as the distribution and abundance (Cranfield et al., 1994; Haddon et al. 1996; Triantafillos, 2008) or growth rates and settlement (Cranfield and Michael, 2001; Conroy et al., 1993). The gametogenesis and gonadal development of NZ clams are not investigated adequately (Grant & Creese, 1995).

Comparatively, the research history of Atlantic surf clams is deep-rooted as mentioned in section 2.2. Ropes (1968) identifies five stages of the reproductive cycles for both male and female by investigating Atlantic surf clams. Furthermore, Ropes (1968) and Fay et al. (1983) discuss the variations of male and female gonads with respect to the five stage of their reproductive cycle. In addition, research studies (Ropes, 1980; Cargnelli et al., 1999) are done to identify exogenous factors influencing the early development of Atlantic surf clams. In the management process of Atlantic surf clam fisheries, which is regulated in accordance with the Fishery Management Plan and the National Standards of the Magnuson Act, life cycle assessments of clams are required for the conservation of those species (Mid-Atlantic Fishery Management Council, 2010). The Mid-Atlantic Fishery Management Council (2010) stresses the importance of life history assessments which ensure the right composition of distinct age classes. These investigations are considered thoroughly before annual allowable catch limits are set for commercial harvesting. The life history assessments of surf clams become more important in Atlantic region since the dramatic reduction of biomass was recognized in New Jersey inshore area and off the coast of the Delmarva Peninsula in 2000s (Mid-Atlantic Fishery Management Council, 2010). Consequently, the amount of industry sponsored research studies was increased. Reproduction, survival, limit distribution, growth and abundance are few concerns related to the life history assessments of Atlantic surf clams (Mid-Atlantic Fishery Management Council, 2010).

Likewise, Joaquim, Matias, Lopes, Arnold and Gaspar (2008b) find the importance of knowledge area in the context of the reproductive cycle of white clams (*Spisula solida*) as a fundamental to improve management strategies and to develop restocking programs in Ireland. The study is conducted to minimize the consequences of intense harvesting, which causes significant inter annual variations in biomass stock and periodic recruitment failure (Joaquim et al., 2008). Other examples of investigating the seasonality of the reproductive systems include: Beninger and Stephan (1985) focusing on *Tapes decussatus* and *Tapes philippinarum*, Gaspar and Monteiro (1998) investigating razor clam (*Ensis siliqua*) and the clam *Venus striatula* in Portugal as well as Gaspar, Ferreira and Monteiro (1999) evaluating *Donax trunculus* in southern Portugal.

There are a few studies (Booth, 1983; Hooker and Creese, 1995; Rapson, 1952; Redfearn, 1974 & Gribben, 2005) which are specific to investigate gametogenic development in NZ. However, there is scepticism about the applicability of past research. Grant and Creese (1995) emphasis that the reproductive cycle of NZ surf clams are investigated to a minimal degree, which indicates that early scholarly studies are inadequate to ensure sustainability of NZ surf clams. Further explaining the limitations, Booth (1983), Rapson (1952) and Redfearn (1974) uses only Condition Index as the methodology to assess the reproductive life cycle of bivalve species. Grant and Creese (1995) stress that the condition index can imply the spawning time, but still an insufficient measure to describe gametogenic development. They articulate the importance of integrating gonad indices to comprehend investigations of reproductive cycles.

Histology of gonad section is first examined in NZ context by Dickie (1986) for *Paphies australis* and another study is done by Grant and Creese (1995) for tautau. Similar methodology is followed by Gribben (2005) to investigate the gametogenic development and spawning of NZ razor clams, which are scientifically known as *Zenatia acinaces*. Although, these studies are based on strong methodological rigours (such as histological techniques), the points of interest are different than Cloudy Bay where commercial harvesting is done currently.

For examples, the reproductive cycle of tautau is investigated by Greenway (1981), and Grant and Creese (1995) for the west coast of the North Island and North eastern

coast respectively. Secondly, the reviews of literature shows (Grant & Creese, 1995; Joaquim et al., 2008b) that reproductive cycles of surf clams vary according to their types. Thus, it is required to investigate the reproductive cycles for other species abundant in NZ.

As the concludary remark for this section, there is a research gap in investigating the three types of surf clam species, diamond shells, moon shells and tautau, with respect to gametogenic and gonadal development in Cloudy Bay. Thus, the current study believes that the investigation of this research will help to minimize the existing gaps in the area of reproductive biology of surf clams.

2.8 Conclusion

In this chapter, the current study first reviewed literature on the biological characteristics of surf clams. The review is made up of chapters that identified different taxonomy levels, feeding behaviours, reproduction cycles and life history of surf clams. The literature review could help to identify several environmental settings that significantly influence surf clam abundance in different ecological zones. According to past studies, surf clams are distributed all over the worlds, but some locations like New Zealand, Mid-Atlantic Zone, South Africa, Japan, Korea and Chile are identified as some locations that commercial surf clam harvesting can be developed due to their higher densities in these countries. Although surf clam harvesting is not well established in New Zealand, there are other regions like Mid-Atlantic zone and Korea where the industry is managed through distinct strategies. From these reviews, the current research study recognized that behaviours of surf clams is locally constructed and hence potential consequences of surf clam fishery should be identified specific to NZ sea life. On the other hand, research studies done in NZ have recognized a significant biomass of surf clams in NZ and hence the potential to become a source of exports. The relevant authorities currently intend to increase the quota allocation of catch limits while consequences are kept to a minimum level. However, without proper surveys on biological characteristics and potential significant impacts to other species and local communities, the development will not be sustainable. Thus, the current study focuses on filling the gaps related to reproductive biology of diamond shells, moon shells and deep water tautau in Cloudy Bay.

Triangle shell (*Spisula aequilatera*)



3 Triangle shell (*Spisula aequilatera*)

3.1 Introduction

As mentioned in the literature review chapter, Diamond shells (*Spisula aequilatera*) has the highest biomass estimate of 7992.6 tons according to the stratified survey done in Manawatu coastal area (Ministry for Primary Industries, 2012). Their abundance coupled with the fastest growth rate (Table 2-2) among NZ surf clam species (Cranfield & Michael, 2001a) make *Spisula aequilatera* economically important in surf clam harvesting.

Spisula aequilatera is the most abundant between the depth of 2-3m (Cranfield & Michael, 2001b) and hence Haddon et al. (1996) categorize them as the shallow water species. However, Cranfield & Michael (2001b) found that they live up to 9m deep. In NZ coastal environments, Diamond shells are dominant in the South Island together with *Macra murchisoni* and *Macra discors* (Cranfield & Michael, 2001b). In addition, a considerable amount of Diamond shells live in some locations of the North Island such as Ohope where 30% of the total biomass is comprised of *Spisula aequilatera* (Cranfield & Michael, 2001b). Cranfield & Michael (2001b) found *Spisula aequilatera*'s most abundant depth ranges as 3-5 and 4-8 m in the North and South Islands respectively.

There are no past studies focused the reproductive biology of *Spisula aequilatera*. *Spisula aequilatera* was introduced into the Quota Management System (QMS) on 1 April 2004 with a TACC of 406 tonnes, which was increased in 2010 up to 725 tonnes based on the estimates of a biomass survey done in SAEs 2 and 3 (Ministry of Fisheries, 2011). As mentioned in section 2.7.1, the current allowances are to be further increased. The feasibility of these increments are based on researches that have made it clear that reproductive biology is one of the major concerns (Ministry of Fisheries, 2011; www.fish.govt.nz, 2010). Thus, the current study's aim is to gather histological evidence of when gametogenesis begins and the later gonadal developmental stages throughout the year. The methods, analysis, findings and discussion related to *Spisula aequilatera* are described in this chapter.

3.2 Materials and methods

This section describes research methods of the current investigation. The section begins with an introduction to the study site and progresses into the sampling procedure. These explanations are followed by environmental parameters, size and growth, histological techniques, condition index and statistical techniques which the investigations are based on. Although the materials and methods are discussed under the chapter for *Spisula aequilatera*, the same methodology is followed for the other two species.

3.2.1 Study sites

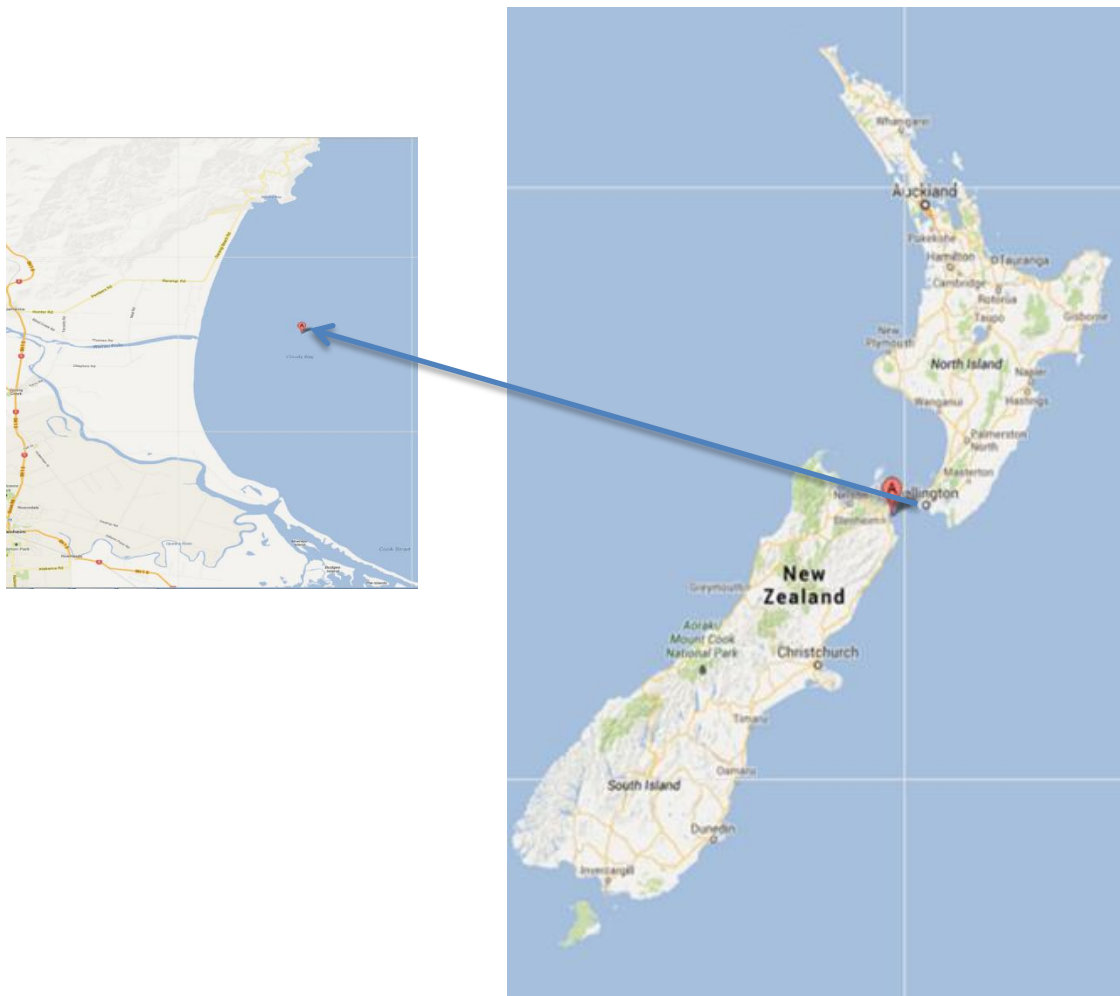


Figure 3-1: The study site of the current study: Cloudy Bay

The investigation of the current study is limited only for a single location which is Cloudy Bay (Figure 3-). The study site is a bay in North-East of New Zealand's South

Island (latitude 41° 26' 56" S and longitude 174° 06' 57" E). Cloudy Bay's habitat consists of extensive mudflats and sand flats. The Wairau and Opawa rivers feed into Cloudy Bay. It is a natural breeding for native surf clams existing in the turbulent food-rich surf zone in a depth of up to 10 metres (H J Cranfield & Michael, 2001b). As described previously, *Spisula aequilatera*, *Dosinia anus* and *Paphies donacina* are recognized as the most suited surf clam species to be grown in Cloudy Bay (H J Cranfield & Michael, 2001b). As mentioned in sections 2.6.1 and 2.6.2, Cloudy Bay is a focal point of research interests which surf clam abundance and growth rates are determined.

3.2.2 Sampling method

Approximately, 90 individuals of each species were collected each month from Cloudy Bay from August, 2012 to July, 2013. Samples were collected by hydraulic dredge, transported to a depuration plant (Cloudy Bay Clams Ltd. Facility in Blenheim) where they were stored in clean seawater at ambient temperatures until being shipped by air to the laboratory at Auckland University of Technology. The samples of *Spisula aequilatera*, *Dosinia anus* and *Paphies donacina* were collected at different depths which were of 3-5m, 5-10m and 2-4 m respectively.

3.2.3 Environmental parameters used

As mentioned in section 2.2, gametogenesis and gonadal development are influenced by many factors such as temperature (ambient and the rate of temperature change), salinity, water currents and availability of food. In the current study, only sea surface temperature was considered. The temperature used for the study was measured randomly at different locations each day at a depth of 100mm below the water surface. The mean temperatures were calculated for each month and used for the analyses of this study. The temperature variation of Cloudy Bay is described in section 3.3.1.

3.2.4 Size and growth

For each species both length and width were measured for the nearest 0.1 mm at the laboratory by using a vernier caliper. The directions that length and width were measured are illustrated in Figure 3-. Based on the sample data, relationships were investigated between length, width and weights.

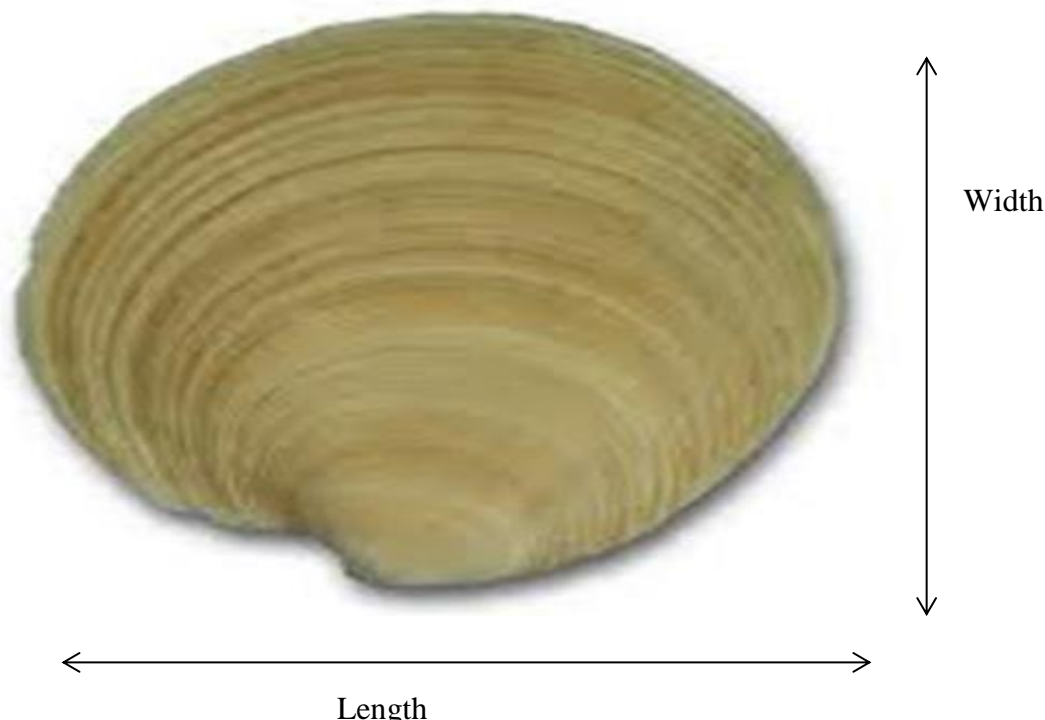


Figure 3-2: Directions of length and width measured

3.2.5 Techniques to investigate the reproductive biology

There are two main techniques used in this study: firstly, the examination of gonad and secondly the development of condition indices. The methods of the current study closely followed those of Drummond et al. (2006), where the authors used this same method to determine the reproductive biology of *Ruditapes philippinarum* from the North-West of Ireland. Since the current study employed both gonad indices and a condition index, this study is different from past NZ research studies which methodologies are based on neither gonad indices (Grant & Creese, 1995) nor a condition index (Booth, 1983; Rapson, 1952; Redfearn, 1974).

3.2.5.1 Histological techniques

The systematic procedure adopted in the current study to investigate the gonad indices follows a similar approach to many past studies (Drummond et al., 2006; Grant & Creese, 1995; Hashizume et al., 2012; Joaquim et al., 2008a).

For the gonad examination, the soft tissues of clams were removed from their shell using a knife and scalpel. The tissue at the top of the foot of the animal was longitudinally cut to provide a transverse section which contains the gonad. The section was fixed in 10% formalin in filtered seawater for 24 hours. The samples were then dehydrated in an ascending series of 70%, 90% and three series of 100%

ethanol solutions, cleared in three of xylene and infiltrated in paraffin wax. The sample was mounted into a cassette with paraffin wax to create a mounting block and then kept at 4° C until the sectioning was begun.

The samples were then sectioned at 5 µm by using a Leica RM2235 microtome. After that, these sections were placed into a water bath of 42° C. At the same time, the sections were bound to an adhesive polylysine slide from the underneath. These slides were dried in a vertical rack and then placed in an oven for about 10-15 minutes at 80° C to remove excessive amount of wax by melting off. The slides were then dehydrated and infiltrated in a descending series of ethanol solutions of 100%, 95%, 95% and 70%, followed by distilled water. The slides were stained in regressive Harris's haematoxylin and then washed with water. After that, the slides were dropped into acid alcohol and again washed with distilled water before the slides were counter-stained with eosin. These were followed by rinsing the slides with distilled water and dipping them 8 times in 2 changes of 95% ethanol. Then, the samples were dipped with a 50:50 mix of absolute alcohol and xylol. After that, two series of xylol were used to clear the samples. After the sections were dried at room temperature for about 10-15 minutes, the slides were fixed with DPX mountant for 24 hours and allowed for setting. At the end of this process, images were taken by using a Leica DM2000 microscope with 10x, 20x and 40x objectives.

Table 3-1: Description of the maturity stages for **male** surf clams, diamond shells, moon shells and deep water tuatua (Source: Drummond et al.(2006))

Development Stage	Description
Stage 0 Resting	Resting Gonad predominantly composed of connective tissue: sex not distinguishable
Stage 1 Early developing	Gonad proliferation started, many follicles with numerous follicle cells, spermatogonia centripetal to follicle walls, spermatocytes present, no spermatids or spermatozoa
Stage 2 Late developing	Spermatogonia, spermatocytes, spermatids and spermatozoa coexisted in follicles; in less developed specimens, there was no dominant cell type; in more developed specimens, the majority of the follicle was filled by spermatids and spermatozoa
Stage 3 Ripe	Follicles predominantly composed of mature spermatozoa with their tails pointing towards the centre of the lumen forming concentric bands or plugs; spermatozoa bands close to the follicle wall in very ripe specimens; follicles neat and orderly in appearance
Stage 4 Partially spent	Spermatozoa clearly visible in a swirling shape and accounting for the greatest portion of cells in the follicle; empty space in some follicles due to release of mature spermatozoa
Stage 5 Spent/Resorbing	Follicles appear broken, scattered and relatively empty; in advanced spent individuals, only residual spermatozoa found and undergoing resorption; presence of phagocytes

Under the histological techniques, the gender and reproductive stage of each surf clam were recognized. There are five stages of the maturity cycle both for male and female surf clams which is described early in section 2.2. These five stages coupled with the sexually undifferentiated stage are used to investigate the reproductive cycle of the three surf clam species in the current study. These six stages are of a modified version of the five scale maturity scale and introduced by Xie and Burnell (1994) first and used in several studies later(Drummond et al., 2006). There were situations that individuals have histological evidences for more than one development stage. Under those circumstances, the maturity stage was assigned by considering the majority of follicles the individuals contained.

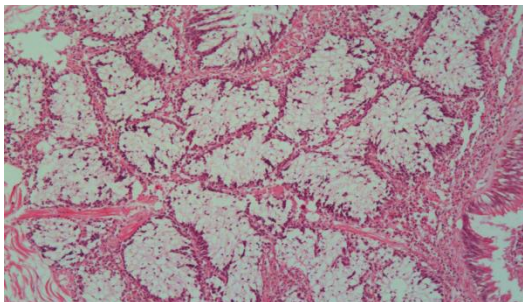
The descriptions and criteria for the six stages are provided separately for male and female reproductive cycles in Table 3-1. Respectively. The tables provide a summary and the distinct maturity stages are discussed in detail in section 2.2.

Table 3-2: Description of the maturity stages for female surf clams, *Spisula aequilatera*, *Dosinia anus* and *Paphies donacina* (Source: Drummond et al.(2006))

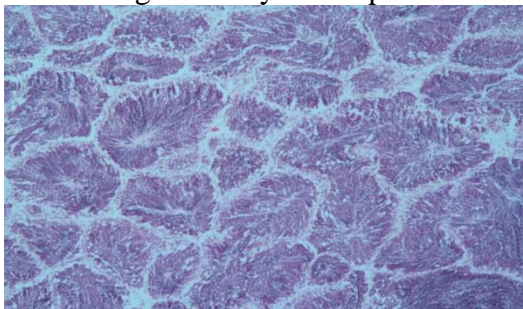
Development Stage	Description
Stage 0 Resting	Resting Gonad predominantly composed of connective tissue: sex not distinguishable
Stage 1 Early developing	Developing Gonad proliferation started; increasing numbers of discernible oocytes in follicle walls; oocytes small; no free oocytes present in the lumen.
Stage 2 Late developing	Free oocytes present in the lumen but accounting for less than half of the total oocytes present in the follicles; attached oocytes equally abundant.
Stage 3 Ripe	Gonad filling large surface area; most oocytes free in the lumen with a polygonal configuration; follicle wall thin.
Stage 4 Partially spent	Number of free oocytes per follicle reduced; some follicles empty having released their gametes; follicle walls breaking down.
Stage 5 Spent/Resorbing	Follicles appear broken, scattered and relatively empty; only residual oocytes found in follicles, most undergoing resorption; numerous phagocytes present.

Figure 3-1 is a graphical illustration by using the images taken under this study for the reproductive cycles of male and female surf clams.

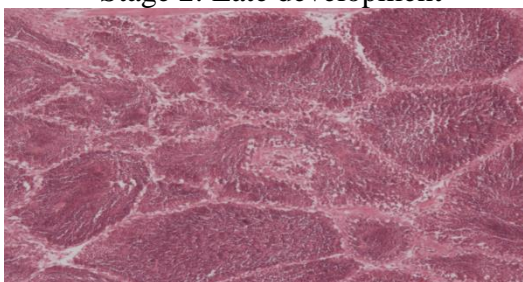
Male



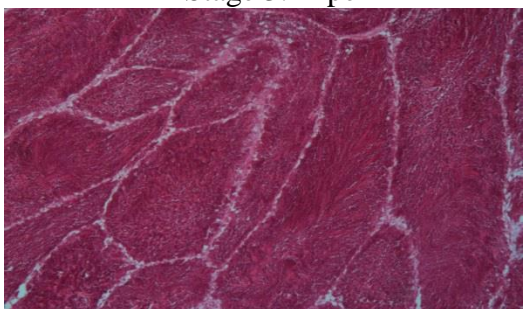
Stage 1: Early development



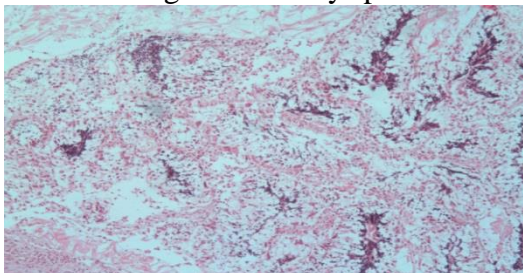
Stage 2: Late development



Stage 3: Ripe

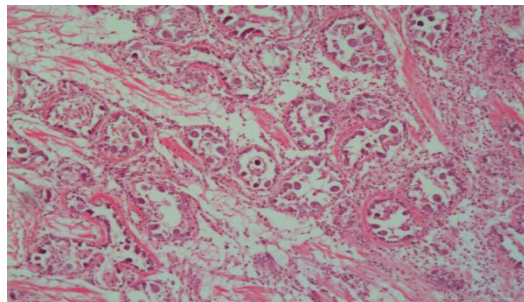


Stage 4: Partially spent

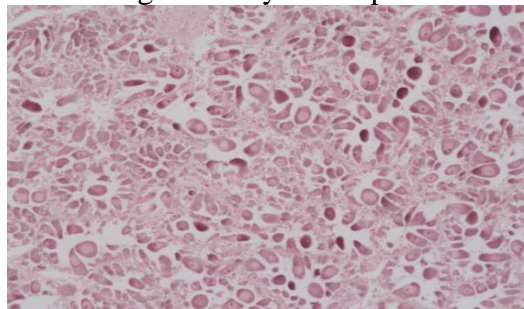


Stage 5: Spent

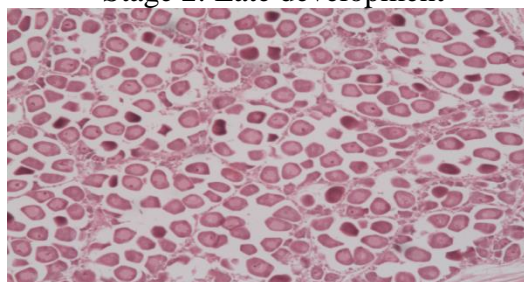
Female



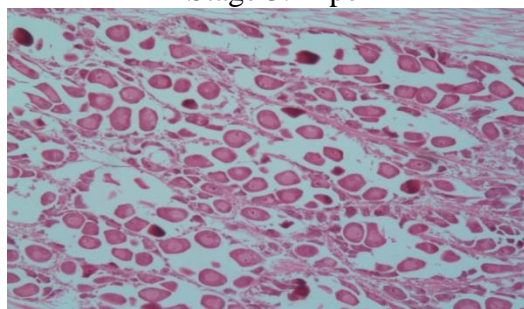
Stage 1: Early development



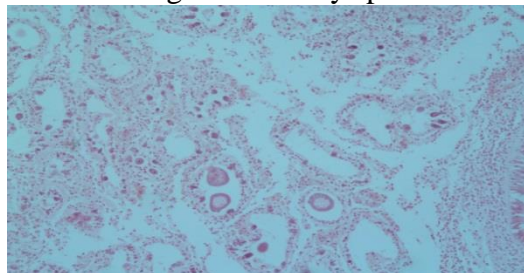
Stage 2: Late development



Stage 3: Ripe



Stage 4: Partially spent



Stage 5: Spent

Figure 3-1: male and female reproductive cycle

3.2.5.2 Condition index

The condition index is a measure widely used in NZ to assess the gametogenic development of surf clams (Booth, 1983; Rapson, 1952; Redfearn, 1974; Grant & Creese, 1995). Although there are limitations in the ability of the index to solely describe the reproductive life cycle of surf clams, it is still useful as it indicates important information of the spawning time (Grant & Creese, 1995). The index compares flesh weight and shell weight in the dry state (Drummond et al., 2006) as shown in Equation 0-1.

Equation 0-1

$$\text{Condition index} = \frac{\text{Dry flesh weight (g)} \times 100}{\text{Dry shell weight (g)}}$$

The investigation included the removal of soft tissues from shells for 30 individuals. Then, both flesh and shells were kept at 80 °C for drying to their constant weights. This way, it was possible to measure the dry tissue and shell weights for surf clam individuals.

3.2.6 Statistics

Statistical analyses were done in order to investigate the followings:

1. To determine the correlation between the reproductive cycles (both male and female), temperature and the condition index.
2. To analyse the seasonal variations associated with the condition index and reproductive stages.
3. To generalize the findings of sex ratios for the three surf clam species.

For the above three investigations, types of statistical techniques, i.e. parametric or non-parametric, were decided after the distributions were checked for normality (Smith, 2012). Actual deviations from normality were determined by using formal tests. Formal tests are the most accurate method to assess normality although none of these methods are exactly definite (Razali & Wah, 2011). Shapiro-Wilk test (SW) test was selected among the four types of formal tests since the selected test can be applied for a wide range of sample sizes varying from small to large (Razali & Wah, 2011). SPSS 18, which is a quantitative analysis tool, was used to check the goodness of fit for a normal distribution.

To determine the correlations, both parametric and non-parametric tests were used according to the types of the distributions. Pearson correlation was used for the variables which were normally distributed and alternative non-parametric tests, Spearson correlations, were used for the non-normal distributions. To determine the seasonal variances in the condition index and reproductive cycles, Kruskal-Wallis tests were done. Chi-square test was done to determine whether the sex ratios between male and female are significantly different from the 1:1 distribution in the population. All these hypotheses were tested at $\alpha=0.05$ significance level.

3.3 Data, results and interpretations

This section includes data, results and interpretations of the current study under the field investigation for *Spisula aequilatera*. The investigations are discussed with particular emphasizes given to the objectives of this study, which are described in section 1.2. The main themes that are discussed throughout the section are exogenous factors, histological evidences, condition indices and size and growth.

3.3.1 Environmental parameters

Under exogenous factors, sea water temperatures were measured and mean central tendencies were calculated monthly as described in section 3.2.3. The variation of temperature is depicted in Figure 3-2. The highest mean temperature occurred for Aug, 2012 - Jul, 2013 at the end of summer in Cloudy Bay which was 18.3 °C for February. From then, temperatures drop down until August when the minimum mean temperature was 10 °C. From that, again temperature increased until February.

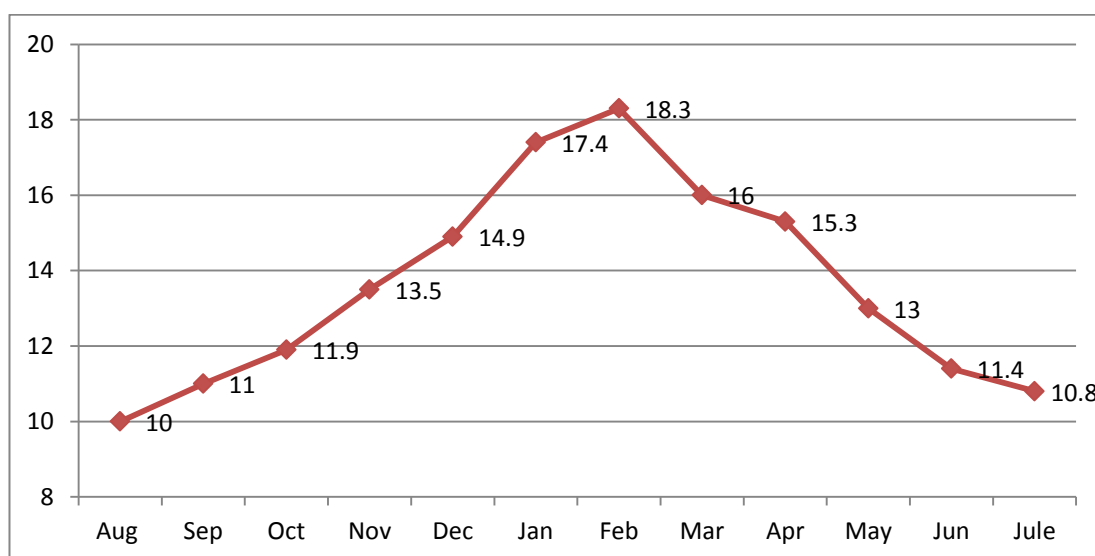


Figure 3-2: Directions of length and width measured

3.3.2 Size and growth

The aim of this section is to provide details of the *Spisula aequilatera* sample harvested from Cloudy Bay in terms of length, width, total wet weight and shell weight as variables. By looking at the descriptive statistics, one can have an idea on the sample that the investigations of this study are based on. Furthermore, this section investigates relationships between the different variables by using statistical techniques.

Table 3-3: Descriptive statistics for the sample of *Spisula aequilatera*

<i>Spisula aequilatera</i>				
	length (mm)	Width (mm)	Total wet weight (g)	Shell weight (g)
N	1108	1108	1108	1108
Mean	38.92	48.75	28.06	14.87
Median	38.60	47.95	26.33	13.89
Mode	36.80 ^a	52.8	27.3	12.10 ^a
Std. Deviation	4.67	5.42	8.61	4.69
Minimum	24.8	35.3	11	4.72
Maximum	73.50	85.9	71.67	38.55

a. Multiple modes exist. The smallest value is shown

Table 3-3 illustrates the descriptive statistics for the sample which was comprised of 1108 individuals. The mean length and standard deviation of the *Spisula aequilatera* sample were 38.92 mm and 4.67mm respectively. Thus, 95% of the lengths of the sample were between 29.77 mm (mean-1.96 x SD) and 48.07mm (mean+1.96 x SD). The smallest and the longest once were 24.8 mm and 73.5 mm correspondingly.

It could be noted from the table that the values for width are greater than the lengths for the descriptive statistics of *Spisula aequilatera* in Cloudy Bay. The mean width was 48.75 mm with a standard deviation of 5.42 mm. Hence, 95% of the sample was comprised of widths between 38.2 and 59.4 mm. The range of the sample was (35.3-85.9 mm) in terms of width.

The total weights for *Spisula aequilatera* varied between 11 and 71.67 g with mean and standard deviation values of 28.06 and 8.61 g, respectively. The data distribution for the total weight was non-normal and hence standard deviation was not used to describe the variance of the sample as similar to the length and weight. Alternatively,

the variance of the total weight was determined by using the quartiles (Table 3-4). For the total weight, 25% of data contained values less than 22.51g and 25% of data was above 31.75g. The 50th percentile, which is the median of the sample, was 26.33 g.

The shell weights of the sample were distributed between the minimum and maximum values of 4.72 and 38.55 g respectively (Table 3-3). The mean central tendency was 14.87 g for the sample of *Spisula aequilatera*. Since, the data distribution was non-normal similar to the total weight, the quartiles were calculated as shown in Table 3-4. For the shell weight, 25% of data contained values less than 11.81g and 25% of data was above 16.73g. The median shell weight of the sample was 13.89 g.

Table 3-4: Quartiles for the total weight and shell weight of *Spisula aequilatera*

Percentiles	Total weight of <i>Spisula aequilatera</i> (g)	Shell weight of <i>Spisula aequilatera</i> (g)
25	22.51	11.81
50	26.33	13.89
75	31.74	16.73

After the sample statistics were calculated, the current study investigated the relationship between the above variables as described in the section below.

3.3.2.1 Relationship between length, width and weights

In this section, all the relationships between variables for *Spisula aequilatera* are described with reference to ‘length’. To have an indication about the relationships between the length and other variables, their plots were observed (from Figure 3-3 to Figure 3-6). Figure 3-6 was drawn after the flesh weights of individuals were calculated based on their total and shell weights. The trend lines were set for all the graphs such that they interpret at (0, 0).

Figure 3-3 indicates a solid linear relationship between length and width in the sample of *Spisula aequilatera*. The majority of the actual data lies on or closer to the trend line drawn for the linear relationship which the coefficient was $m=1.2404$.

Although the relationship was weaker for ‘length versus total weight’ (Figure 3-4) compared to ‘length versus width’, a substantial linear relationship can still be seen. The diversions from the trend line were larger and a fewer points can be seen on or closer to the trend line compared to Figure 3-3. The magnitude of the coefficient of the

trend line was further reduced in the next case, 'length versus shell weight' (Figure 3-5), but the diversions between the actual data and the trend line seems similar to Figure 3-4. The variations between the trend line and actual data were the largest for 'length versus tissue weight'. After these observations related to the sample, the significances of the correlations between these variables were tested for the population by using inferential statistical tests.

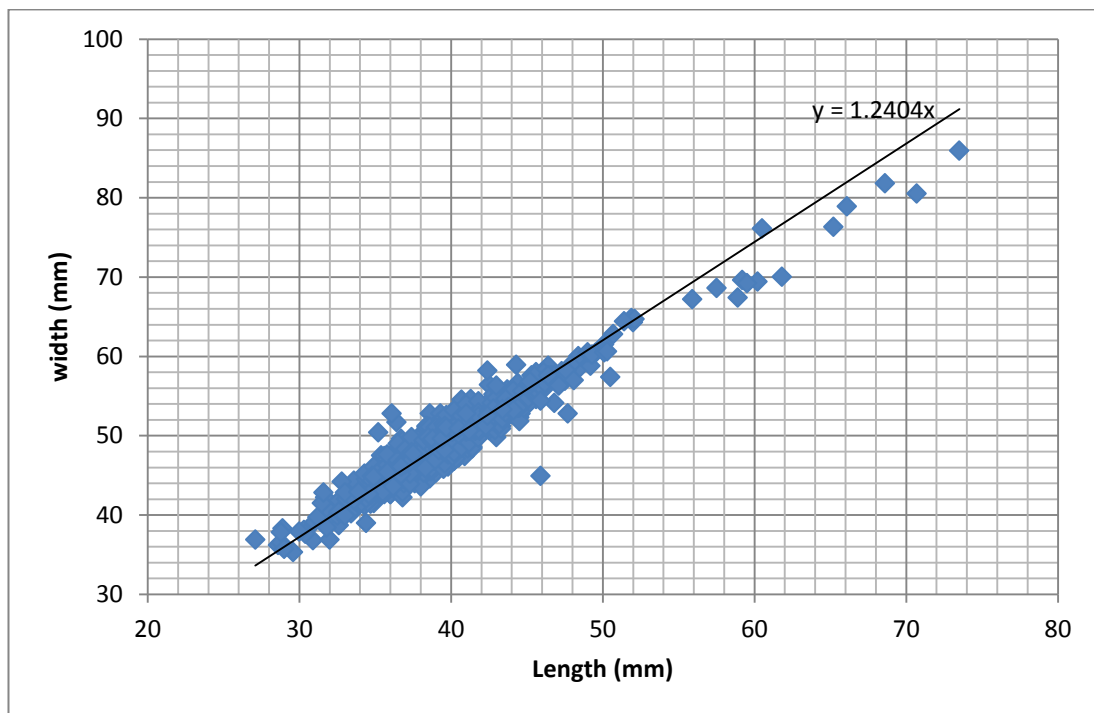


Figure 3-3 :Length versus width: *Spisula aequilatera*

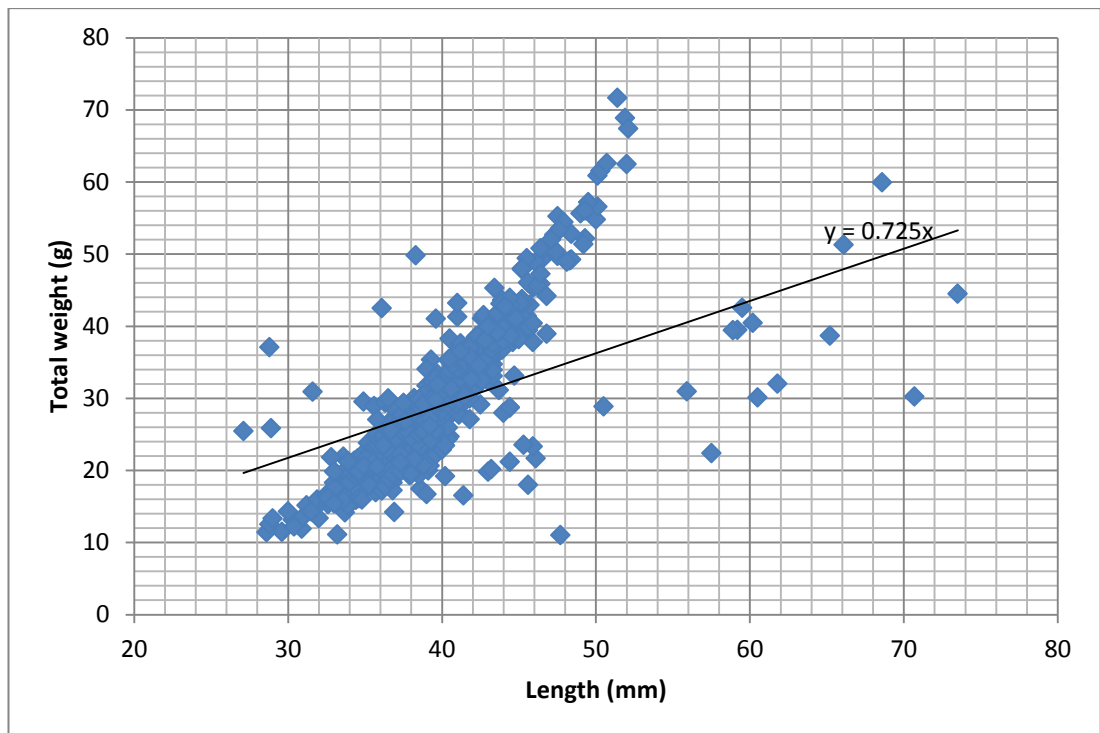


Figure 3-4 :Length versus total weight: *Spisula aequilatera*

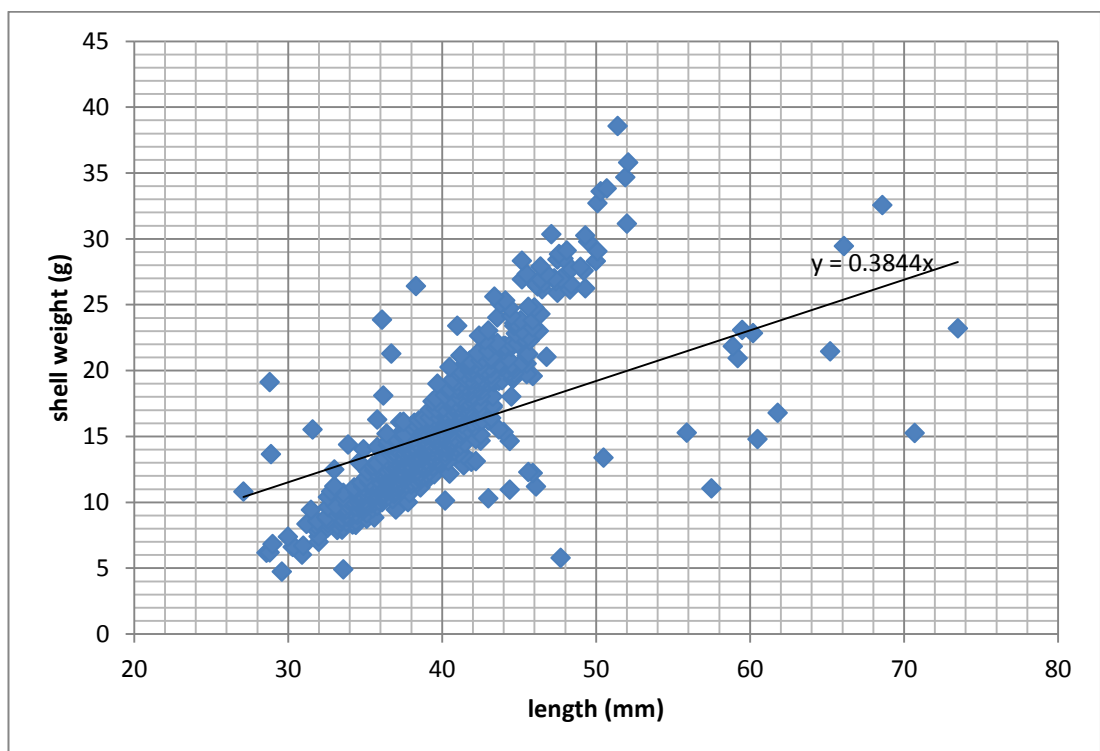


Figure 3-5 :Length versus shell weight: *Spisula aequilatera*

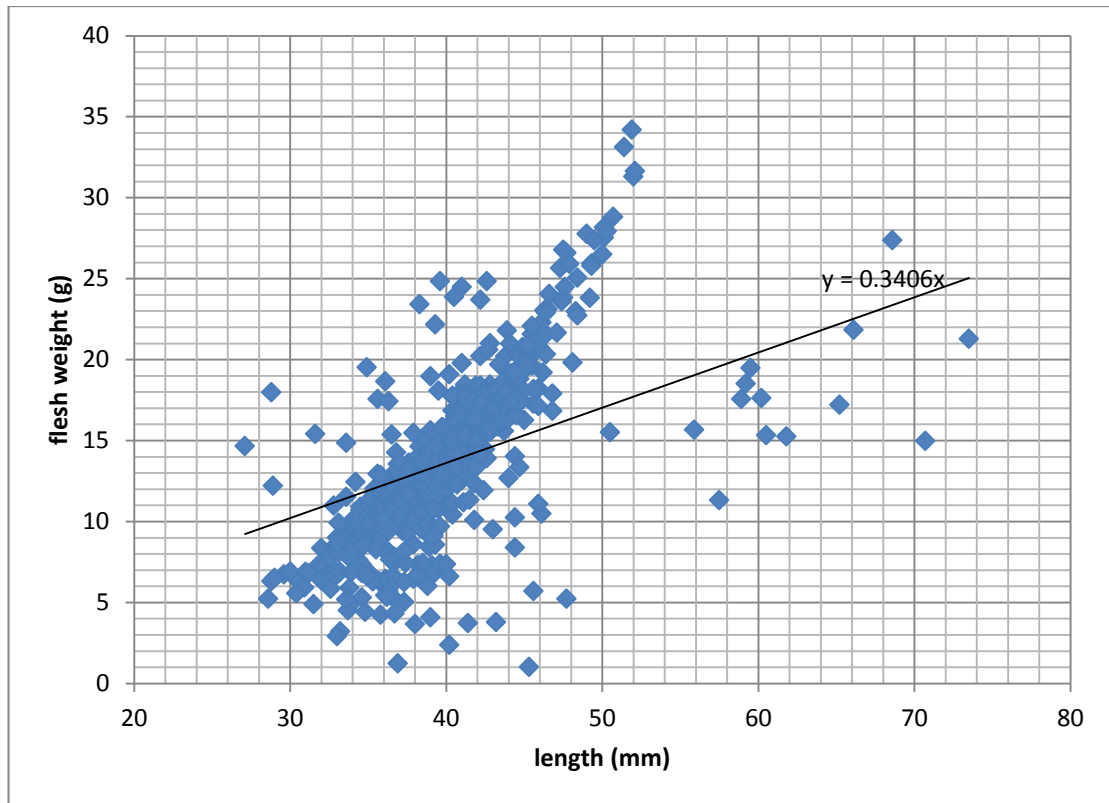


Figure 3-6 :Length versus flesh weight: *Spisula aequilatera*

3.3.2.2 Correlations between length, width and weights in the populations of *Spisula aequilatera* in Cloudy Bay

To test the relationships between length, width and weights, a correlation analysis was done between the variables by using SPSS 18 software package. Since the type of correlation test depends on the nature of distributions, the first step was to assess the normality of the five distributions by SW tests as described in section 3.2.6. The null and alternative hypotheses were set as follow for the SW test:

H_0 : The data distribution is normal.

H_1 : The data distribution is non-normal.

Table 3-5: Normality test for length, width and weights of *Spisula aequilatera*

	Shapiro-Wilk	
	Statistic	Sig.
Total weight of <i>Spisula aequilatera</i>	.850	.000
Shell weight of <i>Spisula aequilatera</i>	.890	.000
Flesh weight <i>Spisula aequilatera</i>	.892	.000
Length of <i>Spisula aequilatera</i>	.963	.096
Width of <i>Spisula aequilatera</i>	.958	.071

Table 3-5 shows SW test statistics for the five variables. The level of significances are more than $p=0.05$ in two cases only which are 'length' ($\alpha=0.096$) and 'width' (0.071). Therefore, the null hypothesis of SW test could not be rejected and the two distributions for 'length' and 'width' were normal. Consequently, Pearson correlation was used to investigate the relationship between 'length' and 'width'. To determine the significance of correlation for other three variables, the non-parametric Spearman correlation was used. Some outliers could be seen in each distribution and hence, those were excluded from the analysis to minimise misinterpretations.

Table 3-6: Correlations between length, width and weights of *Spisula aequilatera* at $\alpha = 0.05$

Type of relationship	Pearson correlation	Spearman correlation
Length versus Shell weight		0.769
Length versus Flesh weight		0.691
Length versus of Total weight		0.872
Length versus Width	0.960	

The null and alternative hypotheses were set as follow for each relationship:

H_0 : There is no significant linear correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r = 0$)

H_1 : correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r \neq 0$)

The results are shown in Table 3-6 for the analysis. The results shows that the four variables have statistically significant positive correlations with 'length' at $\alpha = 0.05$ in the population of *Spisula aequilatera* in Cloudy Bay. The correlation was the highest for width and closer to one ($r=0.960$ and level of significance of 0.000). This was followed by total weight ($r=0.872$ and level of significance of 0.000) and shell weight ($r=0.769$ and level of significance of 0.000). The least correlation was for flesh weight ($r=0.691$ and level of significance of 0.000), but still the correlation was statistically significant at $\alpha = 0.05$. Thus, as the concludary remark for these analyses, width and weights (total, shell and flesh) of *Spisula aequilatera* increase when they grow longer in Cloudy Bay. The shell weight grows slightly higher than the flesh weight ($r=0.769$ versus $r=0.691$) for *Spisula aequilatera* in Cloudy Bay.

3.3.3 Sex ratio

This section describes the sample data, sample sex ratios and interpretations to the population sex ratio based on a chi-square goodness of fit test. These findings are in line with the first objective of this study (section1.2).

Table 3-7: Distribution of surf clams according to sex: *Spisula aequilatera*

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	total
Male	-	17	27	33	27	28	25	22	16	32	30	11	268
Female	-	37	28	23	30	32	26	1	11	8	14	12	222
Stage 0	-	0	0	0	0	0	8	33	15	16	14	22	100
Total	-	54	55	56	57	60	59	56	42	56	58	45	590

As described in section 3.2.2, sampling methods, the surf clams were collected over a year from August, 2012 to July, 2013 on a monthly basis. By observations made through a Leica DM2000 microscope, the sexes of the surf clams were identified individually and the results are shown in Table 3-7. The table shows the distributions for each month for three categories which are male, female and stage 0. As described in section 3.2.5.1, 'stage 0' represents the resting stage which gonad predominantly composed of connective tissue and hence sex not distinguishable. Due to practical disturbances to the sample, histological examination could not be done in August, 2012. For the rest 11 months, the sample contained 590 individuals in total. This sample was comprised of 268 males, 222 females and 100 sexually indistinguishable individuals for *Spisula aequilatera*. The sexually indistinguishable surf clams are highest for the sample of March, 2013 where 59% of the sample belonged to 'stage 0'. There was a hermaphrodite also for *Spisula aequilatera* as shown in Figure 3-7.

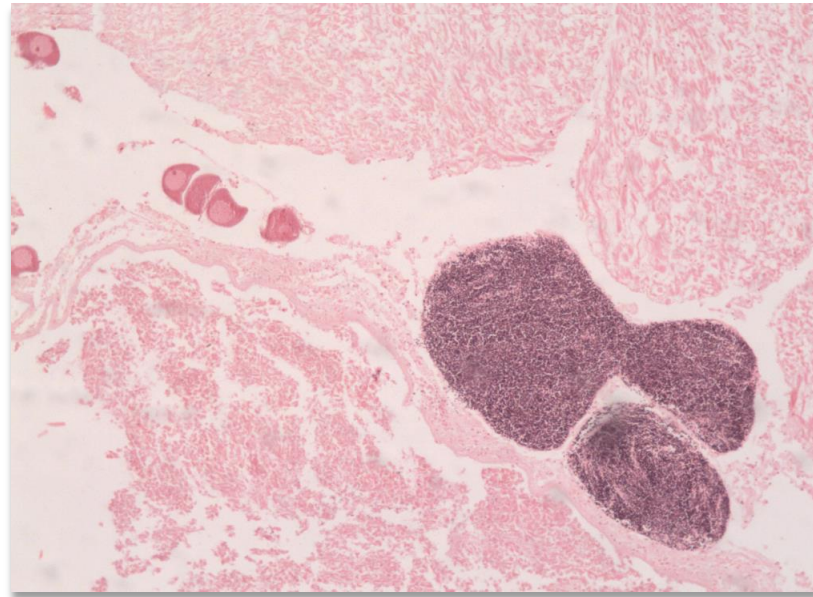


Figure 3-7 : presence of hermaphrodite: *Spisula aequilatera*

Based on the above data, sex ratios were calculated on a monthly basis and as a total. These sex ratios are shown in

Table 3-8. The sex ratios are calculated for the sample after excluding the sexually indistinguishable individuals. The result shows that male: female ratio is 1.21:1 in total for the sample. Extremes in male or female majorities can be clearly observed for four months (September, 2012; March, May, June, 2013) where the sex ratios were more than 2. The extremist sex ratio is for March, 2013 where 59% of the sample contained the individuals of 'stage 0'. The male: female ratio for this month is 22:1. However, the March sample counted only for 5% of the total sample (n=467). Hence, the influence of this extreme is not significant for the total sex ratio. After the total sex ratio is calculated, a statistical analysis was carried out to determine if the sex ratio is significantly different than the

1:1 Distribution in the population.

Table 3-8: Sex ratios for *Spisula aequilatera* in monthly basis and as a total

	Male	:	Female
August, 2012	-		-
September, 2012	1	:	2.18
October, 2012	1	:	1.03
November, 2012	1.43	:	1
December, 2012	1	:	1.11
January, 2013	1	:	1.14
February, 2013	1	:	1.04
March, 2013	22	:	1
April, 2013	1.45	:	1
May, 2013	4	:	1
June, 2013	2.14	:	1
July, 2013	1	:	1.09
Total	1.21	:	1

3.3.3.1 Sex ratio of the population of *Spisula aequilatera*

As described in section 3.2.6, a chi square goodness of fit test was performed to evaluate whether the sample proportion for male: female sexes was significantly different at $\alpha=0.05$ from the hypothesized proportion, 1:1, in the population. The results are shown in Table 3-9. According to the results, the Asymptotic significance is 0.020 ($<\alpha=0.05$) for $X^2=5.568$ and degree of freedom (df) of one. Thus, the related null hypothesis that 'the sex ratio of *Spisula aequilatera* equals to 1:1 in Cloudy Bay' could not be accepted. Therefore, the alternative hypothesis is accepted, which is the sex ratio of *Spisula aequilatera* is significantly different than 1:1. Thus, males are predominant in Cloudy Bay for the population of *Spisula aequilatera*.

Table 3-9: Chi-square goodness of fit test statistics for sex ratio: *Spisula aequilatera*

Chi-Square	5.568		
Df	1		
Asymp. Sig.	.020		
	Observed N	Expected N	Residual
Female	268	245	-23
Male	222	245	23
Total	490		

After the sex ratio was found based on the histological evidences, the individuals were categorized into the distinct reproductive stages.

3.3.4 Sexual cycle

The purpose of determining the reproductive stage of individuals was to investigate seasonal variations in the reproductive cycles of male and female *Spisula aequilatera* in Cloudy Bay. The histological techniques used were explained in section 3.2.5.1. The gonad position is illustrated in

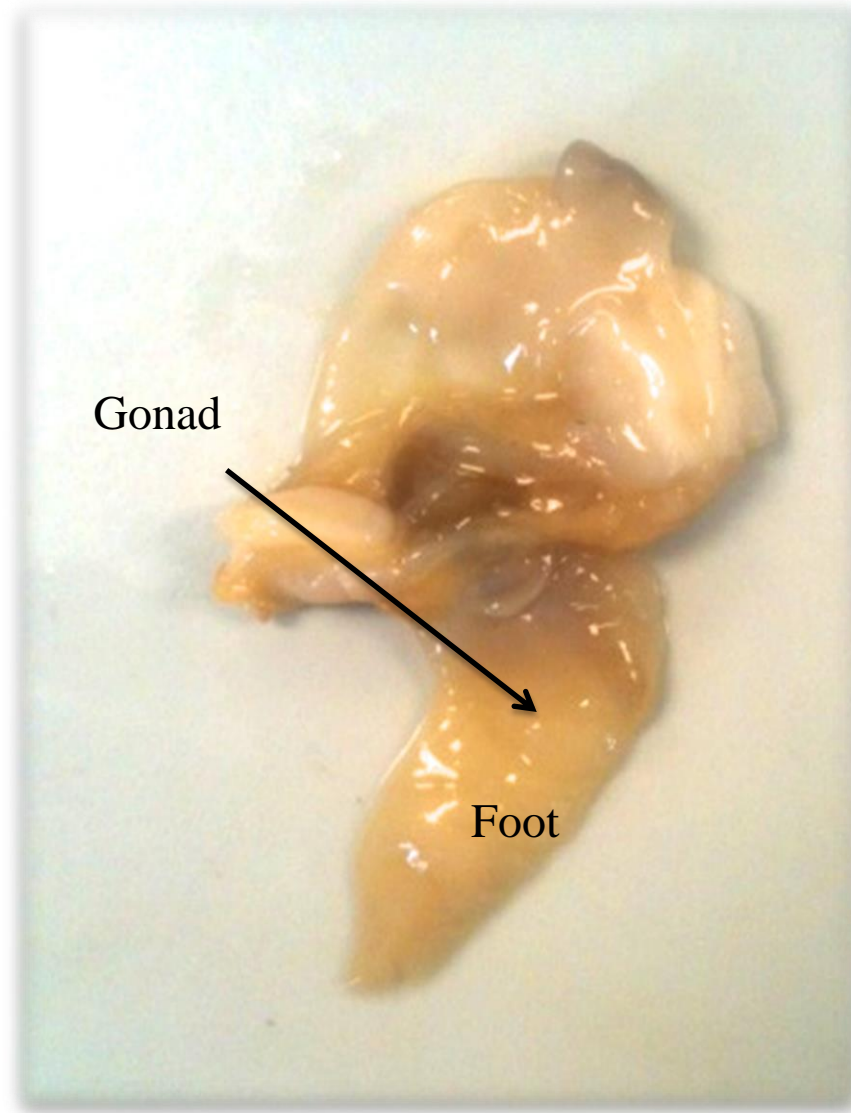


Figure 3-8 : Gonad position: *Spisula aequilatera*

The male and female distributions of the sample based on the six reproductive stages are given monthly basis in Figure 3-9 (a) and (b) respectively. As mentioned previously, there were no histological evidences observed for August, 2012 due to practical difficulties. The sample contained a total of $n=300$. It should be noted that the sexually indistinguishable individuals ($n=78$) were distributed equally between

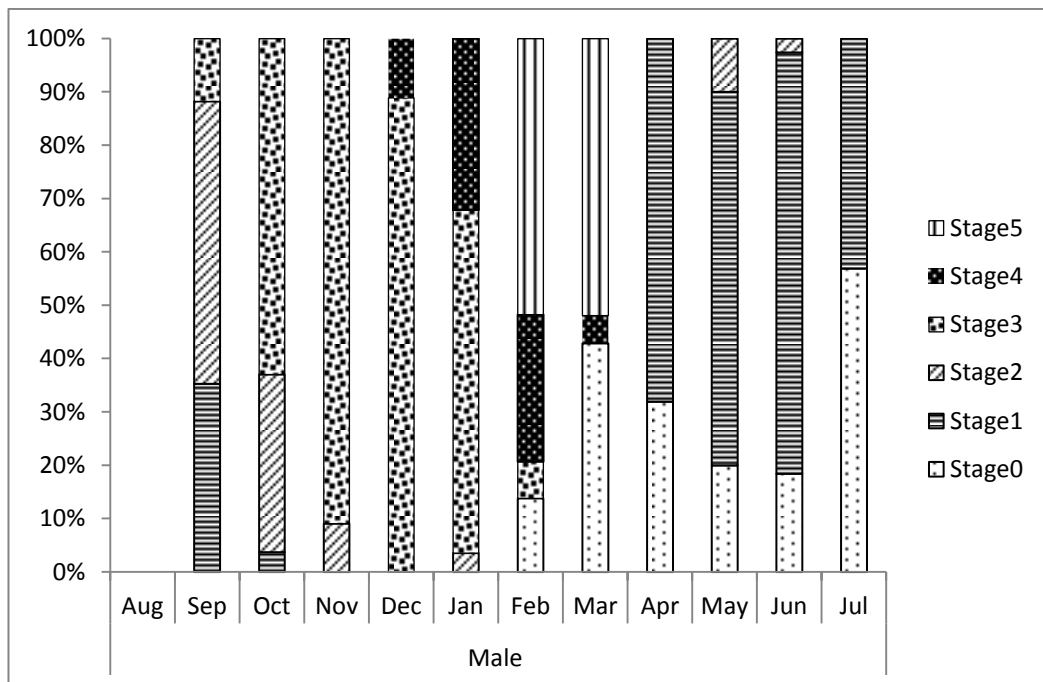
male and female. Thus, there are fractional numbers in the number of surf clams belonging to 'stage 0' for some months.

Male

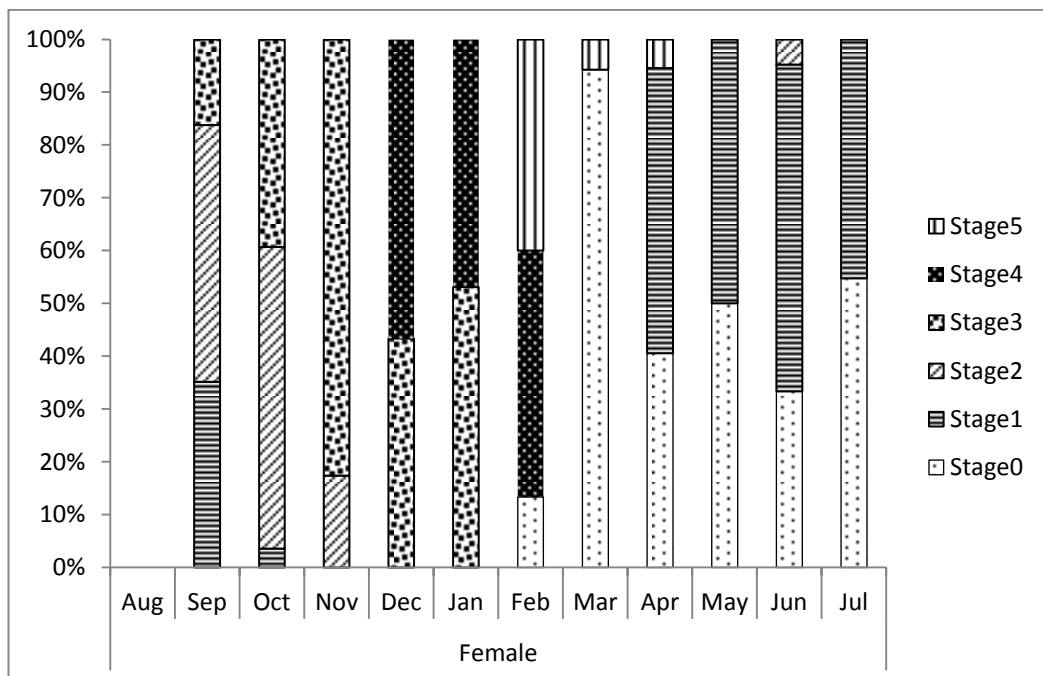
The sample of male *Spisula aequilatera* in September, 2013 was comprised of individuals belonging to three stages which are stage 1 (early developing), stage 2 (late developing) and stage 3 (ripe). The predominant stage was the 'late developing' phase which counted for 53% (n=9). This was followed by the 'early developing' and 'ripe' stages having counted for 35% (n=6) and 12 % (n=2), respectively. In October, the number of male individuals belonging to 'late developing' stage decreased as a percentage (33%). Males with ripe gonads increased up to 63% (n=17), which was 51% increment compared to September. There was only a single male (4%) found still at the 'early developing' stage for October.

The sample extracted for November was comprised only with males belonging to the 'late developing' and 'ripe' stages, while the 'ripe' males were the predominate group counted for 91% (n=30). In December, the majority (n=24, 89%) belonged to the 'ripe' stage while the rest (n=3, 11%) was at the 'partially spent' stage. For January, 2013 also, the predominant group was the 'ripe' phase (n=18, 64%), while the second majority was observed for 'spent' (n=9, 32%). There was one individual (4%) belonging to the 'late development' phase although there were no individuals belonging to this phase in the previous (December) sample.

Males with ripe gonads were dramatically reduced in February and limited only to two (7%). The majority in February was for the 'spent' stage and accounted for 52% (n=15). This was followed by the 'partially spent' and 'resting' stages being counted for 28% (n=8) and 14% (n=4), respectively. In March, there are another important observations which are increments in both the 'spent' (n=20, 52%) and 'resting' (n=16.5, 43%) stages while individuals (n=2, 5%) belonging to the 'partially spent stage' decreased considerably.



(a) Distribution of male surf clams by the reproductive stages: *Spisula aequilatera*



(b) Distribution of female surf clams by the reproductive stages: *Spisula aequilatera*

Figure 3-9 : Distribution of male/female surf clams by the reproductive stages: *Spisula aequilatera*

In April, there were only two categories for male surf clams and these are the 'resting' (n=7.5, 32%) and 'early developing' (n=16, 68%) stages. This observation coupled with the samples of the next two months, shows that individuals belonging to the 'resting' stage reduced steadily (for May n=8 (20%) and June n=7(18%)). On the other hand, the compositions of the 'early development' were gradually increased for May and June such that the percentages of male surf clams were 70% (n=28) and 78% (n=29), respectively. Furthermore, individuals of the 'late development' stage were again apparent in slight amounts for May (n=4, 10%) and June (n=1, 3%).

Surprisingly, in the July sample, the proportion of resting individuals was again increased up to 50% (n=11). The rest of the sample was comprised of the 'late development' stage.

As a summary to the above observations, a few concludary remarks can be made. Firstly, the sample of males shows a few number of individuals belong to the 'ripe' stage in September, but it is not certain whether September is the first month in which ripe males began to emerge due to the absence of sample data for August. It is apparent that males having ripe gonads increasing until December and still becomes the predominant category for January, although the related composition is decreased compared to December. The majority of spawning took place over January and February – New Zealand summer months for male *Spisula aequilatera* in Cloudy Bay. April and May are the months where gametogenesis has begun for males in the. Thus, according to these remarks, seasonal variations were observed in the sample of male *Spisula aequilatera*. To determine the significance of seasonal variations in the populations of males, Kruskal-Wallis test was done with post-hoc tests. The results are shown in section 3.3.4.1 after the reproductive biology of female *Spisula aequilatera* is discussed.

Female

The female sample of September, 2013 also had the similar categories to that of the male sample, but the proportions were different. The females belonged to the 'early development', 'late development' and 'ripe' stages were 35% (n=13), 49% (n=18) and 16% (n=6), respectively.

In October, the number of male individuals belonging to 'late developing' stage decreased dramatically as low as 4% (n=1). The females belonged to both 'late development' and 'ripe' stages increased up to 57% (n=17) and 39% (n=11), respectively. In November, the females were comprised only with two categories: 'late development' and 'ripe' stage. The predominant group was the 'ripe' stage counting for 83% (n=19) of the sample.

Both in December and January, the female sample had individuals belonging only to the 'ripe' and 'partially spent' stages. There was 10% difference in the proportions between the two months such that the ripe females were 43% and 53% respectively for December and January.

In January, ripe gonads were not observed and females belonging to both spent (n=12, 40%) and resting (n=4, 13%) stages were appeared to the first time. The predominant group (n=14, 47%) was the partially spent females. In March, the partially spent group was totally unavailable, while the predominance was taken by the 'resting' stage with 94% of composition. There was only one individual appeared spent.

In April, females belonging to the 'early development' stage appeared and were the majority (n=10, 54%). There were 41% (n=7.5) of females in the 'resting' stage. In May, the composition was 50:50 between the 'early development' and 'resting' stages. In June, one individual appeared in the 'late development' stage. It showed increment in the 'early development' stage (n=13, 63%) while decreasing the composition of the 'resting' group (n=7, 33%).

In the July sample of females, only the 'late development' (52%) and 'resting' (48%) stages were apparent and the compositions were similar to that of the male sample.

As a summary to the female reproductive biology, similar patterns can be seen with a few differences compared to the male sample. In September, October and November, both male and female samples were comprised of similar categories with variations in the proportions. In December, both samples had the same categories (ripe and partially spent), but significant difference was shown such that the percentage of the 'partially spent' is 46% higher in the females. Other similar patterns include that the ripe clams in the sample increasing until January, with the peak in November. The

samples indicate that New Zealand summer months (December and January) are the optimal months for spawning. For both males and females, April is the start of gametogenesis. More importantly, from the male and female samples, seasonal variations into the reproductive cycles are apparent according to the sample. The next section discusses these seasonal variations with respect to the populations of *Spisula aequilatera* in Cloudy Bay.

3.3.4.1 Seasonal variations in the reproductive cycles for *Spisula aequilatera* in Cloudy Bay

Male

In this section, the statistical significance of the seasonal variations in the male reproductive cycle is discussed first for the whole year for the *Spisula aequilatera* population in Cloudy Bay. A Kruskal-Wallis test was performed by using SPSS18. The null and alternative hypotheses were set as follow:

H₀: The male reproductive cycle has the same distribution for the *Spisula aequilatera* population in Cloudy Bay throughout the year.

H₁: The male reproductive cycle has different distributions for the *Spisula aequilatera* population in Cloudy Bay throughout the year.

Table 3-10: Kruskal-Wallis test statistics for the male reproductive cycle of *Spisula aequilatera*

Chi-Square	165.543	
Df	10	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Sep	17	143.65
Oct	27	190.44
Nov	23	205.83
Dec	27	218.83
Jan	28	227.91
Feb	29	243.72
Mar	23	77.09
April	38	180.08
May	40	90.50
June	37	86.97
July	22	63.00
Total	311	

Table 3-10 shows the Kruskal-Wallis test statistics. The asymptotic significance was 0.000 ($\alpha=0.05$) for $X^2=165.543$ and $df=10$. Hence the null hypothesis could be rejected at $\alpha=0.05$. Thus, there are seasonal variations in the reproductive cycle of male *Spisula aequilatera* living in Cloudy Bay. Furthermore, it was possible to determine the degree of variations in mean ranks of reproductive stages due to seasonal variations by dividing the chi-square value from (N-1). This gives 53.4% ($x^2 / (N-1) = 165.543/310$) variations in the male reproductive cycle in Cloudy Bay due to seasonal changes.

Table 3-11: Seasonal variations of reproductive biology of male *Spisula aequilatera* in Cloudy Bay

Difference	Chi-square	df	Degree of variability in mean rank	α value	Significant Y/N
Aug-Sep	-	-	-	-	-
Sep-Oct	13.43	1	30.5	0	Y
Oct-Nov	3.76	1		0.052	N
Nov-Dec	5.91	1	11.8	0.015	Y
Dec-Jan	2.37	1		0.124	N
Jan-Feb	13.7	1	24.2	0	Y
Feb-Mar	23.72	1	45.6	0	Y
Mar-Apr	3.87	1	6.4	0.049	Y
Apr-May	3.72	1		0.054	N
May-Jun	0.23	1		0.632	N
Jun-Jul	6.48	1	11.1	0.011	Y

From the above test, it was not possible to determine where the significant seasonal variations occur in the reproductive cycle of *Spisula aequilatera* population in Cloudy Bay. Thus, as a post-hoc test, Kruskal-Wallis tests were again performed by comparing two consecutive months at once. The results are shown in Table 3-11. There are five months when the variations are significant and another four months where no significant differences found. To identify the similarities and differences, the sample statistics of the male reproductive cycle (Figure 3-9) was observed along with the Kruskal-Wallis test statistics given. Since the data was not available for August, there are no seasonal changes determined for July-Aug-Sep.

Sep-Oct Males at the early developing stage are transferred to the late developing stage whereas the male of the late developing stage are transmitted to the ripe stage. Thus, this is a period of transition for male *Spisula aequilatera* in Cloudy Bay. These transformations can be considered as rapid due to the statistical significance apparent in the analysis. More

specifically, 30.5% of these variations are due to seasonal changes during this period.

- Oct-Nov** The majority is at the ripe stage for the whole period. The minority is transformed from the late development into the ripe stages. Since the difference is not significant, the transformation of minority happens steadily throughout the period.
- Nov-Dec** There is a significant difference during this period such that 11.8% of variations in the mean ranks of reproductive cycles are governed by seasonal changes for male *Spisula aequilatera* in Cloudy Bay. Since most of the males are at the ripe stage for both months relevant to this period, it can be assumed that the majority of these 11.8% variations are related to transformations. The two transformations are late development-to-ripe and ripe-to-partially spawning.
- Dec-Jan** There is no significant difference during this period. According to Figure 3-9, partially spawning continues steadily during this period for *Spisula aequilatera* in Cloudy Bay.
- Jan-Feb** Amount of variations are significant and 24.2% in total due to seasonal changes during this period. According to the temperatures recorded, this period belongs to the highest temperature zone in Cloudy Bay and still the temperatures are increasing. Rapid changes can be detected also by observing Figure 3-9. Males belonging to the ripe stage are dramatically reduced during this period where as the majority is changed from ripe to spent. The majority changes from ripe- to-spent during this time and hence partially spawning is not apparent as a majority in the transformation. Hence theoretically, the occurrence of partially spawning majority can be expected in between this period. At the end of this period, some males are at the resting stage.
- Feb-Mar** The peak temperature occurs during this period. The seasonal variability in the reproductive cycles is the highest (45.6%) for this period for *Spisula aequilatera* in Cloudy Bay. Figure 3-9 provides credence to explain these rapid variations. Individuals of the partially spent stage

were dramatically reduced and hence spawning occurs fast. Consequently, males belonging to the resting stage increased dramatically. Thus, during the whole year, this period can be considered as the most active stage where many changes take place rapidly. During the period, the majority of changes are governed by the spent stage

Mar-Apr Males at the spent stage transferred to the resting stage while other males at the resting stage are transferred to the early development stage. Thus, this period is the transition stage and it is evident that seasonal variations are significant according to the statistical tests. However, the amounts of variations are dramatically reduced compared to the previous two periods (6.4% versus 24.2% and 45.6%).

Apr-May-June Males belonging to *Spisula aequilatera* in Cloudy Bay are mainly at early developing stage throughout these three months. Considerable numbers of males are still at the resting stage. No significant differences for the period and hence the statistical inference is verified. This is the longest period according to the investigation where no considerable seasonal variations are occurred. According to the environmental factors recorded, the temperatures are comparatively low and this is the period which the temperature is gradually dropped down.

Jun-Jul There is a significant seasonal variation (11.8% in mean ranks) during this period in the male reproductive cycle of *Spisula aequilatera*. Sample shows that the proportion of the early development stage is decreased consequently the resting stage is increased for July compared to the June sample. However, this behaviour cannot be described as long as there is no reason related to reproductive activities (such as spawning activities) to increase resting individuals during this period.

Thus, from the above results, it was found that 53.4% variations of the reproductive cycle are governed by seasonal changes for male *Spisula aequilatera* in Cloudy Bay. It is apparent that spawning is mainly occurs during New Zealand summer for males. Further, activities related to reproductive cycle becomes rapid when temperatures increase and the fastest changes occur during the peak temperature. On the other hand, these activities become slower when temperature decreases. During

winter, there are no significant changes which could be observed. From these observations, it can be hypothesised that both temperature and the rate of temperature change may influence the reproductive biology of male *Spisula aequilatera* in Cloudy Bay

Female

The same procedure was followed to male surf clams. Table 3-12 shows the Kruskal-Wallis test statistics. The asymptotic significance was 0.000 ($\alpha=0.05$) for $X^2=194.369$ and $df=10$. The null hypothesis was rejected at $\alpha=0.05$ and hence there are seasonal variations in the female reproductive cycle of *Spisula aequilatera* living in Cloudy Bay. The seasonal variations accounted for 75.5% ($\chi^2 / (N-1) = 194.369/269$), which is a higher variability than that of seen in males.

Table 3-12: Kruskal-Wallis test statistics for the female reproductive cycle of *Spisula aequilatera*

Chi-Square	194.369	
Df	10	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Sep	37	101.32
Oct	21	115.67
Nov	23	153.26
Dec	30	196.43
Jan	32	190.66
Feb	31	169.48
Mar	18	58.11
April	17	34.68
May	16	43.25
June	21	52.50
July	23	55.70
Total	269	

Post-hoc, Kruskal-Wallis, tests were then performed for a pair of consecutive months and the results are shown in Table 3-13. There are five months when the variations are significant and another five months when there are no significant differences; this is a similar pattern to that observed in males. However, minor differences could be seen between the males and females as described throughout this section.

Table 3-13: Seasonal variations of reproductive biology of female *Spisula aequilatera* in Cloudy Bay

Difference	Chi-square	df	Degree of variability	α value	Significant
			in mean rank (%)		Y/N
Aug-Sep	-	-	-	-	-
Sep-Oct	3.773	1		0.052	N
Oct-Nov	17.482	1	40	0	Y
Nov-Dec	21.16	1	40	0	Y
Dec-Jan	0.585	1		0.444	N
Jan-Feb	10.261	1	30	0.008	Y
Feb-Mar	17.247	1	35	0	Y
Mar-Apr	9.931	1	23	0.002	Y
Apr-May	0.961	1		0.456	N
May-Jun	1.281	1		0.258	N
Jun-Jul	1.245	1		0.265	N

Sep-Oct There is no statistical significantly difference in the reproductive cycle during this period for females, which is a different observation for males. By looking at Figure 3-9, it could be noted that the majority is governed by the late development stage for this period. The second major observation is the transformations of early-to-late and late-to-ripe. Since the difference is not significant, from a statistical point of view, the transformation can be assumed as steady throughout the period.

Oct-Nov There is a statistically significant variation of 40% during this time for the females of *Spisula aequilatera* in Cloudy Bay. Females at the late developing stage are transferred to the ripe stage rapidly and consequently the majority is changed from the late development stage to ripe females.

Nov-Dec Similar to males, there is a significant variation in the female reproductive cycles during this period. Seasonal variations govern 40% of the variations in the mean ranks of the reproductive cycle for this period. The two transformations are late development-to-ripe and ripe-to-partially spawning which are similar to transformations that were observed in males.

Dec-Jan There is no statistical significant difference for this period. According to

Figure 3-9, ripe-to-partially spawning continues steadily during this period for female similar to male *Spisula aequilatera* in Cloudy Bay.

- Jan-Feb** Seasonal variations account for 30% of variations in the female reproductive cycle for this period. As mentioned in the male reproductive cycle, this period belongs to the highest temperature zone in Cloudy Bay and still the temperatures are increasing. Rapid changes can be detected also by observing Figure 3-9 which is similar to that of males. Females at the ripe stage are dramatically reduced during this period where as the majority is changed from ripe- to-partially spent. Spent females are the second majority and closer in proportions to the partially spent stage. As a difference to the male reproductive cycle, the partially spawning stage appears as the majority at the end of this period for females. At the end of this period, some females are at the resting stage similar to males.
- Feb-Mar** The peak temperature occurs during this period. The seasonal variability in the reproductive cycles is 35%. According to Figure 3-9, rapid variations can be seen related to the majority and transformation. The majority is changed to the resting stage from the partially spent stage and hence two transformations during this period are partially spent-to-spent and spent-to-resting. Females belonging to the resting stage increased dramatically. Thus, during the whole year, this period can be considered as the most active stage in the female reproductive cycle which is similar to males.
- Mar-Apr** The majority is changed to the early development stage from the resting stage. Thus, another series of female reproductive cycle is started during this period. Seasonal variations account for 23% of the variations in the female reproductive cycle. Temperature is dropping from the peak during this period.
- Apr-May-
June-Jul** Females of *Spisula aequilatera* in Cloudy Bay are mainly at early developing stage throughout these three months while the second majority is at the resting stage. No significant differences were observed during this period making it the longest timeframe when no considerable

seasonal variations have occurred. As mentioned in the male reproductive biology, temperatures are comparatively low (NZ winter occurs) and the temperature is continually dropping lower.

Thus, from the above results, it is apparent that both male and female reproductive cycles are sensitive to seasonal variations. As common to male and female of *Spisula aequilatera* in Cloudy Bay, spawning activities occur during high temperatures-NZ summer. Furthermore, changes to the reproductive cycles are rapid during summer. On the other hand, *Spisula aequilatera* is less active in low temperatures and prefer to be at the resting and early development stages during NZ winter. Thus, temperature seems to be an important factor which influences the reproductive biology of *Spisula aequilatera* in Cloudy Bay. This influence is evaluated in the next section.

3.3.4.2 Correlations between temperature and reproductive cycles for *Spisula aequilatera* in Cloudy Bay

This section investigates the correlations between temperature and the reproductive cycles for *Spisula aequilatera* in Cloudy Bay. Since temperature or the reproductive cycles were non-normally distributed according to the SW tests, non-parametric Spearman correlation was used. The null and alternative hypotheses were set as follow for each relationship:

H₀: There is no significant linear correlation between the male/female reproductive cycle and temperature for *Spisula aequilatera* in Cloudy Bay ($r = 0$)

H₁: There is a significant linear correlation between the male/female reproductive cycle and temperature for *Spisula aequilatera* in Cloudy Bay ($r \neq 0$)

The analysis showed that both reproductive cycles are correlated with temperature at $\alpha=0.05$ significance level for *Spisula aequilatera* in Cloudy Bay. The Spearman correlations were $r=0.520$ and $r=0.498$ for male and female respectively. The higher ranks of reproductive cycles are set for spawning activities (3=ripe, 4=partially spawning and 5=spawning) and the low ranks for less active stages (0=resting,

1=early development and 2=late development). Thus, the positive correlations with temperature mean that spawning activities increase when temperature becomes high.

3.3.5 Condition index for *Spisula aequilatera* in Cloudy Bay

In addition to the histological analyses described above, condition index is widely used to determine the reproductive biology of surf clams. Thus, condition indices were calculated for *Spisula aequilatera* based on the sample consequently inferential statistical tests were done to predict the properties of the population in Cloudy Bay. The indices were calculated by using Equation 0-1 described in section 0. Mean and the standard deviations are shown for each month in Table 3-14.

Table 3-14: Condition Indices for *Spisula aequilatera* in Cloudy Bay

Month	Mean Condition Index	Stand Deviation
Aug	8.00	1.23
Sep	9.27	1.88
Oct	9.12	1.48
Nov	10.23	1.80
Dec	8.62	1.95
Jan	6.84	1.84
Feb	7.62	1.47
Mar	6.35	1.54
Apr	7.31	1.21
May	7.48	1.70
Jun	6.4	1.17
Jul	5.9	1.48

The highest mean monthly condition index was for November ($\mu=10.23$ and $SD=1.80$). According to the histological analyses, the majority of males and females are at the ripe stage in November. Hence, the highest condition index can be expected for this month. While the lowest value ($\mu=5.9$ and $SD=1.48$) was for July and the second lowest was for March ($\mu=6.35$ and $SD=1.54$). These two months correlates with the presence of majority as resting individuals. The low condition index therefore occurs during these months due to empty gonads. The variations of the monthly condition index can be more clearly illustrated graphically as shown in Figure 0-10. By comparing the figure with Figure 3-9, useful remarks could be made as follow.

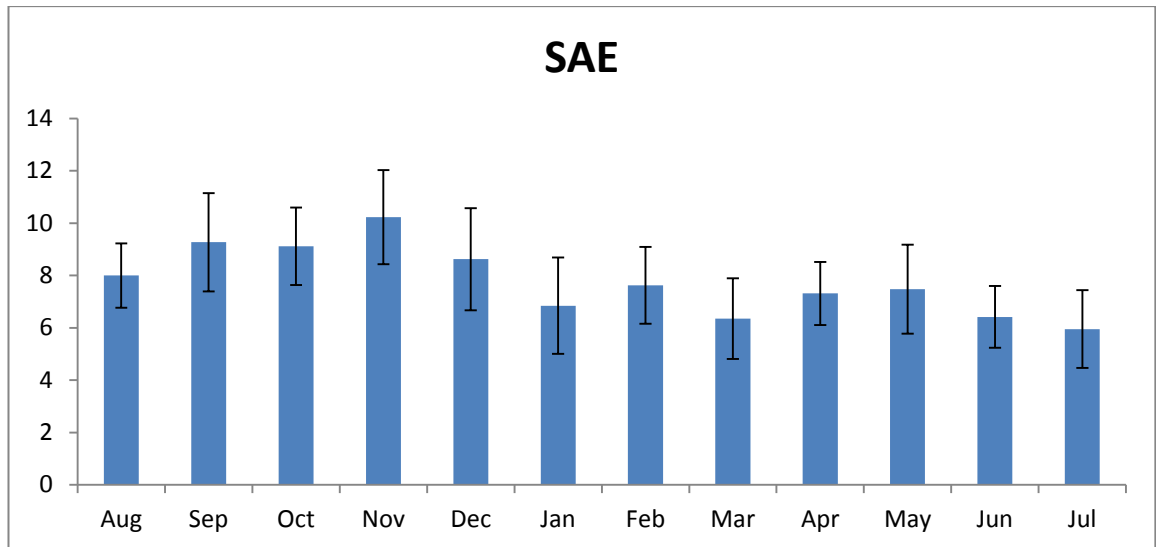


Figure 0-10: Condition Indices for *Spisula aequilatera* in Cloudy Bay

According to Figure 0-10, the condition index increases gradually from August to November when transformed into early-to-late and then late-to-ripe. However, there is no visible difference between September and October in the condition indices. The index then continues to drop down with spawning activities until March. Furthermore, there is an increment in the condition index of February compared to January. However, without statistical inference, it was not possible to determine if the difference was significant. After March, Figure 0-10 shows that the condition index increases gradually with the existence of the early development stage.

It can be observed from the sample data of *Spisula aequilatera* in Cloudy Bay that there is a link between the value of condition indices and the reproductive cycle. Furthermore, seasonal variations are apparent in the condition index similar to the reproductive biology discussed in the previous section.

3.3.5.1 Seasonal variations in the condition index for *Spisula aequilatera* in Cloudy Bay

To determine the significance of these seasonal variations in the condition index, a Kruskal-Wallis test was performed. The null and alternative hypotheses were set as follow:

H_0 : The condition index for the *Spisula aequilatera* population in Cloudy Bay is similar throughout the year.

H₁: The condition index for the *Spisula aequilatera* population in Cloudy Bay varies throughout the year.

Table 0-15 shows the Kruskal-Wallis test statistics. The asymptotic significance was 0.000 ($\alpha=0.05$) for $X^2=106.694$ and $df=9$. The null hypothesis was rejected at $\alpha=0.05$ and hence there are seasonal variations in the condition index throughout the year for *Spisula aequilatera* in Cloudy Bay. The seasonal variations accounted for 37.82% ($\chi^2 / (N-1) = 134.584/358$) of variability in the mean ranks of the condition index.

Table 0-15: Kruskal-Wallis test statistics for the condition index of *Spisula aequilatera*

Chi-Square	134.584	
df	9	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	29	148.55
Sep	30	202.97
Oct	30	208.90
Nov	30	238.33
Dec	30	173.10
Jan	30	91.33
Feb	30	129.07
Mar	30	73.60
April	30	110.60
May	30	123.50
June	30	80.56
July	30	64.98
Total	359	

Through the Post-hoc Kruskal-Wallis tests, it was possible to detect where significant variations occur. Several tests were performed for each pair of consecutive months and the results are shown in Table 0-16. There were seven months in which the variations were significant and another two months that no significant difference occurred.

During Aug-Sep, there is a significant difference in the condition index while there is no significant difference between September and October. With the increase number of ripe individuals in November, the condition index shows a further significant increase. In Table 0-16, the second largest difference is for Nov-Dec and the largest difference is between Dec-Jan. In November, the majority of *Spisula aequilatera* in

Cloudy Bay are at the ripe stage (Figure 3-9) and with partially spawning, it seems that their condition indices reduce significantly throughout this period (Nov-Dec-Jan). Thus, it seems that condition index shows significant differences due to changes (either increase or decrease) in the amount of ripe individuals.

Table 0-16: Seasonal variations of the condition index of *Spisula aequilatera* in Cloudy Bay

Difference	Chi-square	df	Degree of variability	α value	Significant
			in mean rank (%)		Y/N
Aug-Sep	8.741	1	15	0.003	Y
Sep-Oct	0.018	1		0.894	N
Oct-Nov	6.096	1	10	0.014	Y
Nov-Dec	10.104	1	17	0.001	Y
Dec-Jan	11.765	1	20	0.001	Y
Jan-Feb	4.163	1	7	0.041	Y
Feb-Mar	8.569	1	15	0.003	Y
Mar-Apr	4.596	1	8	0.032	Y
Apr-May	0.238	1		0.626	N
May-Jun	7.645	1	12	0.018	Y
Jun-Jul	0.986	1		0.126	N

In February, the condition index appears to show a pattern where it is significantly increased between Jan-Feb and then significantly reduced during Feb-Mar. This remark cannot be described using the reproductive behaviour. Then, there is a significant difference in the index during Mar-Apr when the early development stage starts according to Figure 3-9.

From this analysis, it was found that some of the major patterns in the reproductive cycles can be correlated with changes to the condition index although it is not possible to explain the reproductive biology totally. The major aspects of the reproductive cycles that can be detected are the increase and decrease of ripe gonads, the presence of the majority of ripe individuals, the partially spent and spent periods (although cannot separate the in-between point of two stages), the occurrence of the resting stage as majority and the starting point of the early development stage.

Having determined the above points, the correlation between temperature and the condition index was investigated.

3.3.5.2 *Correlations between temperature and the condition index for Spisula aequilatera in Cloudy Bay*

This section aims to determine the correlations between temperature and condition index for *Spisula aequilatera* in Cloudy Bay. Thus, the data distributions relevant were non-normally distributed according to the SW tests performed, the non-parametric Spearman correlation was used. The null and alternative hypotheses used were as:

H₀: There is no linear correlation between the condition and temperature for *Spisula aequilatera* in Cloudy Bay ($r = 0$)

H₁: There is a linear correlation between the condition index and temperature for *Spisula aequilatera* in Cloudy Bay ($r \neq 0$)

The analysis showed a negative correlation of $r=-0.353$ for *Spisula aequilatera* in Cloudy Bay. The correlation was less than that of the reproductive cycles, but still it was significant at $\alpha=0.05$ significance level. Since the correlation was negative, when temperature increases, the condition index is decreased. Since the spawning happens in summer for *Spisula aequilatera* in Cloudy Bay, the condition index can be reduced in high temperatures due to their reproductive activities.

3.4 Discussion

Literature related to *Spisula Solidissima* was reviewed in both global and NZ perspectives and mentioned in Chapter 2. This literature provide directions towards understand the present gaps on how to sustainably develop surf clams harvesting in NZ coastal areas. The key points discussed in these literature were evaluated under the scope of this study by using a sample of *Spisula aequilatera* from Cloudy Bay. The methodology adopted includes examination of gonads and the development of condition indices, which is a similar approach to many studies done far and wide in the context of surf clam's reproductive biology (eg: Drummond et al., 2006 (Ireland); Grants & Greece, 1995 (New Zealand); Hashizume et al., 2012 (Japan); Joaquim et al., 2008 (Portugal)). The reliability and validity of the findings are based on statistical generalization and no past studies were available to compare the findings relevant to *Spisula aequilatera* from Cloudy Bay or any other NZ coastal environments. In the filed investigation, 95% confidence level was used as the

acceptable confidence level to generalize the findings. The findings of *Spisula aequilatera* in Cloudy Bay are based on a sample having 95% of lengths between 29.77 mm and 48.07 mm.

According to the findings of this study width and weights have strong correlation to 'length'. The highest correlation ($r=0.960$) is related to the width and the value is close to one. Thus, width and length increase almost at similar rates for *Spisula aequilatera* population in Cloudy Bay. Both shell and tissue weights are significantly correlated with 'length' and the correlation for 'shell weight' is higher than that of tissues ($r=0.769$ versus $r=0.691$).

A common fact to all sub-species of *Spisula Solidissima* is that their sex cannot be differentiated via physical appearances. Thus, gonad examination is the only method to recognize their gender (Drummond et al., 2006; Fay et al., 1983; Grant & Creese, 1995; Ropes, 1968). Gonads were examined for a sample of *Spisula aequilatera* by using a microscope as described in section 3.2.5.1. Based on a chi-square goodness of fit test done at 95% confidence level for the sample, this study has investigated that the sex ratio of *Spisula aequilatera* is significantly different than 1:1 distribution in Cloudy Bay and males are predominant. As limitation to the findings of the sex ratio, the current study does not consider age as a parameter. Grant and Creese (1995) states that sex ratio depends on the age and different populations of the same species.

Investigating the reproductive biology of the *Spisula aequilatera* population in Cloudy Bay is in line with the first objective of this study. This objective was achieved by using histological evidences as described in section 3.2.5.1. After sex is differentiated, the individuals of the sample were categorized into distinct stages by using six stages, which are modified versions of the five maturity scale introduced by Xie and Burnell (1994) that incorporate the resting stage (stage 0) into the early development (stage 1), late development (stage 2), ripe (stage 3), partially spent (stage 4) and spent (stage 5). The categorization is based on the images taken from a microscope with 10x, 20x and 40x objectives as described in the test process in section 3.2.5.1. There are several seasonal variations according to the histological examination of this study in both male and female reproductive cycles (section 3.3.4.1). These findings are based on duration of 11 months from Sep, 2012 to Jul,

2013. A summary of the findings are given in Table 3-17 and Table 3- for male and female *Spisula aequilatera* populations, respectively.

By using the information given in the tables, the seasonal variations in the reproductive cycles can be observed in terms of majority and major activities (such as transformations). When significant amount of composition is associated with minorities, the reproductive stage relevant to them is mentioned in Table 3-17 and Table 3-18 together with the majority (eg: Ripe/partially \equiv majority/second majority). Since temperature was used as the exogenous factor in this study, temperature variations are shown in the tables along with the information for the reproductive cycles. In this way, one can observe the variation in temperature and the reproductive biology together. According to the Kruskal-Wallis test at 95% confidence interval, seasonal variations govern 53.4% of the variations of the reproductive cycle for male *Spisula aequilatera* living in Cloudy Bay. Furthermore, seasonal dependency of the female reproductive cycle is higher than that of male (73% versus 53.4%) (section 3.3.4.1).

Through monthly comparisons done using post-hoc Kruskal-Wallis tests, it was possible in this study to investigate where exactly significant seasonal variations occur in the two reproductive cycles (section 3.3.4.1). The findings have shown that the characteristics of male and female reproductive cycles for *Spisula aequilatera* are similar except a few differences.

Table 3-17: summary of the reproductive biology changing with exogenous factors: male *Spisula aequilatera* living in Cloudy Bay

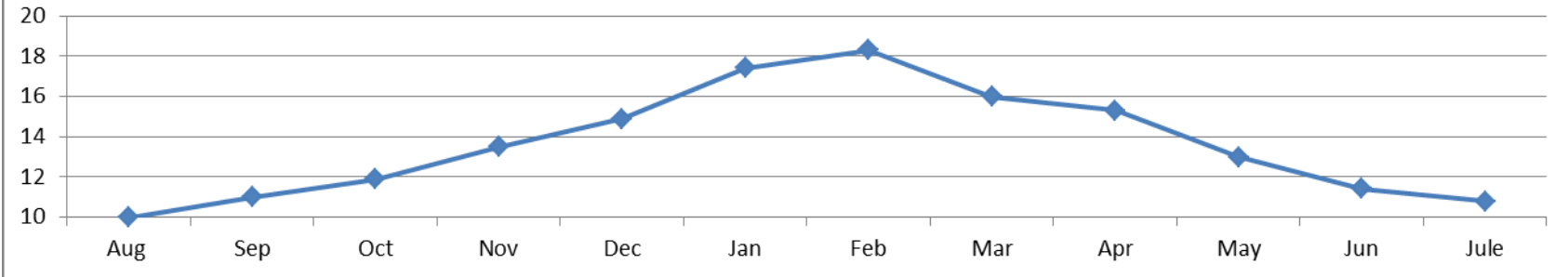
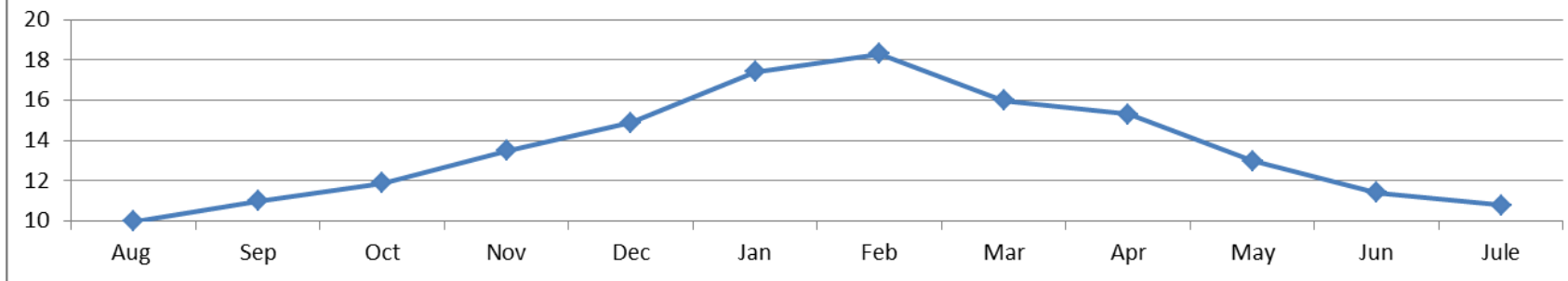
Period		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun			Jul									
Temperature		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun			Jul									
Majority		No data				Late/early development				Ripe/late development				Ripe		Ripe				Ripe/partially spent		Spent/partially spent			Spent/resting		Early/testing			Early development		Early development		Early development=resting
Activities						Majority transformed late-to-ripe and minority early-to-late					Partially spawning begins					Major spawning begins Ripe reduced dramatically			Spawning continues and resting individuals increase			Early development starts No spawning activity Late development begins									There is a significant change of 11.1% during this period but suspicious for sampling errors			
Significance of change		No data			Yes (30.5%)			No	Yes (11.8%)			No	24.2%			45.6%			6.4%			No				There is a significant change of 11.1% during this period but suspicious for sampling errors								

Table 3-18: summary of the reproductive biology changing with exogenous factors: female *Spisula aequilatera* living in Cloudy Bay

Period		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul	
Temperature		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul	
Majority		No data		Late/early development		Late/ripe development		Ripe		Partially spent/ripe		Partially spent/ripe		Partially spent /spent		Resting		Early development /resting		Early development /resting		Early development /resting		Early development=resting	
Activities			No data			Ripe individuals abundant and proportion of late development reduces dramatically		Partially spawning begins				Major spawning begins Ripe reduced dramatically		Spawning almost over and resting predominant		Early development starts									
Significant change		No data		No		Yes (40%)		Yes (40%)		No		30%	Yes		35%	23%	No				No				

A major difference occurs during September-October-November due to the late transformation of females into the ripe stage from the late-development stage. This transformation mainly occurs for males during September-October while females are transformed during October-November. As a whole, both male and female spent their ripe stages in NZ spring (September-October-November) and no spawning activities occur. For the three months, mean temperatures to values as high as 11, 11.9 and 13.5 °C.

For both males and females, partial spawning starts during Nov-Dec when temperature rises from 13.5 to 14.9 °C. Since the exact beginning of partial spawning could not be investigated from monthly data, the required temperature for spawning activities can be estimated as being between 13.5-14.9 °C for *Spisula aequilatera* living in Cloudy Bay. During this period NZ spring ends and summer begins.

The partially spawning stage continues for Nov-Dec-Jan steadily for *Spisula aequilatera* in Cloudy Bay and the major spawning period start during Jan-Feb and continues until Feb-Mar for both male and female. During the major spawning, the mean temperatures are 17.4, 18.3 and 16 °C hence, the required temperature for major spawning can be considered as near 16 °C for *Spisula aequilatera* in Cloudy Bay. Their major spawning period seems to be the shortest (only for two months) among the six stages of the reproductive cycles according to the current study's findings. The major spawning period seems to be even shorter for females as long as almost all the females are at the resting stage in March while majority of males are still at the spent stage. It is apparent that spawning (both partially and major) mainly occurs during NZ summer for *Spisula aequilatera* in Cloudy Bay. The changes in the reproductive cycles are rapid during summer.

The findings of the study illustrate that another series of the reproductive cycles start when New Zealand autumn begins. This happens during Mar-Apr and while the mean temperatures are between 16 and 15.3 °C. Seasonal dependency is further reduced when temperature further decreases so that there are no significant changes happening during Apr-May-June-July (it should be noted that there was a significant change of 11.8% for males during Jun-July, but the increment in resting cannot be understood from reproductive behaviours). This is the longest period without any significant seasonal variations occur for *Spisula aequilatera* in Cloudy Bay. The

latter part of this period belongs to New Zealand winter and hence the temperatures are low and drop down further during this period.

From the investigation of this research, major characteristics of the reproductive biology could be identified except a few limitations. As example for the remaining knowledge gap, the rate of changes in summer is recognized as rapid and some information such as the appearance of partially spawning males as the majority could not be exactly identified from monthly based sample data. Thus, if future studies based on more frequent sampling are carried out for summer months, these gaps can be filled and the findings of this research can be comprehended.

The third objective of this study is to investigate the correlation between exogenous factors and the reproductive cycles (section 1.2). Activities related to the reproductive cycle of surf clams are influenced by several exogenous factors such as temperature, salinity and availability of food (section 2.2.1). These factors are used in past studies in varying extents to investigate the correlations. For example, Grant and Creese (1995) mention that temperature and availability of food are the main exogenous factors that gamete maturation and spawning is influenced. There are other scholars (Drummond et al., 2006; Hashizume et al., 2012) who use both temperature and salinity to evaluate the correlations between the reproductive cycle and external factors. In the scope of this study, only temperature was used as the exogenous factor to evaluate the influence towards the reproductive cycle of *Spisula aequilatera* in Cloudy Bay. Although both ambient temperature and rate of temperature change can influence the reproductive biology of surf clams (Cargnelli et al., 1999), the current study considered only sea water temperatures for the analysis. The mean temperatures are calculated based on the site measurements as mentioned in section 3.2.3.

The Spearman correlations given with temperature at 95% confidence levels were $r=0.520$ and $r=0.498$ for the male and female reproductive cycles, respectively (section 0). Since the spawning activities are represented by the highest two ranks in the scale used to measure the reproductive cycles (4=partially spawning and 5=spawning), the positive correlations indicate that spawning activities increase when temperatures rises. Although the correlation is significant, the coefficients are considerably less than 1. Thus, temperature is not the only factor that variations of

the reproductive cycles are governed. Therefore, other factors that could influence the gametogenesis and gonadal development should be investigated to extend knowledge on the reproductive biology of *Spisula aequilatera* in Cloudy Bay.

The condition index is used as another method to evaluate gametogenesis and gonadal development in surf clams (Grant & Creese, 1995; Joaquim et al., 2008). Hence, the second objective of this study aimed at investigating the condition index and its variations for *Spisula aequilatera* in Cloudy Bay. In this study, condition indices were calculated according to Equation 0-1 based on the dry tissue and shell weights. The findings based on a Kruskal-Wallis test show that there are significant seasonal variations in the condition index for the *Spisula aequilatera* in population. At 95% confidence level, seasonal variations determine 37.82% of variability in the condition index for *Spisula aequilatera* in Cloudy Bay. These variations could be clarified by incorporating the histological observations together.

With the appearance of ripe individuals in the population, the mean condition index increases during Sep-Oct-Nov. The highest condition index is for November when the proportion of ripe individuals is at their highest. When partially spawning begins during Nov-Dec, the condition index reduces. The least mean condition index is for March, where the majority is at the resting stage after major spawning. Thus, the relationship between the condition index and reproductive cycle could be clarified through the findings of this study. The relationship is further apparent when the correlation between the condition index and ambient temperature is considered.

The correlation between the condition index and ambient temperature was found by using statistical analysis to achieve the third objective of this study. The analysis showed a negative correlation where the Spearman coefficient is $r = -0.353$ for *Spisula aequilatera* population in Cloudy Bay. Thus, when temperature increases, the condition index decreases. Since the condition index is directly proportionate to dry tissue weight, the findings indicate that their tissue mass is decreased in high temperatures. This can happen due to spawning activities occurring during high temperatures for *Spisula aequilatera* living in Cloudy Bay.

variations occurring for *Spisula aequilatera* in Cloudy Bay. The latter part of this period belongs to NZ winter and hence the temperatures are low and drop down further during this period.

From the investigation of this research, major characteristics of the reproductive biology could be identified except a few limitations. As example for the remaining knowledge gap, the rate of changes in summer is recognized as rapid and some information such as the appearance of partially spawning males as the majority could not be exactly identified from monthly based sample data. Thus, if future studies based on more frequent sampling are carried out for summer months, these gaps can be filled and the findings of this research can be comprehensive.

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gametogenesis and gonadal development should be investigated to extend knowledge on the reproductive biology of *Spisula aequilatera* in Cloudy Bay.

The condition index is used as another method to evaluate gametogenesis and gonadal development in surf clams (Grant & Creese, 1995; Joaquim et al., 2008a). Hence, the second objective of this study aimed at investigating the condition index and its variations for *Spisula aequilatera* in Cloudy Bay. In this study, condition indices were calculated according to Equation 0-1 based on the dry tissue and shell weights. The findings based on a Kruskal-Wallis test show that there are significant seasonal variations in the condition index for the *Spisula aequilatera* in population. At 95% confidence level, seasonal variations determine 37.82% of variability in the condition index for *Spisula aequilatera* in Cloudy Bay. These variations could be clarified by incorporating the histological observations together.

With the appearance of ripe individuals in the population, the mean condition index increases during Sep-Oct-Nov. The highest condition index is for November when the proportion of ripe individuals is at their highest. When partial spawning begins during Nov-Dec, the condition index reduces. The least mean condition index is for March, where the majority is at the resting stage after major spawning. Thus, the relationship between the condition index and reproductive cycle could be clarified through the findings of this study. The relationship is further apparent when the correlation between the condition index and ambient temperature is considered.

The correlation between the condition index and ambient temperature was found using statistical analysis. This finding was in response to the third objective of this study. The analysis showed a negative correlation where the Spearman coefficient is $r = -0.353$ for *Spisula aequilatera* population in Cloudy Bay. Thus, when temperature increases, the condition index decreases. Since the condition index is directly proportional to dry tissue weight, the findings indicate that their tissue mass is decreased in high temperatures. This can happen due to spawning activities occurring during high temperatures for *Spisula aequilatera* living in Cloudy Bay.

Early studies focusing on reproductive biology of NZ surf calms used the condition index more than histological evidences. Investigating condition indices can be considered as more economical in terms of cost and time when the procedures are

considered (sections 3.2.5.1 and 0). However, there is criticism that condition indices cannot describe reproductive biology in a comprehensive manner (section 2.7.2).

Through the condition index, some patterns could be explained such as the beginning of the early development stage and spawning activities, abundance of ripe individuals and the end of spawning period for the *Spisula aequilatera* population in Cloudy Bay. On the other hand, there are some characteristics which are not apparent in the condition index. These include the margin of partial spawning to major spawning, and beginning of the late development and ripe stage. Thus, the findings of this study agree with the Grant and Creese's (1995) statement that the condition index is insufficient to describe gametogenic development in a holistic manner. However, depending on the scope of an investigation, researchers may still use the condition index as an economical way to investigate the reproductive biology in the *Spisula aequilatera* population in Cloudy Bay. Furthermore, in this study, both findings of histological techniques and condition index provide similar conclusions. Thus, the condition index can be used as a validation method to the findings of histological techniques for *Spisula aequilatera* in Cloudy Bay. Thus, the findings of this study are validated through methodological triangulation, which are both histological techniques and condition index, and statistical generalization.

Ringed dosinia (*Dosinia anus*)



4 Ringed dosinia (*Dosinia anus*)

4.1 Introduction

Ringed dosinia, which is scientifically known as *Dosinia anus*, belongs to the family of venerid. The review of literature includes scholarly studies which are focused on their abundance (Haddon et al., 1996) and growth rate (H J Cranfield & Michael, 2001b) including Cloudy Bay, but major gaps are present around its reproductive biology. Thus, this section aims at providing an understanding already established facts in relation to *Dosinia anus*.

Dosinia anus is more densely populated between depths of 5 and 7m (Haddon et al., 1996). Their living preferences over the depth vary between the locations of the North and the South islands as 5-8m and 6-10m respectively. According to the categorization by Haddon et al. (1996), *Dosinia anus* belongs to deep water group and hence their sizes are larger when they are distributed towards the shoreline. Although *Dosinia anus* is mostly found in northern coastal areas (Great Exhibition Bay, Te Arai and Matakana), Cranfield & Michael (2001) identify them among the top most three species to be grown in Cloudy Bay. Their findings are based on the analysis of growth rates as described in section 2.6.2. Further, Cranfield & Michael (2001) have found that *Dosinia anus* is found with *Dosinia subrosea* in northern locations, but can be distinguished from rougher textures and larger sizes compared to *Dosinia subrosea* (Cranfield et al., 1993).

Related to their biology, there are a few facts which have been found. Cranfield et al. (1993) have found that their spawning occurs during summer months. Their recruitments is believed to be highly dependent between years (Ministry of Fisheries, 2011). The main causes of natural mortality are found to be beach erosion while storms, high temperatures and low oxygen levels in calm summer, excessive amount of freshwater flow and presence of toxic algae (Cranfield & Michael, 2001b).

Dosinia anus is introduced into the Quota Management System (QMS) on 1 April 2004 with a TAC of 112 tonnes, which was increased in 2010 up to 209 tonnes based on a biomass survey done in QMA 2 and 3 (Ministry of Fisheries, 2011). As mentioned in section 2.7.1, the current allowances are considered to be increased.

However, as similar to *Spisula aequilatera*, the future settings for *Dosinia anus* are based only on biomass surveys (Ministry of Fisheries, 2011; www.fish.govt.nz, 2010) and hence their reproductive biology should be investigated as well.

4.2 Materials and methods

The same research methodology as for *Spisula aequilatera* (section 3.2).

4.3 Data, results and interpretations

Similar to *Spisula aequilatera*, this section provides data, results and interpretations for *Dosinia anus* in Cloudy Bay.

4.3.1 Environmental parameters

Temperature was measured monthly at the same place for all the three species. Thus, the temperatures are similar to that of *Spisula aequilatera* (section 3.2.3).

4.3.2 Size and growth

The descriptive statistics are given for the sample of *Dosinia anus* in Cloudy Bay in this section. One aim of this section is to provide an indication of the sample that the findings of the population are based on. Further, the relationships are discussed between length, width and weights (total, shell and tissue). The sample extracted over a year for *Dosinia anus* in Cloudy Bay included 944 individuals (Table 4-1).

Table 4-1: Descriptive statistics for the sample of *Dosinia anus*

<i>Dosinia anus</i>				
	length (mm)	Width (mm)	Total wet weight (g)	Shell weight (g)
N	944	944	944	944
Mean	55.78	57.82	51.17	33.00
Median	55.70	58.10	49.53	31.96
Mode	55.70	57.80 ^a	36.96 ^a	29.72 ^a
Std. Deviation	5.56	5.52	17.57	11.93
Minimum	40.10	40.80	16.78	10.84
Maximum	76.90	77.70	140.95	95.52

a. Multiple modes exist. The smallest value is shown

The sample was comprised of individuals whose lengths were between 40.1 and 76.9 mm. The lengths of the sample were approximately normally distributed with a mean length of 55.78mm and a standard deviation of 5.56. Therefore, the lower and upper

limits of the 95% confidence interval are 44.89(mean-1.96 x SD) and 66.67mm (mean+1.96 x SD) for the sample lengths of *Dosinia anus*, respectively.

The descriptive statistics for the widths showed similar values to those of the length. The sample widths were distributed normally between the minimum and maximum of 40.8 and 77.7 mm, respectively. Both central tendency (mean=57.82) and the variations (SD=5.52) for the widths were also closer to those of the length. The 95% confidence interval was (47.0-68.6mm).

Both weights were distributed non-normally between 16.78 and 140.95 g. Their quartiles were used as the most appropriate measure to describe the variance (Table 4-2). For the total weight mean and median, central tendencies were 51.17 and 49.53 g (Table 4-1). There were several mode values and hence mode was not considered as a measure of central tendency for the weights. For the total weight, 25% of data contained values less than 42.02 g and 25% of data was above 59.14g. In the sample, individuals were densest between the 42.02 (25th) and 49.53 g (50th).

The mean and median sample central tendencies for the shell weights were 33 and 31.96 g, respectively (Table 4-1). The tables further show that 25% of data points were distributed between 10.84 (minimum (Table 4-1)) and 26.85 g (25th percentile (Table 4-2)). Another 25% were between 38.7 (75th percentile) and 95.52 g (maximum).

Table 4-2: Quartiles for the total weight and shell weight of *Dosinia anus*

Percentiles	Total weight of <i>Spisula aequilatera</i> (g)	Shell weight of <i>Spisula aequilatera</i> (g)
25	42.02	26.85
50	49.53	31.96
75	59.14	38.70

4.3.2.1 Relationship between length, width and weights

This sections aims at evaluating the relationships between the dimensions by using sample statistics. Similar to *Spisula aequilatera*, all the relationships are described with respect to 'length' for the *Dosinia anus* sample. In addition to total and shell weights, this section considers the tissue weight, which is the difference between total and shell weights. These weights are in wet basis.

The relationships between the variables are shown from Figure 4-1 to Figure 4-4 for width, total weight, shell weight and tissue weight. The trend lines are set such that the intercept at the origin (0,0).

The strongest linear relationship was for ‘length versus width’ and the coefficients was $m=1.037$. Thus, the sample data showed that width of *Dosinia anus* is slightly higher than length. The significance of this difference is further discussed in the next section. The distance between the actual data and the theoretical distribution, which is the trend line, are small according to the illustration given in Figure 4-1.

The second strongest relationship was for ‘length versus total weight’ where the coefficient was $m=0.956$ (Figure 4-2). The distances between the actual and theoretical distributions are higher than the relationships discovered for ‘length versus width’. These distances become even higher for ‘length versus shell weight’ (Figure 4-3) and ‘length versus tissue weight’ (Figure 4-4) and consequently the coefficients of the linear relationships are reduced to $m=0.918$ and $m=0.341$, respectively. The significance of these four linear relationships is discussed in the next section related to the *Dosinia anus* population in Cloudy Bay.

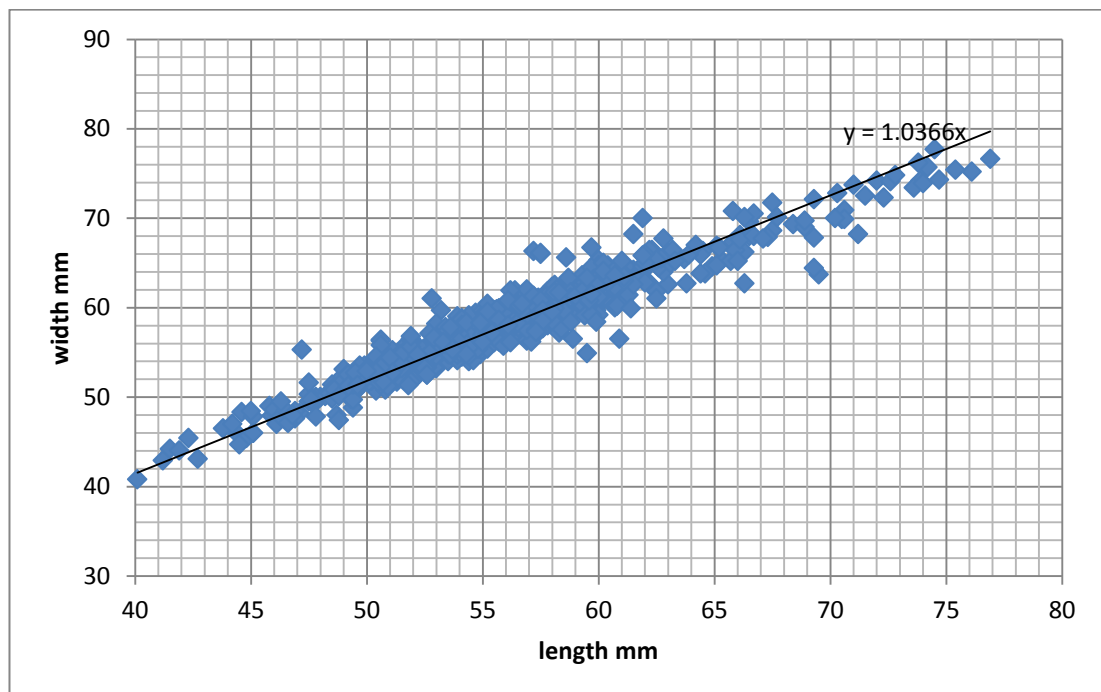


Figure 4-1 :Length versus width: *Dosinia anus*

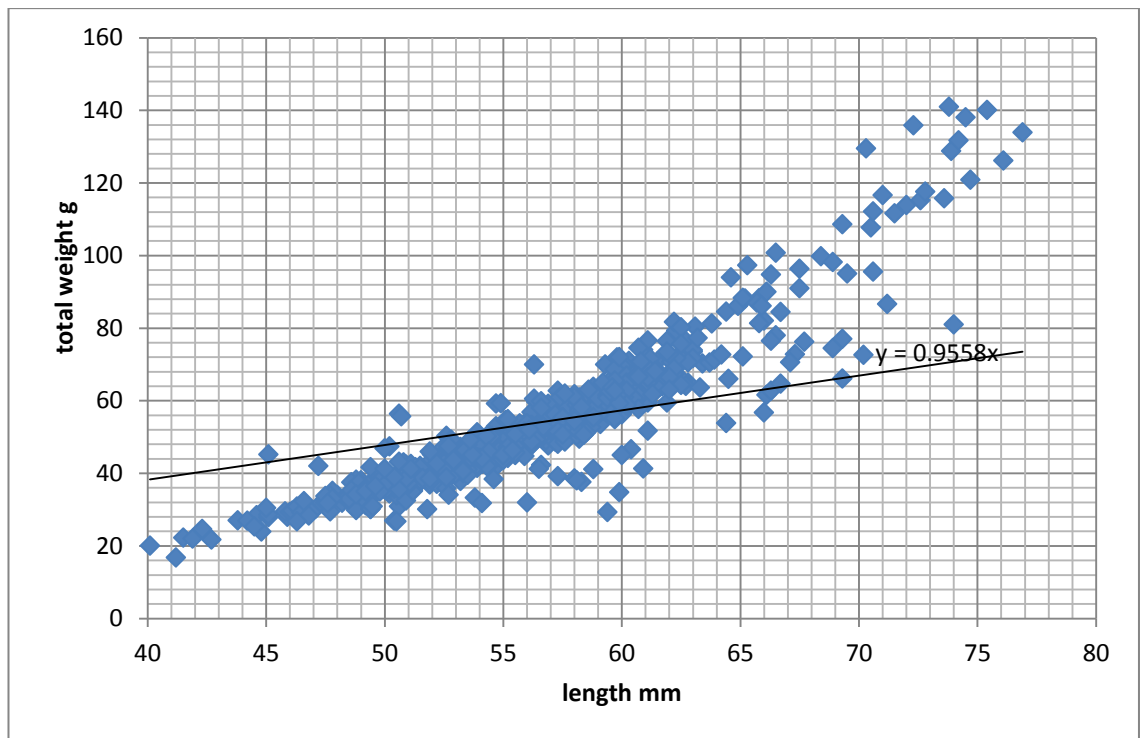


Figure 4-2 :Length versus total weight: *Dosinia anus*

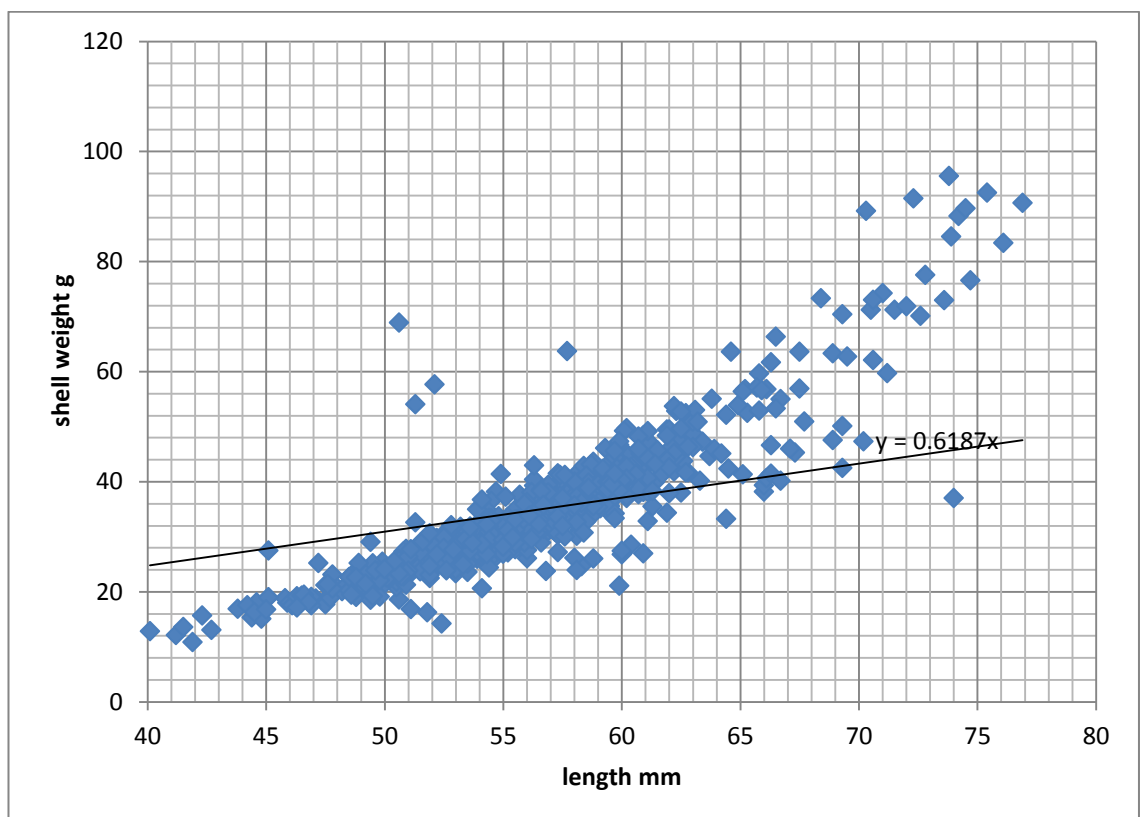


Figure 4-3 :Length versus shell weight: *Dosinia anus*

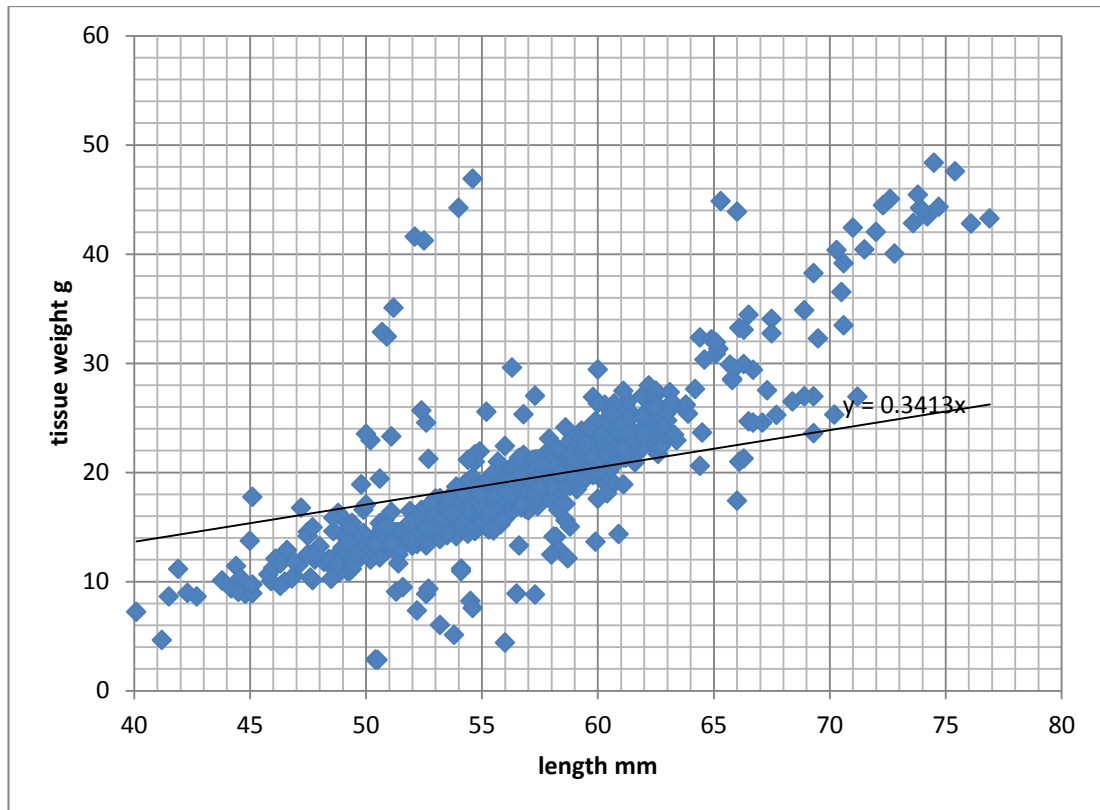


Figure 4-4 :Length versus flesh weight: *Dosinia anus*

4.3.2.2 Correlations between length, width and weights in the populations of *Dosinia anus* in Cloudy Bay

Based on the sample statistics, relationships between the dimensions were investigated for the *Dosinia anus* population in Cloudy Bay by using inferential statistics via SPSS 18.

Since all the descriptive statistics were closer for the lengths and widths for *Dosinia anus*, it was found whether length and width are equal in individuals for the population in Cloudy Bay. For the normal distributions, a paired sample t-test was carried out using SPSS 18. The null hypothesis is that ‘the dimensions of length and width are same for individuals in *Dosinia anus* population in Cloudy Bay’. The significance was less than $\alpha=0.05$ and hence the null hypothesis was rejected. Thus, although the descriptive statistics are closer, their width and length for individuals are significantly different. Since, the description of the sample shows that the widths are higher than lengths, *Dosinia anus* has larger widths than length.

The correlations were then determined between the variables. The data distributions were normal for width and length, and non-normal for the weights as described previously and summarized in Table 4-3. Thus, Pearson correlation was used between length and weight while other correlations were found via the use of Spearman correlation. Similar to the analyses of *Spisula aequilatera*, some outliers were removed from the analyses to minimize errors in interpretations.

Table 4-3: Normality test for length, width and weights of *Dosinia anus*

	Shapiro-Wilk		
	Statistic	Sig.	Interpretation
Total weight of <i>Dosinia anus</i>	.860	.000	Non-normal
Shell weight of <i>Dosinia anus</i>	.864	.000	Non-normal
Flesh weight <i>Dosinia anus</i>	.879	.000	Non-normal
Length of <i>Dosinia anus</i>	.991	.075	Normal
Width of <i>Dosinia anus</i>	.988	.066	Normal

The null and alternative hypotheses were set as follow for each relationship:

H₀: There is no significant linear correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r = 0$) for *Dosinia anus* in Cloudy Bay’.

H₁: There is a significant linear correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r \neq 0$) for *Dosinia anus* in Cloudy Bay’.

The analysis was done using SPSS 18 and the results are shown in Table 4-4. According to the results, all the variables had statistically significant linear correlations with length at $\alpha = 0.05$ significance level. The correlations in the descending order are $r=0.958$ for width; $r=0.906$ for total weight; $r=0.886$ for shell weight and $r=0.821$ for tissue weight. Thus all the dimensions are strongly correlated with length for *Dosinia anus* living in Cloudy Bay.

Table 4-4: Correlations between length, width and weights of *Spisula aequilatera* at $\alpha = 0.05$

Type of relationship	Pearson correlation	Spearman correlation
Length versus Shell weight		0.886
Length versus Tissue weight		0.821
Length versus of Total weight		0.906
Length versus Width	0.958	

4.3.3 Sex ratio

During the histological examinations, gender was identified individually for a sample of n=535 of *Dosinia anus* in Cloudy Bay. The procedure is described in section 3.2.5.1. The ultimate aim of this analysis is to evaluate the sex ratio of the *Dosinia anus* population in Cloudy Bay. The process is systematically described in this section from the sample statistics to interpretation to the population.

Table 4-5: Sample distribution of surf clams according to sex: *Dosinia anus*

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	total
Male	25	25	35	24	-	17	9	7	33	24	25	21	245
Female	26	32	25	23	-	21	21	14	16	31	28	20	257
Stage 0	0	0	0	0	-	0	12	17	1	3	0	0	33
Total	51	57	60	47	-	38	42	38	50	58	53	42	535

Table 4-5 illustrates the composition of the sample in monthly basis for male, female and ‘stage 0’. The latter were found to be sexually indistinguishable individuals due to empty gonads. The observations were taken over a year similar to the other two species studies and the timeframe was from Aug, 2012 to Jul, 2013. Due to practical disturbances, there are no observations for Dec, 2012. For the whole period, the sample includes 494 individuals. The majority of the sample were females and n=257 in total. This was followed by males having n=245 and hence the difference between male and females are marginal in total. There were 33 individuals belonging to the stage 0. The stage 0 individuals were highest during Feb and Mar, 2012 such that 28.5% and 44.7% proportions are belonged to the category, respectively. This may indicate the major spawning period for *Dosinia anus* population in Cloudy Bay.

To see the proportions of males and females in the sample, sex ratios were calculated on a monthly basis and illustrated in Table 4-6. There were no sexually indistinguishable individuals considered when calculating the sex ratios. The analysis showed that the male: female sex ratio is 1.05:1 in the sample. In the sample, the highest sex ratio was for Feb, 2013 with a ratio of 2.33:1 in favour of males. The second highest sex ratio is for April, 2013 where females were abundant with 2.06:1 sex ratio. Although males were predominant in more months, their sex ratios were not that favourable overall. Thus, sample shows that the population may be distributed evenly, but the generalization should be done via the use of inferential statistics.

Table 4-6: Sex ratios for *Dosinia anus* in monthly basis and as a total

	Male	:	Female
August, 2012	1.04	:	1
September, 2012	1.28	:	1
October, 2012	1	:	1.4
November, 2012	1	:	1.04
December, 2012		:	
January, 2013	1.24	:	1
February, 2013	2.33	:	1
March, 2013	2	:	1
April, 2013	1	:	2.06
May, 2013	1.3	:	1
June, 2013	1.12	:	1
July, 2013	1.05	:	1
Total	1.05	:	1

4.3.3.1 Sex ratio of the population of *Dosinia anus*

The sex ratio for the Cloudy Bay *Dosinia anus* populations was determined by using a chi-square goodness of fit test, which was done by using SPSS 18. The null and alternative hypotheses were set as follows:

was performed to evaluate whether the sample proportion for male: female sexes was significantly different ($\alpha=0.05$) from the hypothesized proportion, 1:1, in the population.

The null and alternative hypotheses were set as follow for each relationship:

H_0 : Male: female ratio for *Dosinia anus* is 1:1 in Cloudy Bay.

H_1 : Male: female ratio for *Dosinia anus* is not 1:1 in Cloudy Bay.

The test showed that the Asymptotic significance is 0.608 ($>\alpha=0.05$) for $X^2=0.264$ and degree of freedom (df) was one. Since the level of significance is greater than the accepted level of significance in this study, the related null hypothesis could not be rejected. Therefore, both males and females are equally distributed in the population of *Dosinia anus* in Cloudy Bay.

Table 4-7: Chi-square goodness of fit test statistics for sex ratio: *Dosinia anus*

Chi-Square				.264
Df				1
Asymp. Sig.				.608
	Observed N	Expected N	Residual	
Female	257	251	-6	
Male	245	251	6	
Total	502			

4.3.4 Sexual cycle

This section discusses the reproductive cycles of male and female *Dosinia anus* in Cloudy Bay. The ultimate aim of this section is to evaluate the seasonal variations and relationships of it to exogenous factors. The analysis is based on histological evidences, and the procedure is described in section 3.2.5.1. The gonad position is depicted in Figure 4-5.

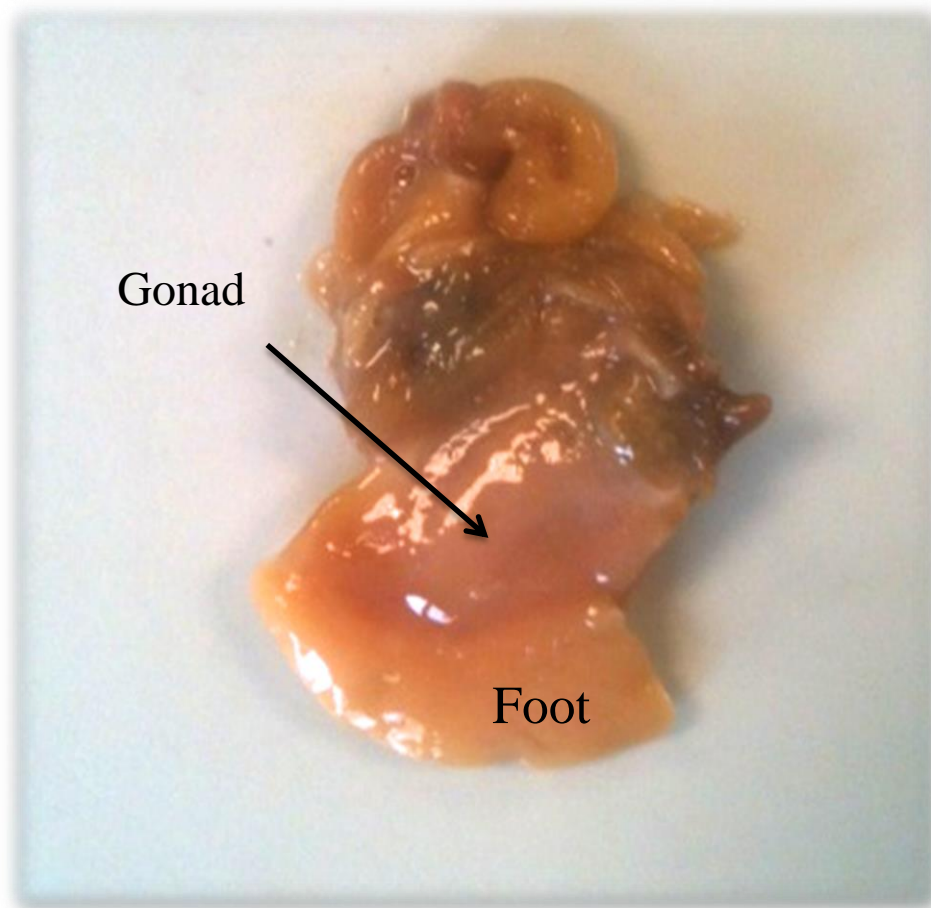


Figure 4-5 : Gonad position: *Dosinia anus*

The six stage scale was used similar to *Spisula aequilatera*. The proportions of individuals belonging to each stage are illustrated in Figure 4-6 (a) and (b) for male and females, respectively, for each month. The analysis is done for 11 months from August, 2012 to Jul, 2013 except for Dec, 2012. When the sexually indistinguishable individuals were found, they were distributed evenly for male and females, which is the same approach for *Spisula aequilatera*.

Male

The male sample considered under this analysis was comprised of n=261.5 individuals. A fractional number was derived due to the distribution of stage 0 individuals based on a 1:1 hypothetical distribution. In August, 2012, there were 25 males which belonged to stages 3, 4 and 5 having 15 (60%), 9 (36%) and 1 (4%) individuals, respectively (Figure 4-6 (a)). Thus, both partially and major spawning could be observed in August although the majority is at the ripe stage. In September sample, stage 3 individuals are reduced into 8 (32%) while stage 5 males were increased into 8 (32%). As a percentage, the composition of stage 4 was maintained at 36% similar to the August sample. In October sample, stage 3 males were further reduced while both belonging to stages 4 and 5 were increased. The relevant proportions for stages 3, 4 and 5 were 11% (n=4), 46% (n=16) and 43% (n=15). A remarkable increment of stage 4 individuals could be observed in November, which the proportion was 75% (n=18). Although both males in stage 3 and stage 5 are reduced, the reduction in the latter stage was dramatic compared to the previous month's sample (7% reduction versus 23%). There is no sample for December, 2012 observed in this study.

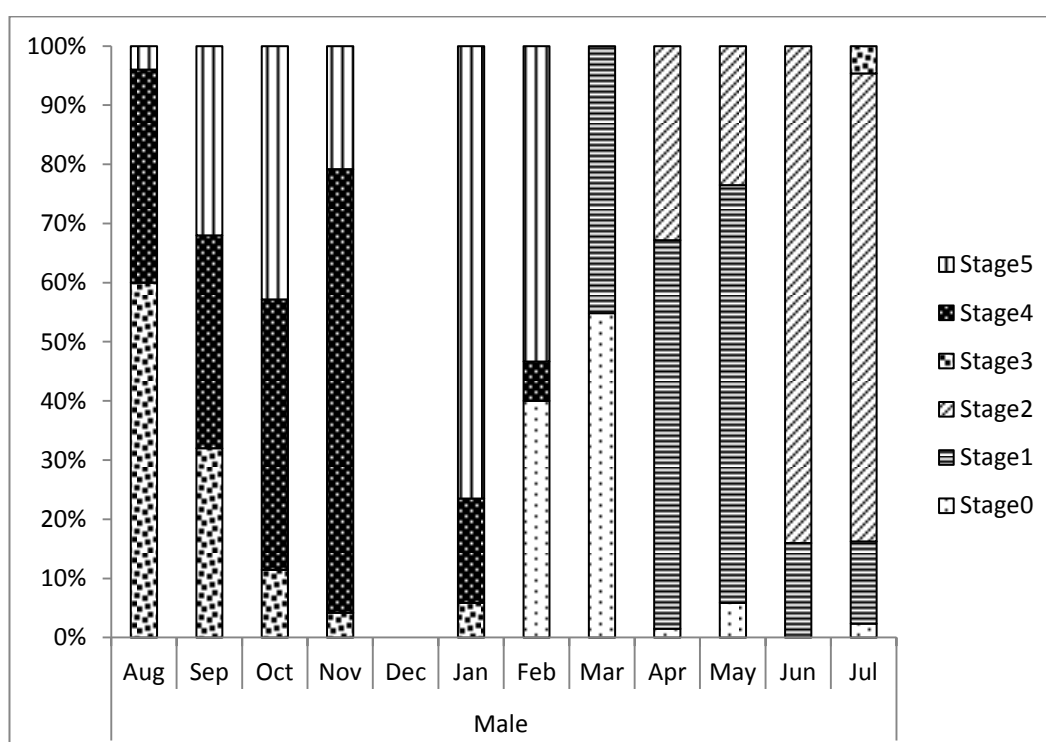
In January, 2013, the proportion of stage 3 individuals was (5.8% and n=1) almost same as that of November. Stage 4 individuals were reduced from 75% to 17.6% (n=3) during Nov-Jan. Consequently, stage 5 males were increased in the sample up to 76.5% (n=13). In the February sample, there are no ripe once and partially spawning was also reduced into 6.7% (n=1). The majority and the second majority were respectively for the spent (53%, n=8) and resting (40%, n=6) stages. February was the latest month that spawning activities were observed.

In March, the distribution of males between the resting and early development was 55:45. Thus, in March, another series of the male reproductive cycle was started. In

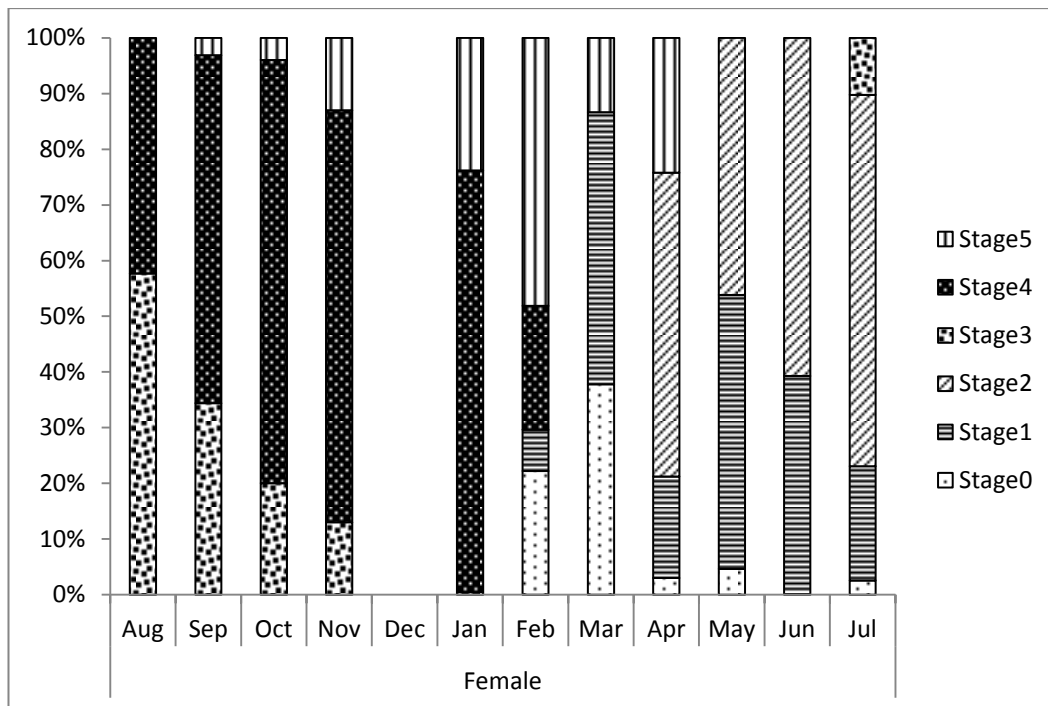
April, the late development stage was started such that the compositions of early to late was 3:2. Until May, individuals at the resting stage were apparent and 5.9% (n=1.5) as a composition for May. For this sample, the composition or early to late individuals was also 3:2. The June sample was comprised only with the early and late development stages and males at the late development stage were abundant (84%, n=21).

In the July sample, one male individual appeared at the ripe stage (14.3%) and hence the ripe stage is started for male *Dosinia anus* in Cloudy Bay. The majority was at the late development stage (81%) and the second majority was for the early development stage (4.7%).

As a remark for the sample observations, seasonal variations were evident in the male reproductive cycle for *Dosinia anus* in Cloudy Bay. Further, there were differences to the observations to *Spisula aequilatera* such as the longevity of spawning activities according to the sample observations. The similarities and differences between the species are discussed in Chapter 6.



(a) Distribution of male surf clams by the reproductive stages: *Dosinia anus*



(b) Distribution of female surf clams by the reproductive stages: *Dosinia anus*

Figure 4-6 : Distribution of male/female surf clams by the reproductive stages: *Dosinia anus*

Female

Female reproductive cycle for *Dosinia anus* sample is depicted in Figure 4-6 (b). In August, 2012, the sample was comprised of only partially spawning (57.7% and n=15) and ripe (42.3% and n=11) individuals. There was no individual belonging to the major spawning stage as seen in males. Indeed, the first appearance of major spawning (3.12% and n=1) was for the September sample for females. In the September sample, the proportion of the ripe stage was decreased (34.3% and n=11) and parallel to that the partially spawning stage becomes the majority (62.5% and n=20). This trend was further continued for October, such that the composition of the sample for the partially spawning, ripe and spent was 20% (n=5, 76% (n=19) and 4% (n=1), respectively. Similar to the males, there was no sample observed for December.

The female sample of January showed considerable differences to the male sample. In January, the majority of the females were at the partially spawning stage still whereas spent individuals were abundant for the males. The composition of the female sample was 76.2% (n=16) and 23.8% (n=5) for the partially spawning and spent stages, respectively.

Out of the six stages, there were four that could be observed in the female sample for February. The majority belonged to the spent stage and the proportion was 48% (n=13) of the total. Both resting and partially spawning individuals were equally distributed so that the proportions are 22% (n=6) per each. There were two females which the stage was the early development. Thus, another female reproductive cycle was observed earlier than for males which the early development stage was discovered in March.

In the March sample for females, the early development stage was the most abundant (48%, n=11). This was followed by the resting and spent stages having 37% (n=8.5) and 13% (n=3) proportions respectively in the sample. As a difference to the male sample, major spawning was still continuous for females.

Major spawning was observed at last for April which the female sample had 24% (n=4) of individuals. The majority of the sample was at the late (54.5%, n=9) development stage. The rest of the sample was comprised of individuals at the early development (18%, n=3) and resting (3%, n=0.5) stages.

In May and June, the types of stages present in the female sample were same as for the males, but their proportions were different. In May, the proportions of the early (49%, n=16) and late development (46%, n=15) stages were almost similar for females. There were 4.6% (n=1.5) of individuals at the resting stage still for May.

In June, only two stages were apparent and which are the early and late development stage. The majority was at the late development stage such that the ratio of late: early was 3:2.

Similar to the male sample, there were ripe individuals (10%, n=2) appeared in the female sample for July. The first and second majorities were for the late (70%, n=14) and early development stages (20%, n=4), respectively.

According to the sample observations for males and females, the major difference was with regards to their major spawning. The major spawning period for females is longer than males according to the sample (Sep-Apr for females versus Aug-Feb for males). For both cycles, seasonal variations were evident and hence the significance of seasonal variations was found through statistical generalization.

4.3.4.1 Seasonal variations in the reproductive cycles for *Dosinia anus* in Cloudy Bay

In this section, the significance of the seasonal variations observed according to the sample observations are generalized into the *Dosinia anus* population in Cloudy Bay. Two Kruskal-Wallis tests were performed by using SPSS18 for males and females separately. The null and alternative hypotheses were set as follow:

H₀: The male/female reproductive cycle for *Dosinia anus* in Cloudy Bay has no seasonal variations throughout the year.

H₁: The male/female reproductive cycle for *Dosinia anus* in Cloudy Bay has seasonal variations throughout the year.

The test results and subsequent interpretations are discussed for males and females under the following sub-sections.

Male

Table 4-8 illustrates the test statistics for the Kruskal-Wallis test for males. The asymptotic significance for the null hypothesis was less than $\alpha=0.05$ for $X^2=207.608$ and $df=10$. Hence, the null hypothesis was rejected and consequently the alternative hypothesis was accepted. Thus, there are significant variations in the male reproductive cycle for *Dosinia anus* in Cloudy Bay. Further, the seasonal variations influence for 84.9% ($\chi^2 / (N-1) = 207.608/244$) of variations in the mean ranks of the reproductive cycle for male for *Dosinia anus*, which is higher than the dependency for male *Spisula aequilatera* in Cloudy Bay (53.4% versus 84.9%).

Table 4-8: Kruskal-Wallis test statistics for the male reproductive cycle of *Dosinia anus*

Chi-Square	207.068	
Df	10	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	25	143.82
Sep	25	170.70
Oct	35	185.30
Nov	24	176.75
Dec	-	-
Jan	17	205.50
Feb	9	214.61
Mar	7	27.50
April	33	45.67
May	24	41.13
June	25	73.28
July	21	76.24
Total	245	

From the above test, it was impossible to determine where differences occur. Thus, several post-hoc Kruskal-Wallis tests were performed by comparing two consecutive months. Table 4-9 summarized the results and interpretations for each pair of months. Since the data is not available for December, seasonal variations were found between November and January. There were six situations that seasonal variations were significant at 95% confidence level. Further, there were five other situations that seasonal variations are not significant. By considering these results with the sample observations for males (Figure 4-6 (a)), the following remarks could be made.

Table 4-9: Seasonal variations of reproductive biology of male *Dosinia anus* in Cloudy Bay

Difference	Chi-square	df	Degree of variability in mean rank %	α value	Significant Y/N
Aug-Sep	6.268	1	12.8	0.012	Y
Sep-Oct	2.276	1		0.131	N
Oct-Nov	1.288	1		0.256	N
Nov-Dec-Jan	10.07	1	25	0.002	Y
Jan-Feb	0.618	1		0.432	N
Feb-Mar	14	1	93	0.000	Y
Mar-Apr	5.456	1	13.9	0.048	Y
Apr-May	0.453	1		0.501	N
May-Jun	16.878	1	35.1	0.000	Y
Jun-Jul	0.253	1		0.615	N
Jul (2013)- Aug (2012)	35.133	1	78.1	0.000	Y

Aug-Sep There is a significant seasonal variation of 12.8% in the male reproductive cycle in the *Dosinia anus* population living in Cloudy Bay. Figure 4-6 (a) shows that the amount of seasonal variations accounts for the reduction of ripe individuals and the augmentation of major spawning. During this period, partially spawning remains comparatively stable. Spawning activities for the *Dosinia anus* population begins at lower temperatures compared to *Spisula aequilatera* in Cloudy Bay, which this difference is discussed in Chapter 6.

Sep-Oct There are no significant seasonal variations in the male reproductive cycle for this period for *Dosinia anus* in Cloudy Bay. Figure 4-6 (a) shows that ripe individuals are decreased and major spawning is increased, which is similar to the differences found for the previous period. Thus, these changes can be assumed as gradually continued for Sep-Oct.

Oct-Nov Figure 4-6 (a) shows the similar constitute for Oct and Nov in terms of the types of stages, but partially spawning is increased for November. However, the statistical analysis showed that these differences are no significant in the population.

Thus, during NZ spring (Sep-Oct-Nov), there are no significant variations in the male reproductive cycle the *Dosinia anus* population living in Cloudy Bay. During

this period, spawning activities happens both as partial and major and ripe individuals are gradually decreased.

Nov- Jan

Since there was no sample for December, 2012, the comparison was made between January, 2013 and November, 2012. The analysis showed that the amount of seasonal variations in the reproductive cycle of the *Dosinia anus* population living in Cloudy Bay is 25%. According to the sample, major spawning activities are increased dramatically during this period while both partially spawning and ripe individuals are decreased. The ripe stage seems to be almost over at the end of this period. Since the summer has begun during this period, augmentation in major spawning activities can be considered as triggered by increasing temperatures.

Jan-Feb

As With regards to differences between the two months, partially spawning activities are reduced and the resting stage has begun at the end of this period in the sample. As per similarities, the majority is for the spent stage in both months belonging to this period. Since the variations are not statistically significant, the transformations (partially spawning-to-spent; spent-to-resting) happened during this period can be assumed as gradual for the population of *Dosinia anus* in Cloudy Bay.

Feb-Mar

The highest degree of variability in mean ranks for the *Dosinia anus* population is for this period and is 93%. Since the peak temperature belongs to this period, the highest variability can be assumed as due to temperature, which is a similar conclusion to *Spisula aequilatera* in Cloudy Bay. Both partially and major spawning activities disappear during this period; for males, another series of reproductive cycle is started at the end of this period; the majority is changed from the spent stage to resting.

According to the investigation of this study, *Dosinia anus* males in Cloudy Bay continue spawning activities throughout NZ Summer (December- January- February) and discontinue their spawning activities at the end of Summer.

Mar-Apr There is a 13.9% amount of variations in the male reproductive cycle of *Dosinia anus* in Cloudy Bay between March and April. Individuals at the resting stage are reduced dramatically during this period. The late development stage is started and the majority is changed from the resting to the early development stage. With decreased temperatures, the amount of seasonal variation is reduced by about 79% compared to the previous duration, which is observed for *Spisula aequilatera* in Cloudy Bay also.

Apr-May There is no significant difference between the male reproductive cycle for *Dosinia anus* in Cloudy Bay between April and May.

In NZ Autumn (March-April-May), the early development stage is abundant. At the beginning of Autumn, the early development stage is started and continued to grow until the end of Autumn. At the mid of Autumn, the late development stage is started.

May-June There is a significant difference of 35.1% during this period for male *Dosinia anus* in Cloudy Bay. At the beginning of NZ winter (June), the late development stage is increased dramatically compared to May consequently the early development stage is reduced.

June-July During this period, there is no significant change for the male reproductive cycle. However, at the end of this period, Figure 4-6 (a) showed evidence to support the beginning of the ripe individuals.

July-Aug There was no sample for August which belonged to the same year. Thus, Aug, 2012 sample was used as an approximation to compare the seasonal variations between July and August. There are significant changes in the male population of *Dosinia anus* in Cloudy Bay for this period and which the amount is 78.1%. These differences were apparent in the sample observations. In August, the early and late development stages are disappeared; both partially and major spawning is begun; the proportion of the rip stage is dramatically increased.

Thus, at the last month of NZ winter (August), spawning activities are in progress for male *Dosinia anus* in Cloudy Bay.

Female

This section described the seasonal variations for the female population of *Dosinia anus* in Cloudy Bay. A summary of the Kruskal-Wallis test is shown in Table 4-11. The asymptotic significance was less than $\alpha=0.05$ for $X^2=175.009$ and $df=10$. Thus, the null hypothesis that ‘there are no seasonal variations in the female reproductive cycle of the *Dosinia anus* population’ could be rejected. Consequently the female reproductive cycle has significant seasonal variation. Seasonal variations account for 69% in the reproductive cycle, which is lower than that of *Dosinia anus* males (84.9%).

Table 4-10: Kruskal-Wallis test statistics for the female reproductive cycle of *Dosinia anus*

Chi-Square	175.009	
Df	10	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	26	142.94
Sep	32	159.38
Oct	25	168.88
Nov	23	178.61
Dec	-	-
Jan	21	193.17
Feb	19	219.71
Mar	14	68.39
April	16	103.69
May	31	45.71
June	28	51.75
July	18	70.61
Total	253	

From the post-hoc Kruskal-Wallis test done to each pair of months, it was possible to identify where significant differences occur in the female reproductive cycle. The results are summarized in Table 4-11. There were six months which seasonal variations were significant and another five months without significant variations. Similar to *Dosinia anus* males, remarks were made by using these results along with Figure 4-6 (b).

Table 4-11: Seasonal variations of reproductive biology for female *Dosinia anus*

Difference	Chi-square	df	Degree of variability in mean rank %	α value	Significant Y/N
Aug-Sep	3.391	1		0.066	N
Sep-Oct	1.323	1		0.250	N
Oct-Nov	1.189	1		0.276	N
Nov-Dec-Jan	1.231	1		0.248	N
Jan-Feb	7.821	1	20	0.005	Y
Feb-Mar	14.342	1	44.8	0.000	Y
Mar-Apr	5.754	1	19.8	0.016	Y
Apr-May	7.828	1	17	0.005	Y
May-Jun	0.885	1		0.347	N
Jun-Jul	5.814	1	12.9	0.016	Y
Jul-Aug	30.539	1	71	0.000	Y

**Aug-Sep-
Oct-Nov-
Jan** From August to January, there are no significant variations between two consecutive months. Figure 4-6 (b) shows that spawning activities are increased gradually while the proportion of the ripe stage is reduced. Regarding differences to the male cycle, there were no significant variances in the population for the periods of Aug-Sep and Nov-Jan which the male cycle has significant seasonal variability. The intensity of major spawning activities seems to be less compared to males. Thus, during NZ spring (Sep-Oct-Nov) and early summer (Dec-Jan), there are no significant variations in the female reproductive cycle of *Dosinia anus* living in Cloudy Bay.

Jan-Feb There is a statically significant variation in the reproductive cycle during this time for the females of *Dosinia anus* in Cloudy Bay. Seasonal variations account for 20% of variability in the man ranks of the reproductive cycle for this period. During this period, the majority is changed from the partially spawning to the spent stage. As individual at the resting and early development stage are shown at the end of this period, spawning activities should be rapid.

Feb-Mar For the period of the highest temperatures, 44.8% of the variations are found in the female reproductive cycle for this period. Figure 4-6 (b) shows that the proportion of major spawning individuals is reduced considerably

and partially spawning activities have disappeared. The majority is changed from the spent stage to the early development stage at the end of this period. Compared to the male reproductive cycle, the seasonal variability is less for females during the highest temperatures (93% versus 44.8%).

Thus, in mid and late summer, there are significant seasonal variations in the female reproductive cycle. At the end of this period, only partial spawning is discontinued and still major spawning happens for females only. However, most major spawning activities for females belong to this period.

Mar-Apr Seasonal variations significantly influence the reproductive cycle of the female population of *Dosinia anus* in Cloudy Bay according to the findings of this study. In total, 19.8% of variability in the man ranks of the reproductive cycle is due to seasonal changes. Figure 4-6 (b) shows that the proportions of resting and early development stages are reduced dramatically. Individuals at the late development stage are first appeared and occupied the majority in April.

Apr-May There is a significant variance in the female reproductive cycle for this period, and hence the behaviour during this period is different from that of male. Spawning activities are disappeared at the end of this period whereas this happens two months before for males. The composition is almost taken by individuals of the early and late development stages.

There is no statistically significant difference for this period. The composition is taken by the early and late development stages. This observation is also different for males where the reproductive cycle has significant variations during this time. The difference is due to the more rapid early-to-late transformation happening in the male cycle.

May-Jun

In NZ Autumn (March-April-May), the early and late development activities are predominant for females, which is a similar conclusion to males. Spawning activities are gradually decreased during this time for female *Dosinia anus* in Cloudy Bay.

Jun-Jul

There is a 12.9% variation for this period in the main ranks of the reproductive cycle. Figure 4-6 (b) shows that individuals of the early development stage are reduced while the late development stage is increased. Furthermore, similar to males, the first ripe individuals appeared at the end of this period.

**Jul
(2013)-
August
(2012)**

Since there was no sample for August, 2013, the comparison was done between Jul, 2013 and Aug, 2013, which is a similar approach to male sample. The seasonal variability is highest for the female reproductive cycle during this period and is 71% of total variability in mean ranks. These variations are accounted for by the disappearance of early and late development activities and beginning of partially spawning in August. Even then, there are no major spawning activities in the female reproductive cycle and this is slightly different from the male reproductive cycle in August.

Thus, during NZ winter (August), there are three transformation activities in the female reproductive cycle which are early-to-late, late-to-ripe and ripe-to-partially spawning.

As a conclusion to the above results, significant seasonal variations are evident for both male and female reproductive cycles of *Dosinia anus* in Cloudy Bay. The next section evaluates correlations with temperature for seasonal variations in both cycles.

4.3.4.2 Correlations between temperature and reproductive cycles for *Dosinia anus* in Cloudy Bay

The correlations were found by using SPSS 18. Since the data distributions were non- normally distributed, the non-parametric alternative test of Pearson correlation, which is Spearman, was done. The null and alternative hypotheses were set as follow for both males and females:

H₀: There is no significant linear correlation between the male/female reproductive cycle and temperature for *Dosinia anus* in Cloudy Bay ($r = 0$)

H₁: There is a significant linear correlation between the male/female reproductive cycle and temperature for *Dosinia anus* in Cloudy Bay ($r \neq 0$)

According to the analysis, both cycles had significant correlations at $\alpha=0.05$ significance level. The Spearman correlations were similar for males and females ($r=0.319$). Thus, with increasing temperatures, ripe, partially and major spawning activities are increased for *Dosinia anus* in Cloudy Bay. The influence of temperature seems less compared to *Spisula aequilatera* in Cloudy Bay and further discussion are done to clarify differences in Chapter 6.

4.3.5 Condition index for *Dosinia anus* in Cloudy Bay

This section discusses the condition index for *Dosinia anus*. The section discusses the sample data first and then inferential statistics are described to interfere to the population. Mean and the standard deviations of the condition indices are given for each month in Table 4-12. There were no samples for August and December.

Table 4-12: Condition Indices for *Dosinia anus* in Cloudy Bay

Month	Mean Condition Index	Stand Deviation
Aug		
Sep	6.45	1.56
Oct	7.67	1.97
Nov	5.70	1.82
Dec		
Jan	5.83	0.77
Feb	5.64	0.82
Mar	5.16	0.87
Apr	5.35	0.65
May	5.15	0.66
Jun	5.22	0.52
Jul	5.09	0.81

The highest mean monthly condition index was for October according to the available data. The man and standard deviation of the condition index for October is 7.67 and 1.97, respectively. From then to until July, the index seemed to decrease and the lowest values could be observed during March-July with marginal differences in the mean values. This could happen due to the presence of resting individuals during Feb-April, but their condition index did not increase with the development of another cycle. Thus, the variation of the condition index with the reproductive cycles for *Dosinia anus* was not related as it was for *Spisula aequilatera* sample.

During September to October, the condition index increased, but absence of sample data for August made it impossible to evaluate the changes between July-August and September.

The variation of the condition index for the sample is graphically shown in Figure 4-7 for *Dosinia anus*. According to the figure, the seasonal variations of the condition index were lower than for *Spisula aequilatera*. To evaluate the significance of seasonal variations in the population statistical tests were performed.

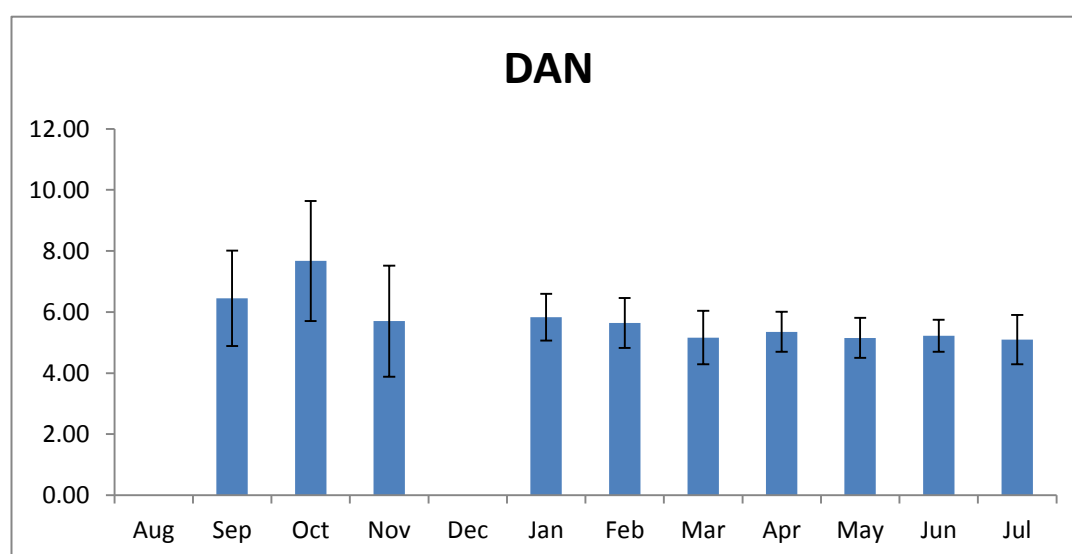


Figure 4-7: Condition Indices for *Dosinia anus* in Cloudy Bay

4.3.5.1 Seasonal variations in the condition index for *Dosinia anus* in Cloudy Bay

The null and alternative hypotheses were set for a Kruskal-Wallis test as follow to evaluate the seasonal variations in the condition index for *Dosinia anus* in Cloudy Bay:

H_0 : The condition index for the *Dosinia anus* population in Cloudy Bay is similar throughout the year.

H_1 : The condition index for the *Dosinia anus* population in Cloudy Bay varies throughout the year.

Table 4-13 shows the asymptotic significance was 0.000 ($<\alpha=0.05$) for $X^2=72.047$ and $df=9$. Thus, there are significant seasonal variations in the condition index throughout the year for the *Dosinia anus* population in Cloudy Bay. The amount of

variability in the mean ranks of the condition index is 28 % ($x^2 / (N-1) = 72.047 / 256$).

Table 4-13: Kruskal-Wallis test statistics for the condition index of *Dosinia anus*

Chi-Square	72.047	
df	9	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	-	-
Sep	30	178.67
Oct	25	211.84
Nov	23	126.26
Dec	-	-
Jan	24	152.29
Feb	21	136.43
Mar	24	99.08
April	25	109.96
May	27	94.85
June	28	90.36
July	30	95.17
Total	257	

Table 4-14: Seasonal variations of the condition index of *Dosinia anus* in Cloudy Bay

Difference	Chi-square	Degree of variability	α value	Significant
		in mean rank (%)		Y/N
Aug-Sep	-	-	-	-
Sep-Oct	8.064	15	0.005	Y
Oct-Nov	12.527	27	0.000	Y
Nov-Dec	-	-	-	-
Dec-Jan	-	-	-	-
Jan-Feb	0.709		0.400	N
Feb-Mar	3.313		0.069	N
Mar-Apr	0.490		0.484	N
Apr-May	0.996		0.318	N
May-Jun	0.150		0.699	N
Jun-Jul	0.490		0.484	N

Post-hoc Kruskal-Wallis tests were done to evaluate significant variations among consecutive months (Table 4-14). For the available data, significant seasonal variability in the condition index could be found only for Sep-Oct and Oct-Nov. These differences could happen due to changes in ripe individuals and spawning

activities, but not clearly distinguish from the histological evidences. From January to July, there were no seasonal variations between consecutive months. Thus, although seasonal variations were found, the condition index does not become a good indicator to describe the reproductive cycle for *Dosinia anus* in Cloudy Bay.

4.3.5.2 Correlations between temperature and the condition index for *Dosinia anus* in Cloudy Bay

This section evaluates the correlations between temperature and the condition index for *Dosinia anus* in Cloudy Bay. Non-parametric Spearman correlation was used to test the hypotheses which are given below:

H₀: There is no linear correlation between the condition and temperature for *Dosinia anus* in Cloudy Bay ($r = 0$)

H₁: There is a linear correlation between the condition index and temperature for *Dosinia anus* in Cloudy Bay ($r \neq 0$)

The analysis gave a significant negative correlation of $r=-0.281$ for the condition index of *Dosinia anus* in Cloudy Bay. Thus, in summer months, their condition index is low. However, the sample data does not provide evidence to interrelate this with reproductive biology.

4.4 Discussion

The findings for *Dosinia anus* are discussed along with the objectives of this study under this section. These findings are based on a sample having 95% of lengths between 44.89 mm and 66.67 mm. There was no past study done for *Dosinia anus* in Cloudy and hence statistical techniques were used widely to construct reliability and validity to the findings.

According to the findings at 95% confidence level, length and width of *Dosinia anus* in Cloudy Bay are strongly correlated such that the Spearman correlation coefficient is $r=0.958$. Total weight of the population has the second highest correlation ($r=0.906$) among the variables considered. Thus, length can be considered as an indirect measure for other physiological parameters.

Under the histological examinations, gonads were examined through a microscope according to the procedure is described in section 3.2.5.1. Statistical inference

through a chi-square goodness of fit test showed that the population distribution is not significantly different from 1:1 distribution for males and females. Thus, the *Dosinia anus* and *Spisula aequilatera* populations have different properties in Cloudy Bay in terms of the sex ratio.

To achieve the first objective of this study, the reproductive cycles of male and female *Dosinia anus* were investigated through histological techniques which are described in section 3.2.5.1. The categorization was based on the six-stage scale introduced by Xie and Burnell (1994). Based on the sample harvested for a year, seasonal variations were evident and hence the variability in the reproductive cycles for the population was found by using Kruskal-Wallis tests. The summary of the results are shown in Table 4-15 and Table 4-16 for male female, respectively. The summary includes the majority at different seasons, major activities and significant variations among consecutive months. Similar to *Spisula aequilatera*, the second majority is sometimes given after the abundant stage when the proportions are considerable. The only exogenous factor in this study was temperature and which is given in the tables to indicate the influence from external factors toward the reproductive cycles.

In the population, both male and female reproductive cycles have significant seasonal variations such that the variability is higher for male (84.9% versus 69%). According to these findings, *Dosinia anus* males in Cloudy Bay are more sensitive to seasonal variations than *Spisula aequilatera* (84.9% versus 53.4%). The sensitivity of females is slightly higher for *Spisula aequilatera* females than *Dosinia anus* (73% versus 69%).

Table 4-15: summary of the reproductive biology changing with exogenous factors: male *Dosinia anus* living in Cloudy Bay

Table 1. Reproductive biology of male <i>Dosinia anus</i> during the breeding season. Data were collected from 1998 to 2000. The data were pooled for all years. The data were													
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Table 4-16: summary of the reproductive biology changing with exogenous factors: female *Dosinia anus* living in Cloudy Bay

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug																											
<table><caption>Reproductive Biology Data (Estimated from Graph)</caption><thead><tr><th>Month</th><th>Value</th></tr></thead><tbody><tr><td>Aug</td><td>10</td></tr><tr><td>Sep</td><td>11</td></tr><tr><td>Oct</td><td>12</td></tr><tr><td>Nov</td><td>13.5</td></tr><tr><td>Dec</td><td>15</td></tr><tr><td>Jan</td><td>17.5</td></tr><tr><td>Feb</td><td>18.5</td></tr><tr><td>Mar</td><td>16</td></tr><tr><td>Apr</td><td>15.5</td></tr><tr><td>May</td><td>13</td></tr><tr><td>Jun</td><td>11.5</td></tr><tr><td>July</td><td>10.5</td></tr></tbody></table>														Month	Value	Aug	10	Sep	11	Oct	12	Nov	13.5	Dec	15	Jan	17.5	Feb	18.5	Mar	16	Apr	15.5	May	13	Jun	11.5	July	10.5	
Month	Value																																							
Aug	10																																							
Sep	11																																							
Oct	12																																							
Nov	13.5																																							
Dec	15																																							
Jan	17.5																																							
Feb	18.5																																							
Mar	16																																							
Apr	15.5																																							
May	13																																							
Jun	11.5																																							
July	10.5																																							
significance change		No		No		No	Yes (20%)		Yes (44.8%)		Yes (19.8%)		Yes (17%)		No		No		Yes (71%)																					
activities		Major spawning begins					partially-to-major spawning		Resting/early development appear		Spent-to-resting Resting-to-early		late development begins		Major spawning stops		early-to-late and ripe begins		late and early disappear, ripe increase and partially spawning begins																					
majority		Ripe/ partially spawning		partially spawning/ ripe		Partially spawning/ spent	Partially spawning		Spent/ resting Partially spawning		early development/ Resting		Early development/ spent		Early/ late development		Late /early development		Late development																					

As one of the major differences, females were in major spawning for a longer period than of males (males: Aug-Feb, females: Sep-Apr). In overall, their reproductive cycles do not seem much different. In New Zealand Spring (September-October-November), both males and females are primarily at partial and major spawning. During these months, temperature increases as 11, 11.9 and 13.5 °C. Therefore, *Dosinia anus* in Cloudy Bay begins their spawning activities at lower temperatures than *Spisula aequilatera* living in Cloudy Bay. According to the sample data (Figure 4-6), the intensity of spawning increases with rising temperatures. Similar to *Spisula aequilatera*, *Dosinia anus* in Cloudy Bay is mostly active during New Zealand summer. Although there is no sample for December, most intense major spawning could be seen for both male and females. At the end of summer, considerable amount of males and females are at the resting stage. In March, which is the beginning of New Zealand autumn, another series of the reproductive cycle begins and hence similar to *Spisula aequilatera* in Cloudy Bay. During autumn and winter, *Dosinia anus* is mainly at the early and late development stages. In the end of winter (August), their spawning activities start when the temperatures are low as 10 °C.

To achieve the third objective of this study, correlations were found between exogenous factors and the reproductive cycles of *Dosinia anus* living in Cloudy Bay. As described previously, the scope of this study is limited only for a single environmental factor, which is sea surface temperature. According to the hypothesis testing done at $\alpha=0.05$ significance, male and female reproductive cycles were significantly correlated such that the non-parametric correlation was $r=0.319$ for both. Thus, similar to *Spisula aequilatera*, spawning activities are high when temperatures increase for *Dosinia anus* in Cloudy Bay. These correlations seem lower than that of the *Spisula aequilatera* population. Thus, partial and major spawning activities happen at lower temperatures as found under histological examinations.

In the previous section, the seasonal variations in *Dosinia anus* were found to be higher than *Spisula aequilatera* in Cloudy Bay. On the other hand, their sensitivity to temperature, which this study considers as one factor that causes seasonal variations, is lower than that of *Spisula aequilatera*, even though still significant. Therefore, the influence from other exogenous factors (such as salinity, availability

of food) towards *Dosinia anus* can be hypothesised as higher than for *Spisula aequilatera* in Cloudy Bay.

In this study, the condition index was calculated for *Dosinia anus* to achieve the second objective of this study. The methodology is quantitative and critics developed for its limited usage to evaluate gametogenesis and gonadal development in surf clams (Grant & Creese, 1995; Joaquim et al., 2008).

Similar to the reproductive cycles, a Kruskal-Wallis test showed that there are significant seasonal variations in the condition index for the *Dosinia anus* population in Cloudy Bay. The findings were based on a sample harvested throughout a year, except for August and December. At 95% confidence level, the seasonal variability in the condition index was 28%. These variations were mainly due to the differences between Sep-Oct and Oct-Nov. However, there was not strong evidence like in *Spisula aequilatera* to describe the variations of condition index in the *Dosinia anus* population by using histological evidences. Thus, this study finds the condition index as inadequate to describe the reproductive biology of *Dosinia anus* living in Cloudy Bay. Further, to achieve the third objective of this study, correlations were found between the condition index and temperature for *Dosinia anus*. The analysis showed a negative correlation which the Spearman coefficient is $r=-0.281$ for the *Dosinia anus* population in Cloudy Bay. Thus, similar to *Spisula aequilatera*, the condition index decreases with rising temperatures.

Deep water Tuatua (*Paphies donacina*)



5 Deep water Tuatua (*Paphies donacina*)

5.1 Introduction

Deep water tautau is a colloquial term used for the type of surf clam species that is scientifically known as *Paphies donacina*. *Paphies donacina* belongs to the family of Mesodesmatidae which the other two species are toheroa (*Paphies ventricosum*) and pipi (*Paphies australis*) (Grant & Creese, 1995; www.fish.govt.nz, 2012). The review of literature provides credence that *Paphies donacina* is the mostly investigated type of surf clam specie in NZ. Past investigations are focused on their abundance (H. J. Cranfield & Michael, 2001a), growth rate (H J Cranfield & Michael, 2001b) as well as their reproductive cycle (Grant & Creese, 1995). However, the focus of investigation is only limited to the abundance and growth rate with respect to Cloudy Bay environment where the current study's site is located. The following sections provide a comprehensive view of literature to identify the scope and limitations of past studies.

The life history of *Paphies donacina* is investigated by Cranfield and Michael (1993). As they determined, the duration of planktonic larval development varies about two to three weeks. According to the authors' articulation, this time duration allows larvae to disperse across a wide area, f other exogenous factors allow. There juveniles are highly mobile (Cranfield & Michael 1993) and this is a controversial observation to Ropes (1980) who finds that juveniles of surf clams do not prefer to move frequently via the examination of Atlantic surf clams. Furthermore, when *Paphies donacina* grows up, they tend to move from intertidal habitats to lower intertidal and shallow sub tidal environments (www.fish.govt.nz, 2010).

The length-weight relationship is found for tuatua based on two samples taken from East Auckland and southern (probably Dunedin) coastal areas, but www.fish.govt.nz (2010) stresses that the applicability of this relationship is debatable for other populations. *Paphies donacina* grow up to 40-70 mm of a shell length when they are about 3-year-old and become 50-80mm of maximum when the age is 5 years (www.fish.govt.nz, 2010). Thus, their growth seems to be rapid during first three years. Maximum length and growth rates are found to be highly dependent of surrounding environmental conditions(H. J. Cranfield & Michael, 2001a).

Paphies donacina is widespread in the South Island, north coast of Stewart Island the lower part of the north Island (Grant & Creese, 1995) as mentioned in section 2.6.1. More specifically, in the same section, it is mentioned that Nahuka, Kapiti Coast, and Pegasus Bay are major habitats for *Paphies donacina* in addition to Cloudy Bay (H J Cranfield & Michael, 2001b). Cloudy Bay is found as more suitable for *Paphies donacina* than Kapiti Coast in terms of their asymptotic size although faster growth can be seen in Kapiti Coast (section 2.6.2).

Paphies donacina is customary and recreationally important to New Zealanders from long time ago and Pegasus Bay and Canterbury are specific locations that mainly handpicking of *Paphies donacina* is done for recreational purposes (Ministry of Fisheries, 2011). *Paphies donacina* is introduced into the Quota Management System (QMS) on 1 April 2004 with a TACC of 168 tonnes, which was supposed to be increased in 2010 up to 629 tonnes based on a biomass survey (Ministry of Fisheries, 2011). As mentioned in section 2.7.1, the current allowances are far below the potential. These future settings are based only biomass surveys (www.fish.govt.nz, 2010) and hence the current study believes that investigations focused on their reproductive biology are required to set sustainable fishery regulations.

There are two studies which are already done for *Paphies donacina* as described in section 2.7.2, which are Greenway (1981), and Grant and Creese (1995). These two studies are based on the west coast of the North Island and North eastern coast respectively. As a result of these surveys, some of the facts are found for their spawning cycles. As an example, between September and November and between February and April are recognized as two main spawning areas of *Paphies donacina* in north-eastern New Zealand (www.fish.govt.nz, 2012). The current study evaluates these properties with respect to the Cloudy Bay environment.

5.2 Materials and methods

The same research methodology as for *Spisula aequilatera* (section 3.2).

5.3 Data, results and interpretations

This section provides data of the sample, results and interpretations to the population for *Paphies donacina* in Cloudy Bay.

5.3.1 Environmental parameters

The measurements taken for temperature are mentioned previously in section 3.2.3 under the results for *Spisula aequilatera*.

5.3.2 Size and growth

Table 5-1 shows the descriptive statistics for *Paphies donacina* sample harvested from Cloudy Bay. The sample was taken from August, 2012 to Jul, 2013 monthly basis and had 972 individuals in total.

Table 5-1: Descriptive statistics for the sample of *Paphies donacina*

<i>Paphies donacina</i>				
	length (mm)	Width (mm)	Total wet weight (g)	Shell weight (g)
N	972	972	972	972
Mean	48.55	74.08	51.35	31.47
Median	48.50	74.10	48.82	29.76
Mode	49	70	46 ^a	34
Std. Deviation	5.556	8.285	16.703	11.021
Minimum	23	32	11	7
Maximum	77	99	138	92

a. Multiple modes exist. The smallest value is shown

The lengths of individuals were normally distributed approximately between the maximum and minimum values of 77 and 23mm, respectively. The mean central tendency was 48.55mm with 5.556 mm standard deviation. Thus, the lower and upper limits of the sample lengths are 37.66 (mean-1.96 x SD) and 59.44mm (mean+1.96 x SD).

The sample widths were also normally distributed approximately with a mean of 74.08 mm and 8.285 mm of a standard deviation. The 95% confidence interval of the width is (57.84, 90.32mm). The maximum and minimum values for the sample width were 32 and 99mm.

Neither total nor shell weights were normally distributed. Hence, in this study, quartiles were used to describe the variance of the sample. The mean and median

central tendencies were 51.35 and 48.82g, respectively (Table 5-1). 25% of the data for total weight were between 32 and 40.53g (Table 5-2). Another 25% of data points were between 58.92 and 138g.

The sample mean and median central tendencies for shell weights were 31.47 and 29.76g, respectively. These data were distributed between 7 (minimum) and 92g (maximum). From them, 25% were below 24.19g and another 25% were above 39.02g.

Table 5-2: Quartiles for the total and shell weights of *Paphies donacina*

Percentiles	Total weight of <i>Spisula aequilatera</i> (g)	Shell weight of <i>Spisula aequilatera</i> (g)
25	40.53	24.19
50	48.82	29.76
75	58.95	36.02

5.3.2.1 Relationship between length, width and weights

By using the sample data described above, the relationships were predicted via the use of graphical means. Length was used as the benchmark and its relations to width (Figure 5-1), total (Figure 5-2), shell (Figure 5-3) and tissue (Figure 5-4) weights are illustrated below. Trend lines were added for each relationship by setting the intercept at (0, 0).

Similar to the two species discussed before, the strongest linear relationship could be observed between length and width. The coefficients was $m=1.524$ and hence the highest figure among the three species investigated in this study. The distances between the actual data and the theoretical linear distribution are small according to the illustration given in Figure 5-1.

The second highest linear coefficient was for ‘length versus total weight’. The coefficient was $m=1.0766$ and the data points were considerably deviated from the theoretical linear distribution (Figure 5-2). For shell and tissue weights, the relationships are further reduced. Consequently the coefficients were reduced to $m=0.660$ (Figure 5-3) and $m=0.416$ (Figure 5-4).

The significance of these four linear relationships is discussed in the next section related to the *Paphies donacina* population in Cloudy Bay.

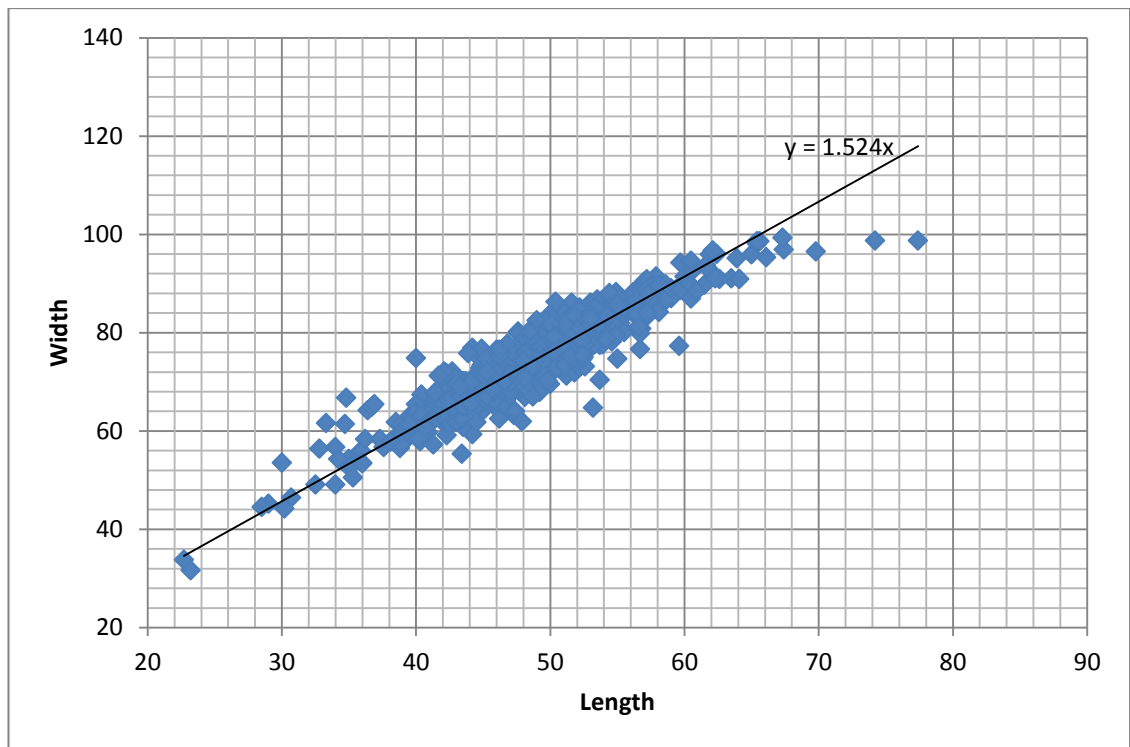


Figure 5-1 :Length versus width: *Paphies donacina*

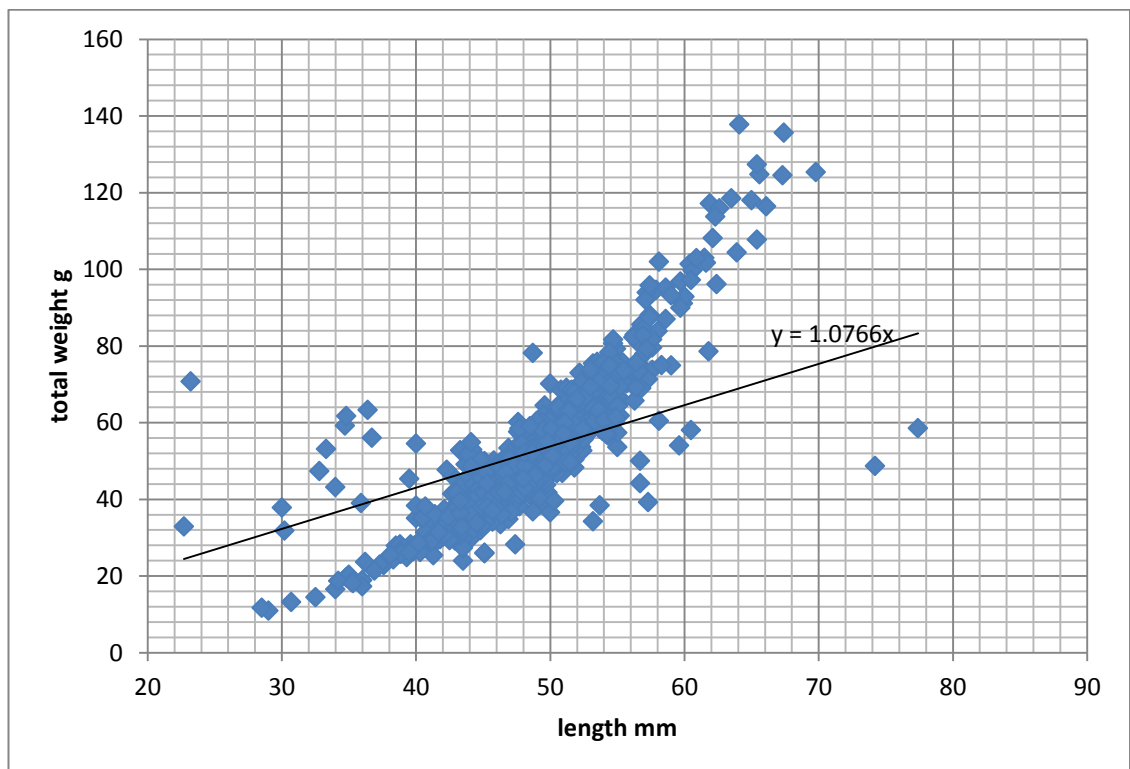


Figure 5-2 :Length versus total weight: *Paphies donacina*

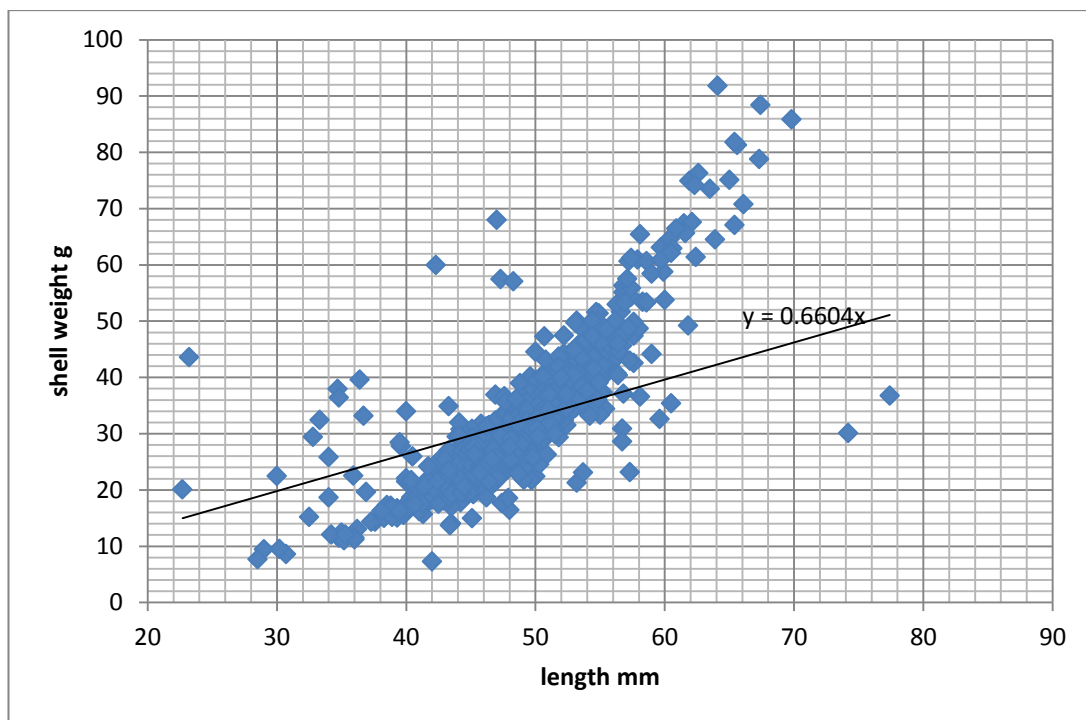


Figure 5-3 :Length versus shell weight: *Paphies donacina*

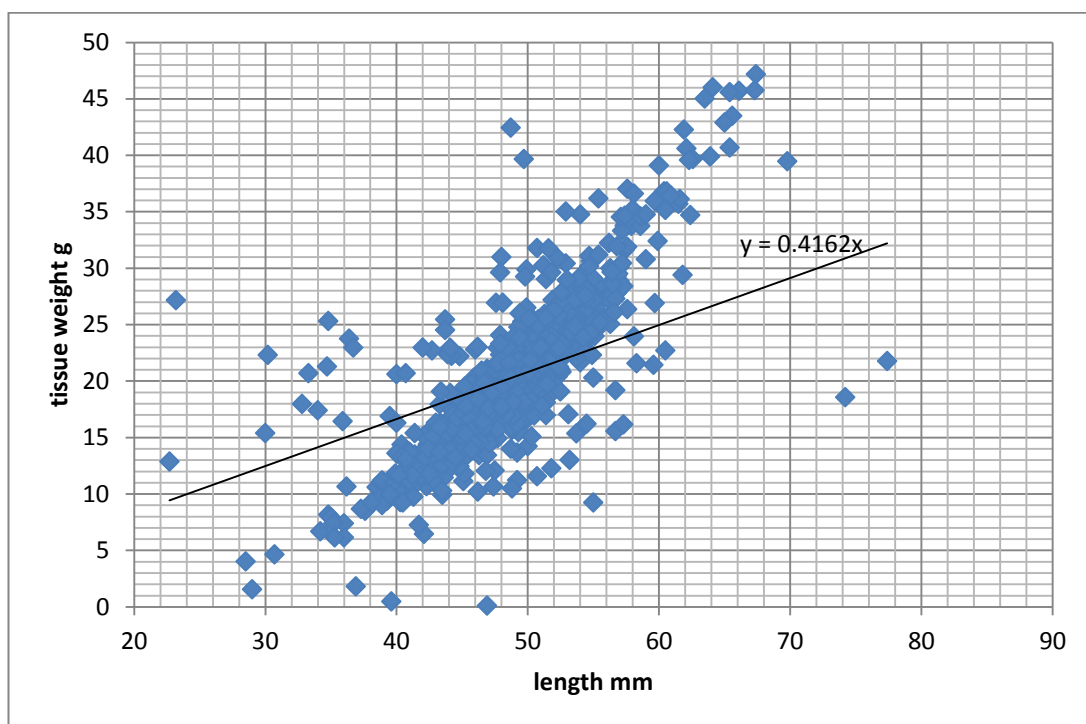


Figure 5-4 :Length versus flesh weight: *Paphies donacina*

5.3.2.2 Correlations between length, width and weights in the populations of *Dosinia anus* in Cloudy Bay

Correlations between the variables were found by using SPSS 18. The sample descriptions given in the above section showed the presence of some outliers and hence some data points were removed from the analysis for each variable to increase accuracy.

As a preliminary step to decide the type of analysis (parametric or non-parametric), normality assessments were done by using a SW test. These test results are summarized in Table 5-3 with the interpretations at $\alpha=0.05$ significance level. The data distributions of length and width were normal and hence Pearson correlation was used as the most appropriate test. For the weights, non-parametric Spearman correlation was used.

Table 5-3: Normality test for length, width and weights of *Paphies donacina*

	Shapiro-Wilk		
	Statistic	Sig.	Interpretation
Total weight of <i>Paphies donacina</i>	.965	.000	Non-normal
Shell weight of <i>Paphies donacina</i>	.982	.000	Non-normal
Flesh weight <i>Paphies donacina</i>	.916	.000	Non-normal
Length of <i>Paphies donacina</i>	.985	.052	Normal
Width of <i>Paphies donacina</i>	.990	.076	Normal

The null and alternative hypotheses were set as follow for each relationship:

H₀: There is no significant linear correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r = 0$) for *Paphies donacina* in Cloudy Bay’.

H₁: There is a significant linear correlation between the dependent variable (width, total weight, shell weight and flesh weight) and length ($r \neq 0$) for *Paphies donacina* in Cloudy Bay’.

The results are shown in Table 5-4. All the variables were significantly correlated with length at $\alpha = 0.05$ significance level. The highest correlation was for width and which was $r=0.924$. The second highest correlation was for total weight ($r=0.891$) and those were followed by shell and tissues weight which the Spearman correlations were $r=0.872$ and $r=0.744$, respectively. Thus, the difference between the correlation

coefficients for shell and tissue weights were the highest for *Paphies donacina* among the three species studied in this study.

Table 5-4: Correlations between length, width and weights of *Spisula aequilatera* at $\alpha = 0.05$

Type of relationship	Pearson correlation	Spearman correlation
Length versus Shell weight		0.872
Length versus Tissue weight		0.744
Length versus of Total weight		0.891
Length versus Width	0.924	

5.3.3 Sex ratio

Through histological evidences, gender was identified for a sample of n=581 for *Paphies donacina* in Cloudy Bay. The procedure of testing is similar to that of *Spisula aequilatera*, which was described in section 3.2.5.1. In addition to male and female, there were individuals at the 'stage 0' where sex was indistinguishable. Furthermore, there was one hermaphrodite in the sample which is shown in Figure 5-5.

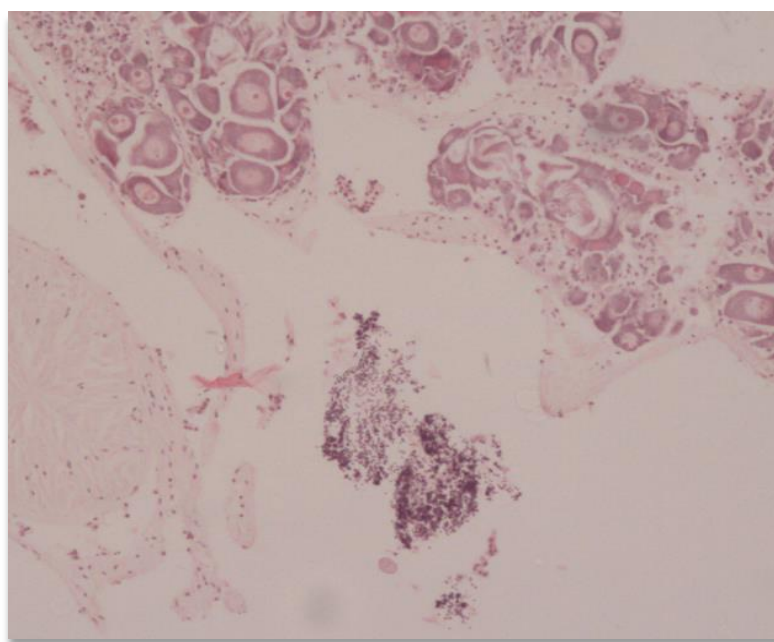


Figure 5-5 : presence of hermaphrodite: *Paphies donacina*

The results are summarized monthly basis for the sample in Table 5-5.

Table 5-5: Sample distribution of surf clams according to sex: *Paphies donacina*

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	total
Male	12	14	28	20	32	27	19	20	23	28	32	19	274
Female	17	21	26	13	28	31	27	24	22	31	17	19	276
Stage 0	16	4	0	0	0	0	0	0	0	0	3	8	31
Total	45	39	54	33	60	58	46	44	45	59	52	46	581

There were only 31 surf clams belonging to the 'stage 0'. These species were found for the period of Jun-Sep and the maximum number of them was for August (n=16, 35.6%). There were 274 and 276 individuals for male and female, respectively, and hence the proportions are almost similar. The ratios between males and females for the sample were calculated on a monthly basis and illustrated in Table 5-6. In this analysis, sexually indistinguishable organisms were not considered.

Compared to other two species, the monthly sex ratios for the sample were closer to 1:1 distribution. The highest deviation was for June, 2013 where the sex ratio of male: female was 1:88:1. As a total, the distribution was different from 1:1 distribution if the third decimal point is considered. To generalize the sex ratio into the Cloudy Bay population, inferential statistics were done.

Table 5-6: Sex ratios for *Paphies donacina* monthly basis and as a total

	Male	:	Female
August, 2012	1.00	:	1.42
September, 2012	1.00	:	1.50
October, 2012	1.08	:	1.00
November, 2012	1.54	:	1.00
December, 2012	1.14	:	1.00
January, 2013	1.00	:	1.15
February, 2013	1.00	:	1.42
March, 2013	1.00	:	1.20
April, 2013	1.05	:	1.00
May, 2013	1.00	:	1.11
June, 2013	1.88	:	1.00
July, 2013	1.00	:	1.00
Total	1.000	:	1.007

5.3.3.1 Sex ratio of the population of *Paphies donacina*

A chi-square goodness of fit test was done to generalize the sex ratio of the population using SPSS 18. The null and alternative hypotheses were set as follows:

The null and alternative hypotheses were set as follow for each relationship:

H₀: Male: female ratio for *Paphies donacina* is 1:1 in Cloudy Bay.

H₁: Male: female ratio for *Paphies donacina* is not 1:1 in Cloudy Bay.

The Asymptomatic significance was 0.623 ($>\alpha=0.05$) for $X^2=0.256$ and degree of freedom (*df*) of one. Since the level of significance of the test was greater than $\alpha=0.05$, the related null hypothesis could not be rejected. Thus, both males and females are equally distributed in the population of *Paphies donacina* in Cloudy Bay, which the finding is similar to *Dosinia anus*.

Table 5-7: Chi-square goodness of fit test statistics for sex ratio: *Paphies donacina*

Chi-Square				.256
Df				1
Asymp. Sig.				.613
	Observed N	Expected N	Residual	
Female	276	275	-1	
Male	274	275	1	
Total	550			

5.3.4 Sexual cycle

Through gonad examination, the stages of the reproductive cycles were identified for individuals in the sample of *Paphies donacina* from August, 2012 to Jul, 2013. The six stage scale was used similar to *Spisula aequilatera*. Their gonad position is shown in Figure 5-6.

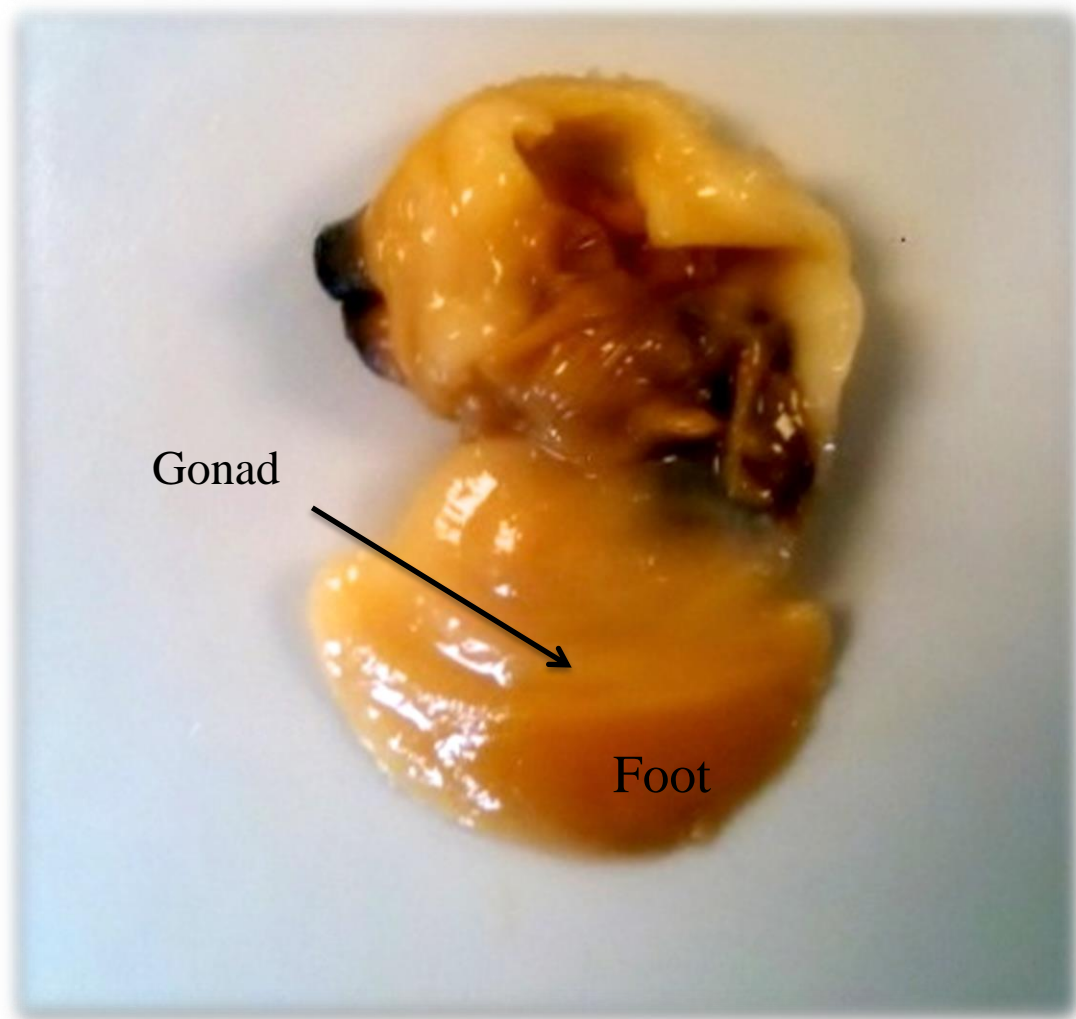


Figure 5-6 : Gonad position: *Paphies donacina*

The distribution of the sample is graphically illustrated in Figure 5-7 (a) and (b) for male and female, respectively. Similar to other two species, the sexually indistinguishable organisms were divided evenly between the males and females as an approximation.

Male

The male sample was comprised of $n=289.5$ organisms in total. The fractional numbers are since of the 1:1 distribution of 'stage 0' individuals.

In August, 2012, there were 20 males which belonged to stages 0, 1 and 5 (Figure 5-7 (a)). Since there were no organisms in the early development stage for July, 2013, August can be considered as the beginning of a new series of the reproductive cycles for male *Paphies donacina* in Cloudy Bay. The sample contained two individuals

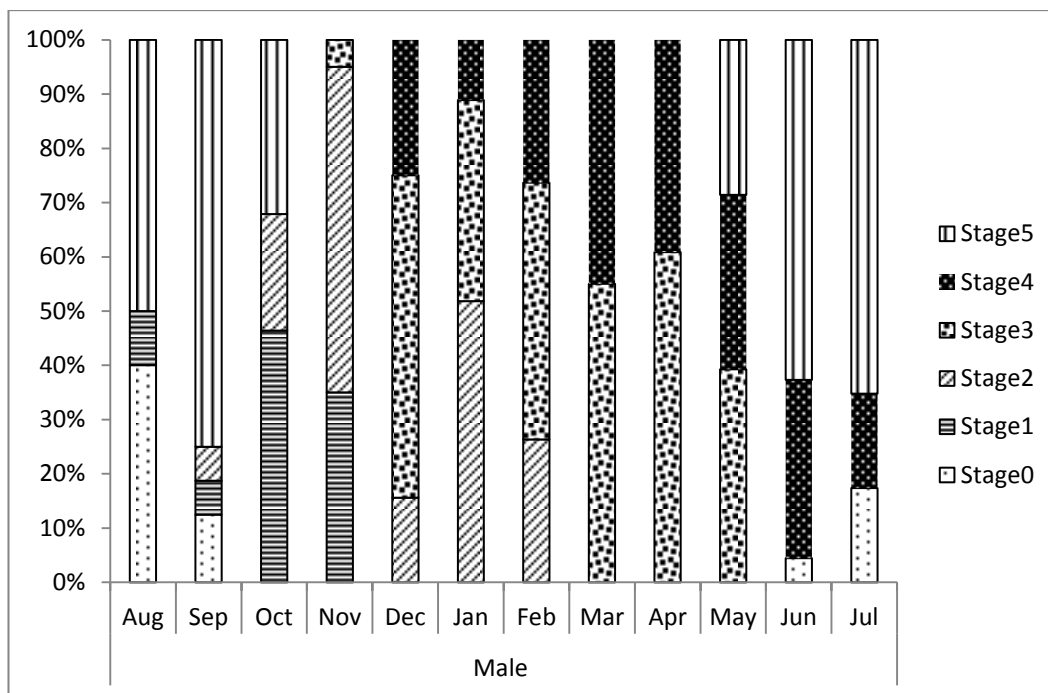
(10%) for the early development stage. The majority was for the spent stage (n=10, 50%) and it was followed by resting individuals (n=8, 40%). In September, 75% of the sample was comprised of the stage 5. Both early and late development stages had one individual per each (6.25%). Only 12.5 % (n=2) individuals belonged to the resting stage. In October, the majority was changed from the spent stage to the early development stage (n=13, 46.4%) compared to the previous two months. This was followed by the organisms of the stages '0' and '2' which the proportions were 21.4% (n=9) and 32.2% (n=6). In November, ripe individuals could be observed to the first time and contained only one being (5%). The majority was changed to the late development stage (n=12, 60%) from the early development stage (n=7, 35%) compared to the previous month.

In December, partially spawning activities could be discovered (n=8, 25%). The ripe stage had the majority and which was 59.3% (n=19) from the total sample of *Paphies donacina*. The rest (n=5, 15.7%) was for the late development stage. In January, the type of species discovered were same in terms of their stages, but the proportions of the stages '2', '3', '4' were 51.9%, 37% and 11.1%, respectively. In February also only stages '2' (n=5, 26.3%), '3' (n=9, 47.4%), '4' (n=5, 26.3%) could be observed.

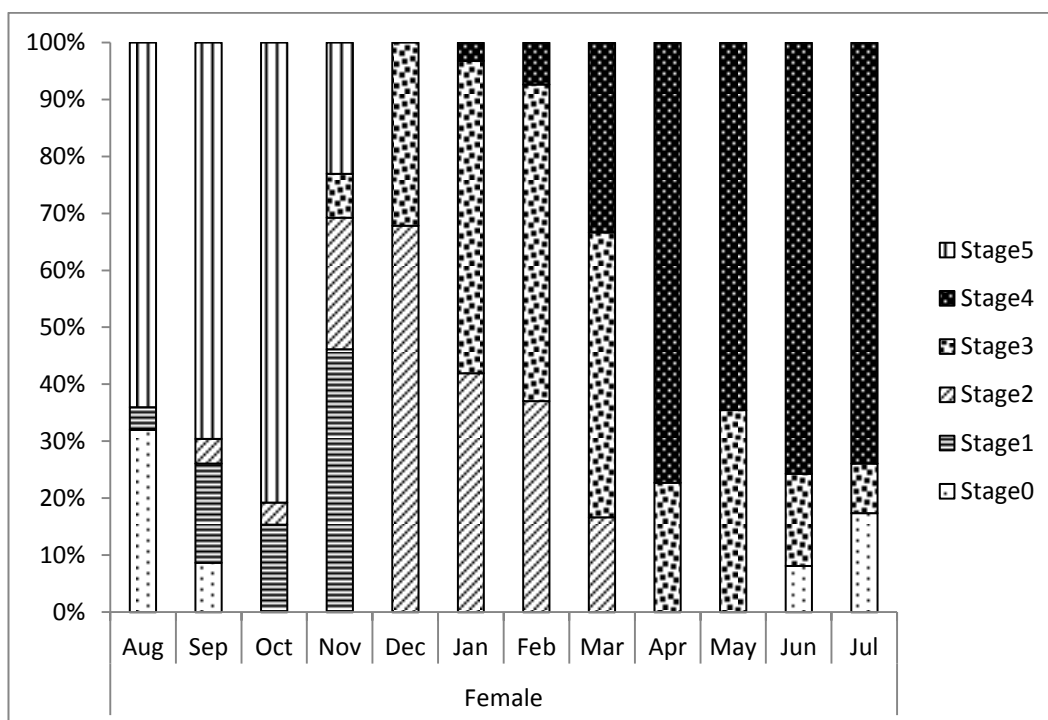
In March, the late development stage was disappeared and the whole sample contained only ripe and partially spawning organisms having a ratio of 11:9 (n_{Total}=20). This ratio was changed to 6:4 (n_{Total}=23) in April for males. May is the first month that major spawning occurred (n=8, 28.7%). Ripe and partially spawning individuals had 39.2% (n=11) and 32.1% (n=9), respectively.

In June, ripe males were not available and instead there were three individuals at resting and they were evenly distributed between males and females (4.5% per each group). Spawning activities in terms of partially and major were 32.8% (n=11) and 62.7% (n=21), respectively. The last month observed in this study contained 65.2% (n=15) organisms having major spawning. The partially spawning and resting stages had 17.4% (n=4) per each category.

As a total, the spawning activities were apparent throughout the year for the males in the sample except in November. During summer, spawning activities were comparatively the lowest and only partially spawning activities could be observed for males. This is different from observations made in for other two species.



(a) Distribution of male surf clams by the reproductive stages: *Paphies donacina*



(b) Distribution of female surf clams by the reproductive stages: *Paphies donacina*

Figure 5-7 : Distribution of male/female surf clams by the reproductive stages: *Paphies donacina*

Female

The female sample is depicted in Figure 5-7 (b). Similar to the males observed, the beginning of a new reproductive cycle can be considered as August for females according to the sample distribution. As other similarities to the male sample, the

majority and the second majority were for the spent (n=16, 64%) and resting (n=8, 32%) stages, respectively. The first appearance of females belonging to the late development stage (n=1, 4.3%) was for September and hence showed similarity to the male sample. The majority was at the spent stage (n=16, 69.6%) and this was followed by the individuals of the stages '1' and '0' having the proportions of 17.4% (n=4) and 8.7% (n=2), respectively.

In October, individuals at the resting stage were removed and 80.8% (n=21) of the species in the sample were having major spawning activities. The compositions of the early (n=4, 15.4%) and late development (n=1, 3.8%) stages remained almost similar to the previous month.

A major difference between the samples for November was the presence of the spent stage (n=3, 23.1%) in the female sample only. Ripe females were appeared similar to males in this month (n=1, 7.7%). Both early (n=6, 46.1%) and late (n=3, 23.1%) development stages were increased in terms of proportions compared to the previous month's sample.

There were no partially spawning activities for December and hence the samples of males and females showed differences. The sample was comprised of individuals at the late (n=19, 67.9%) and early (n=9, 32.1%) development stages. In addition to these two stages, partially spawning activities (n=1, 3.2%) could be observed in the January sample. The majority and the second majority were for the ripe (n=17, 54.9%) and late development (n=13, 41.9%) stages in January. In February, the stages were similar to those of January and the proportions of stages '2', '3', and '4' were 37% (n=10), 55.6% (n=15) and 7.4% (n=2), respectively. In March, the corresponding proportions of stages '2', '3', and '4' were 16.7% (n=4), 50% (n=12) and 33.3% (n=8), respectively. During NZ summer, the seasonal changes in the sample of females were the lowest compared to other seasons and which was similar to the males.

In April and May, the samples contained individuals belonging to the ripe and partially spawning stages. For April, the proportions of ripe and partially spawning individuals were 22.7% (n=5) and 77.3% (n=17), and those were 35.5% (n=11) and 64.5% (n=20) for May, respectively. In May, only for males, major spawning activities observed.

The June sample did not have any individual at the spent stage. There are some individuals at the resting stage due to the assumptions made in the study that species at stage 0 are divided equally among males and females. Thus, the assumption is doubtful here and the sample may provide credence to support that female major spawning starts later and is shorter than males. In July sample also, there were no individual observed for the spent stage. For June and July, nearly 75% of the samples were for the partially spawning while the rest is at the ripe stage.

According to the sample descriptions provided for males and females, *Paphies donacina* seems to behave differently than other two species considered in this study. The differences are described in detail under chapter 6 after the sample findings are generalized to the population *Paphies donacina* in Cloudy Bay.

5.3.4.1 Seasonal variations in the reproductive cycles for *Dosinia anus* in Cloudy Bay

Seasonal variations are generalized for the population of *Paphies donacina* in Cloudy Bay by using the sample data described above. Kruskal-Wallis tests were performed for males and females by using SPSS18. The null and alternative hypotheses were set as follow:

H_0 : The male/female reproductive cycle for *Paphies donacina* in Cloudy Bay has no seasonal variations throughout the year.

H_1 : The male/female reproductive cycle for *Paphies donacina* in Cloudy Bay has seasonal variations throughout the year.

The test results and interpretations are discussed under the sub-headings below.

Male

Table 5-8 summarizes the results of the Kruskal-Wallis test for males. The level of significance for the hypothesis test was 0.000 ($\alpha=0.05$) for $X^2=119.745$ and $df=11$. Thus, the null hypothesis could be rejected and hence there are significant seasonal variations in the male reproductive cycle throughout the year. The amount of seasonal variations was 42.2% ($x^2 / (N-1) = 119.745 / 284$) in the man ranks of the male reproductive cycle and hence the lowest seasonal changes among the three species.

Table 5-8: Kruskal-Wallis test statistics for the male reproductive cycle of *Paphies donacina*

Chi-Square	119.745	
Df	11	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	21	122.86
Sep	16	191.69
Oct	28	102.39
Nov	20	47.40
Dec	32	122.41
Jan	27	91.80
Feb	19	116.97
Mar	20	144.93
April	23	141.02
May	28	174.38
June	32	225.14
July	19	234.00
Total	285	

To evaluate where the significant differences occur, post-hoc Kruskal-Wallis tests were done for each pair of succeeding months (Table 5-9). There were only six periods which the seasonal variations were significant at 95% confidence interval.

Table 5-9: Seasonal variations of reproductive biology of male *Paphies donacina* in Cloudy Bay

Difference	Chi-square	df	Degree of variability in mean rank %	α value	Significant Y/N
Aug-Sep	3.589	1		0.058	N
Sep-Oct	4.509	1	10	0.034	Y
Oct-Nov	0.278	1		0.598	N
Nov- Dec	29.755	1	58	0.000	Y
Dec-Jan	7.763	1	13	0.005	Y
Jan-Feb	3.454	1		0.063	N
Feb-Mar	3.834	1	10	0.050	Y
Mar-Apr	0.148	1		0.701	N
Apr-May	4.778	1	9.6	0.029	Y
May-Jun	13.344	1	23	0.000	Y
Jun-Jul	0.999	1		0.318	N
Jul (2013)- Aug (2012)	0.318	1		0.915	N

- Aug-Sep** No significant seasonal change in the male reproductive cycle for *Paphies donacina* in Cloudy Bay. The majority is at major spawning during this period. The late development first appeared at the end of this period (Figure 5-7 (a)).
- Sep-Oct** There is 10% significant seasonal variability in the mean ranks of male reproductive cycle for this period. The difference account for by the dramatic increase in the early development stage, reduction in major spawning and disappearance of the resting stage during this period.
- Oct-Nov** There are two major transformations according to the sample data during this period and these are spent-to-early development and early development-to-late development. However, in the population, these transformations cannot account for significant seasonal variation and hence the transformation can be considered as gradual during this period (Figure 5-7 (a)).

By considering the above conclusions, only during mid-Spring there is a significant seasonal variability in the reproductive cycle for male *Paphies donacina* in Cloudy Bay. During this period, major spawning is gradually decreased consequently a new series of the reproductive cycle begins for males.

- Nov-Dec** 58% of the variability in the mean ranks of the male reproductive cycle is due to seasonal changes during this period and the highest alteration for the whole year. Partially spawning is started for males and ripe individuals are dramatically increased at the end of this period (Figure 5-7 (a)). Furthermore, there are no individuals belonging to the early development stage at the end of this period and the proportion of the late development stage is also reduced.

Dec- Jan There is 13% of variability in the male reproductive cycle of *Paphies donacina* in Cloudy Bay. However since there is no early development of individuals at the beginning of this period, the dramatic increment in the late development stage cannot be explained. Furthermore, February sample shows near similarities to the December sample. Hence, this difference between the two months may happen due to sampling errors. Considering this fact, the seasonal variability occurred for this period was neglected and seasonal variations were found between December and February samples.

Dec-Feb For this period, a post-hoc Kruskal-Wallis test gave a level of significance for the null hypothesis as 0.650 ($>\alpha=0.05$) for $X^2=0.206$ and $df=1$. Thus, it was not possible to reject the null hypothesis and hence for the period between Dec and Feb, there is no seasonal variability of the reproductive cycle. According to Figure 5-7 (a), partially spawning activities continues steadily while the late-to-ripe transformation happens for male *Paphies donacina* in Cloudy Bay.

Feb-Mar There is a 10% variation in the mean ranks of the male reproductive for this period. The variations are due to the disappearance of the late development stage and the transformations, late-to-ripe and ripe-to-spawning, continuing during this time.

According to the above findings, male *Paphies donacina* in Cloudy Bay has least variability during the summer period (December- January- February). Only partially spawning activities can be observed, but the respective proportions represent the minority in the sample. Thus, male *Paphies donacina* in Cloudy Bay behaves differently than other two species.

Mar-Apr There is no significant variability during this period.

Apr-May There is a significant variability of 9.6% in the mean ranks of the male reproductive cycle between April and May. Figure 5-7 (a) shows that the amount of variations is governed by the beginning of major spawning at the end of this period.

Thus, in NZ Autumn (March-April-May), male *Paphies donacina* in Cloudy Bay spend most of their time at the partially spawning and ripe stages. At the end of autumn, these species begins their major spawning.

May-June During this period, ripe individuals disappear. Major spawning activities are increased rapidly. Some of the species are at the resting stage at the end of this period. Altogether, these changes account for 23% of variability in the mean ranks of the male reproductive cycle of *Paphies donacina* in Cloudy Bay.

June-July There is no significant difference for this period in the male reproductive cycle. Partially and major spawning are continued steadily during this period.

July-Aug The comparison was done between the samples of Jul, 2013 and Aug, 2012 for this period. There was no significant variability that could be found for this period. Although there are a few individuals in the August sample, main activities are the two transformations, which are spent-to-resting and partially spawning-to-spent.

According to these interpretations done to the population, spawning activities of male *Paphies donacina* in Cloudy Bay are densest during NZ winter (Jun-Jul-Aug) and hence can be considered as more active. This is a different observation compared to other two species. During NZ winter, males gradually finish one series of the reproductive cycle. Another series is started at the end of winter.

Female

Table 5-10 illustrates the results of the Kruskal-Wallis test done for female *Paphies donacina* in Cloudy Bay. The level of significance of the test was lower than $\alpha=0.05$ for $X^2=119.106$ and $df=11$ and hence there is a significant seasonal variability in the reproductive cycle of female *Paphies donacina*. According to the results $41.9\% (x^2/(N-1) = 119.106 / 284)$ variability in the mean ranks of the reproductive cycle is governed by the seasonal changes throughout the year, which is almost similar to that of males.

Table 5-10: Kruskal-Wallis test statistics for the male reproductive cycle of *Paphies donacina*

Chi-Square	119.106	
Df	11	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	25	157.68
Sep	23	174.52
Oct	26	200.60
Nov	13	84.62
Dec	28	70.45
Jan	31	88.11
Feb	27	93.65
Mar	24	121.92
April	22	159.02
May	31	151.18
June	16	229.47
July	19	235.18
Total	285	

The results of the Post-hoc Kruskal-Wallis tests are given in Table 5-11. There were some changes compared to the male reproductive cycle which the changes are described in the interpretations below.

Table 5-11: Seasonal variations of reproductive biology of female *Paphies donacina* in Cloudy Bay

Difference	Chi-square	df	Degree of variability in mean rank %	α value	Significant Y/N
Aug-Sep	0.774	1		0.379	N
Sep-Oct	1.053	1		0.305	N
Oct-Nov	10.023	1	26.4	0.002	Y
Nov- Dec	2.006	1		0.157	N
Dec-Jan	4.177	1	7	0.041	Y
Jan-Feb	0.284	1		0.594	N
Feb-Mar	5.619	1	11.2	0.018	Y
Mar-Apr	9.734	1	21.6	0.002	N
Apr-May	0.975			0.323	N
May-Jun	8.964	1	20	0.000	Y
Jun-Jul	0.466	1		0.495	N
Jul (2013)- Aug (2012)	22.36	1	56	0.667	Y

Aug-Sep Similar to the male population, there is no significant seasonal change in the female reproductive cycle for *Paphies donacina* in Cloudy Bay during

this period. The majority is at major spawning during this period and the late development first appeared at the end of this period (Figure 5-7 (b)).

Sep-Oct In terms of stages, both males and females are similar for September and October. However, there is no significant seasonal variability in the female reproductive cycle and hence difference from males. It could be seen from the sample that major spawning is continued steadily while major spawning is reduced and the proportion of the early development stage is increased in the male cycle. Thus, the difference between the two cycles could be explained from the sample data (Figure 5-7 (b)).

Oct-Nov There is a significant variation that contributes to 26.4% variability in the mean ranks of female reproductive cycle for *Paphies donacina* in Cloudy Bay. At the end of this period, individuals at the early development stage increase and ripe individuals are apparent in the sample data. Major spawning activities are reduced dramatically.

By considering the above conclusions, only during the latter part of spring, there is a significant seasonal variability in the reproductive cycle for female *Paphies donacina* in Cloudy Bay. During this period, major spawning is gradually decreased and a new series of the reproductive cycle is begun for females, which is similar to males.

Nov-Dec There are differences in the sample descriptions, but those differences are not statistically significant in the population. Two transformations are late-to-ripe and early-to-late. These transformations can be considered as steady due to the non-significant difference shown in the analysis. At the end of this period, there is no any spawning activity happening and hence different from males where partially spawning is begun at the end of this period.

Dec- Jan There is a significant seasonal variation of 7% in the female reproductive cycle. During this period, individuals of the late development stage reduce consequently the proportion of ripe organisms is increased. Furthermore, partially spawning is started for females at the end of this period.

Jan-Feb There is no statistically significant variability in the female reproductive cycle for this period.

Feb-Mar There is an 11.2% variation in the mean ranks of the female reproductive cycle between February and March. The variations are due to the two transformations which are late-to-ripe and ripe-to-spawning. The late development stage is still apparent at the end of this period which is a different observation to males.

According to the above findings, female *Paphies donacina* in Cloudy Bay spend NZ summer period (December- January- February) mainly at the late development, ripe and partially spawning activities.

Mar-Apr There is a significant variability which counts for 21.6% in the mean ranks of the female reproductive cycle during this period. During this period, partially spawning activities are increased dramatically and the late development stage is disappeared.

Apr-May There is no significant variability in the reproductive cycle.

Thus, in NZ Autumn (March-April-May), female *Paphies donacina* in Cloudy Bay spend most of their time at the partially spawning and ripe stages which is a similar conclusion for males. However, only for males, there is no major spawning at the end of autumn.

May-June During this period, ripe individuals are reduced considerably consequently partial spawning activities are increased at the end. These seasonal variations account of 20% dependency in the man ranks of the female reproductive cycle for *Paphies donacina* in Cloudy Bay.

June-July There is no significant difference for this period in the female reproductive cycle. Partially spawning individuals are abundant throughout the period.

July-Aug Similar to males, the comparison was done between the samples of Jul, 2013 and Aug, 2012. The highest seasonal variations occur during this period, which is 56% in the mean ranks of the reproductive cycle.

Partially spawning is disappeared and major spawning is evident at the end of this period. A new series of female reproductive cycle is begun similar to males.

According to these interpretations done to the population, partially spawning activities are abundant for female *Paphies donacina* in Cloudy Bay in NZ winter (Jun-Jul-Aug). At the end of winter, major spawning happens rapidly. Thus, the correlation between temperature and the reproductive cycles seem to be different than other two species considered in this study.

5.3.4.2 Correlations between temperature and reproductive cycles for *Paphies donacina* in Cloudy Bay

The correlations between temperature and the reproductive cycles were found by using SPSS 18. For the non- normal distributions, the non-parametric (Spearman) correlations were found. The null and alternative hypotheses were set as follows:

H₀: There is no significant linear correlation between the male/female reproductive cycle and temperature for *Paphies donacina* in Cloudy Bay ($r = 0$)

H₁: There is a significant linear correlation between the male/female reproductive cycle and temperature for *Paphies donacina* in Cloudy Bay ($r \neq 0$)

The analysis showed that both male and female cycles had statistically significant correlations with temperature. The Spearman correlations were $r_{\text{male}} = -0.386$ and $r_{\text{female}} = -0.511$. Only for *Paphies donacina*, negative correlations were found in this study and hence their reproductive cycles behave differently than other two species. Spawning activities are thus triggered at low temperatures for *Paphies donacina* in Cloudy Bay and during high temperatures they are at idle at the development and ripe stages.

5.3.5 Condition index for *Paphies donacina* in Cloudy Bay

In addition to histological evidences, condition indices were calculated for the *Paphies donacina* sample in Cloudy Bay. The calculations were done by using

Equation 0-1. The mean and standard deviations are shown for each month in Table 3-14.

Table 5-12: Condition Indices for *Paphies donacina* in Cloudy Bay

Month	Mean Condition Index	Stand Deviation
Aug	5.09	0.81
Sep	9.12	1.67
Oct	8.84	1.24
Nov	-	-
Dec	10.29	2.76
Jan	9.41	1.28
Feb	10.71	2.31
Mar	9.41	1.28
Apr	9.36	2.02
May	9.07	1.34
Jun	7.63	0.95
Jul	8.49	1.03

The highest and the second highest mean monthly condition indices were observed for February ($\mu=10.71$ and $SD=2.31$) and December ($\mu=10.29$ and $SD=2.76$), respectively. From the observations of Figure 5-7(a) and (b), males and females are at the late development and ripe stages at during this time. Spawning activates are comparatively low and no resting individuals were present in the sample. Based on these observations, the highest values can be expected during this time.

The lowest values were during June, July and August, where both reproductive cycles were comprised of most of partial and major spawning activities. Furthermore, the lowest value is for August ($\mu=5.09$ and $SD=0.81$). when the individuals of the resting stage were highest for both male and female throughout the year. Thus, low weights can be expected due to empty gonads. These variations are clearly shown in Figure 5-8.

Due to above remarks, it can be hypothesised that the condition index can provide useful information about the reproductive biology of *Paphies donacina* in Cloudy Bay. Thus, the seasonal variations of the condition indices were investigated for the population by using statistical techniques as described below.

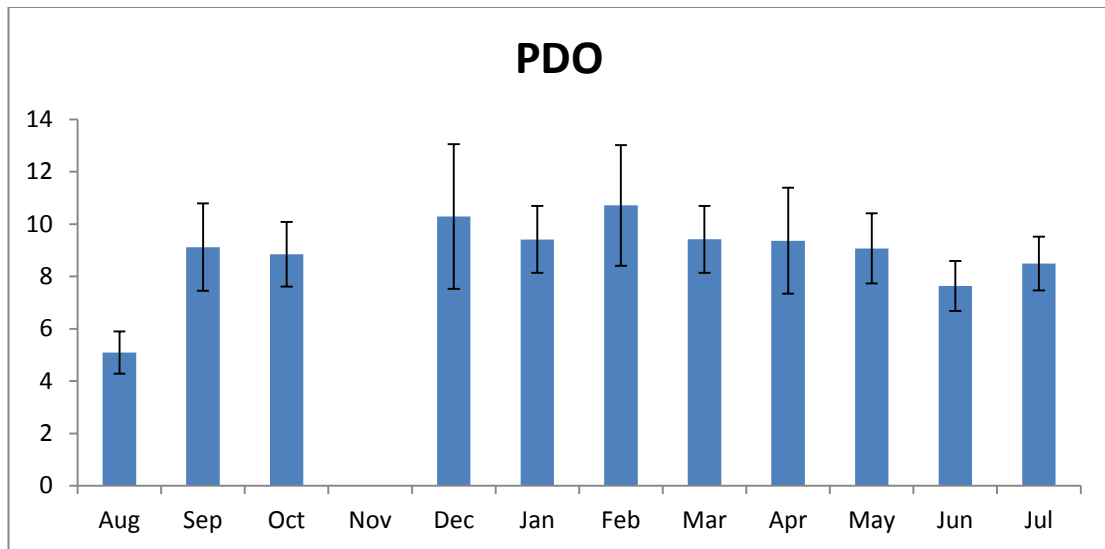


Figure 5-8: Condition Indices for *Paphies donacina* in Cloudy Bay

5.3.5.1 Seasonal variations in the condition index for *Paphies donacina* sample in Cloudy Bay

To evaluate the significance of seasonal variations in the condition index in *Paphies donacina*, a Kruskal-Wallis test was done. The null and alternative hypotheses of the test were as follows:

H_0 : The condition index for the *Paphies donacina* population in Cloudy Bay is similar throughout the year.

H_1 : The condition index for the *Paphies donacina* population in Cloudy Bay varies throughout the year.

The asymptotic significance for the hypothesis test was 0.000 ($\alpha=0.05$) for $X^2=106.694$ and $df=9$, and hence the null hypothesis was rejected. Thus, there are seasonal variations in the condition index throughout the year for the *Paphies donacina* population in Cloudy Bay. These seasonal variation count for 21.9% ($\chi^2/(N-1) = 65.752/298$) variability in the mean ranks of the condition index for *Paphies donacina* in Cloudy Bay.

Table 5-13: Kruskal-Wallis test statistics for the condition index of *Paphies donacina*

Chi-Square	65.752	
Df	10	
Asymp. Sig.	.000	
Month code	N	Mean Rank
Aug	30	142.67
Sep	30	166.67
Oct	5	196.60
Nov	-	-
Dec	29	214.55
Jan	25	171.20
Feb	26	170.08
Mar	30	148.27
April	34	127.82
May	30	187.03
June	30	112.40
Jul	30	62.87
Total	299	

Table 5-14: Seasonal variations of the condition index of *Paphies donacina* in Cloudy Bay

Difference	Chi-square	df	Degree of variability	α value	Significant
			in mean rank (%)		Y/N
Aug-Sep	1.770	1		0.183	N
Sep-Oct	0.889	1		.346	N
Oct-Nov	-	-	-	-	-
Nov-Dec	-	-	-	-	-
Dec-Jan	2.314	1		0.058	N
Jan-Feb	7.618	1	12.3	0.006	Y
Feb-Mar	1.038	1		0.308	N
Mar-Apr	1.045	1		0.307	N
Apr-May	0.091	1		0.763	N
May-Jun	15.466	1	25	0.000	Y
Jun-Jul	10.483	1	17.8	0.001	Y
Jul (2013)- Aug(2012)	18.256	1	30.9	0.000	Y

The results of the post-hoc tests are summarized for each pair of months in Table 5-14. For four months there were statistically significant variations for the *Paphies donacina* population. By observing Figure 5-7 (a) and (b), the difference between January and February can be due to increase in ripe individuals. During May-August, significant reductions are due to partial and major spawning activities, but the increment between June and July cannot be described through the reproductive

biology. Thus, only a few facts can be predicted from the use of condition index for *Paphies donacina* in Cloudy Bay.

5.3.5.2 Correlations between temperature and the condition index for *Paphies donacina* in Cloudy Bay

The aim of this section is at evaluating the correlation between temperature and the condition index for *Paphies donacina* in Cloudy Bay. Non-parametric Spearman correlations were found by testing the following hypotheses.

H₀: There is no linear correlation between the condition and temperature for *Paphies donacina* in Cloudy Bay ($r = 0$)

H₁: There is a linear correlation between the condition index and temperature for *Paphies donacina* in Cloudy Bay ($r \neq 0$)

The analysis showed a positive correlation of $r=0.252$ for *Paphies donacina* and hence this is different compared to the other species that had negative correlations to temperature. Thus, increasing temperatures cause them to increase in the condition index. Since the condition index proportionate to dry tissue weight, temperature and tissue weight are correlated positively.

5.4 Discussion

As mentioned in section 2.7.2, *Paphies donacina* is the most widely evaluated surf clam species in NZ in terms of reproductive biology. However, there is no study where the findings are focused on Cloudy Bay environment. Thus, this study was conducted to evaluate gametogenesis and gonadal development of *Paphies donacina* living in Cloudy Bay. In this discussion, the findings are discussed along with other scholar's findings which are done for other environmental settings, wherever information is available. Thus, variations between different populations can be identified. The findings are based on a sample having 95% lengths between 37.66 and 59.44mm. Based on this sample, which was extracted over a year, properties of the population investigated through statistics as described below to achieve the objectives of the current study.

Physiological properties considered include length, width and weights in terms of total and shells. Similar to other two species, the highest correlation was between length and width, which equals to $r=0.924$. The second correlation was for total weight and length ($r=0.891$). These were followed by the relationships of shell tissue weights with the correlations of $r=0.872$ and $r=0.744$, respectively. Thus, all the physiological properties considered are strongly correlated to their lengths and hence the independent variable can be considered as a good measure to represent width and weights of *Paphies donacina* in Cloudy Bay.

Gonads were examined through a microscope to identify sex according to the procedure described in section 3.2.5.1. The sample was evenly distributed to the third decimal point and statistical inference through a chi-square goodness of fit test generalized the findings into *Paphies donacina* that the population distribution is not significantly different from 1:1. Thus, the distribution of *Paphies donacina* is similar to the *Dosinia anus* population in Cloudy Bay. Furthermore, the sex ratio is similar to the Omaha Bay tautau population (Grant & Creese, 1995). However, their sex ratio can be different between distinct age groups, which this study did not consider as a variable. Grant and Creese (1995) mention that *Paphies australis* are predominant with females at their juvenile stage (length is less than 40mm).

After the sex was distinguished through the histological evidences, the sample was categorized into sex stages by using the scale introduced by Xie and Burnell (1994). The aim of this investigation was to evaluate seasonal variations in the reproductive cycles for *Paphies donacina* living in Cloudy Bay to achieve the first objective of this study. A summary of the Kruskal-Wallis and post-hoc tests are given in Table 5-15 and Table 5-16 for male and female, respectively. Particular emphasizes are given to the majority, major activities and significance of seasonal variability throughout the year.

In the population, both male and female reproductive cycles have significant seasonal variations and the variability in the man ranks of the reproductive cycles are nearly 42% for both. Thus, the least seasonal variability in the reproductive cycle is for *Paphies donacina* among the three species studies in Cloudy Bay. Accordingly, partially or major spawning activities could be observed throughout the year for both cycles except for one month. This is a similar conclusion to Grant and Creese (1995)

whose findings indicate that gametogenesis of tautau in Omaha Bay continues throughout the year without particular period for resting.

During the latter part of the NZ spring, major spawning is gradually decreased consequently a new series of the reproductive cycle begins for males and females. During this period, temperature gradually increases and hence negative influence can be seen towards spawning activities. Thus, their behaviour is different than other two species. During summer, no major spawning activities were observed in the samples. Partially spawning occurs, but less intense compared to other seasons. Thus, NZ summer becomes the least active face in terms of spawning activities for *Paphies donacina* living in Cloudy Bay.

In NZ Autumn (March-April-May), *Paphies donacina* in Cloudy Bay gradually increases their partially spawning activities and males start major spawning. Thus, decreasing temperatures cause to become them more active in terms of reproductive activities. Female spent stage is considerably shorter according to the sample such that males are at the spent stage during May-Oct whereas females are only for Aug-Nov. Although these durations are different, in terms of temperature, both species continue major spawning at temperatures as low as 10 °C and as high as 13.5 °C. Statistical analyses showed that both male and female cycles had statistically significant correlations with temperature in the population. The Spearman correlations were $r_{\text{male}}=-0.386$ and $r_{\text{female}}=-0.511$ and hence *Paphies donacina* is different than other two species which have positive relations. Thus, there is an influence from temperature to have less spawning activities in NZ summer. This finding is different than other scholars' findings which the study sites for tautau are different. As example, *Paphies sub-traingulata* stops their spawning activities in the coldest winter months (Grant & Creese, 1995) which indicates a positive relationship between spawning and temperature. Furthermore, for tautau, minimum threshold temperature is specified by Grant and Creese (1995) as 15 °C, but *Paphies donacina* in Cloudy has only partially spawning activities during the periods of that high temperatures. Their major spawning is mainly between 10-13.5 °C according to this study's findings.

Since spawning activities happen 11 months per year for male and female, there may be other factors that influence their gametogenesis and gonadal development in

addition to temperature. Thus, future studies are required to explain the seasonal variation in the reproductive cycles with environmental factors.

[illegible]

Table 5-16: summary of the reproductive biology changing with exogenous factors: female *Paphies donacina* living in Cloudy Bay

	Aug	Sep		Oct	Nov	Dec		Jan	Feb		Mar		Apr		May		June		July		Aug																										
<div><table><caption>Reproductive Biology Data (Estimated from Graph)</caption><thead><tr><th>Month</th><th>Value</th></tr></thead><tbody><tr><td>Aug</td><td>10</td></tr><tr><td>Sep</td><td>11</td></tr><tr><td>Oct</td><td>12</td></tr><tr><td>Nov</td><td>13.5</td></tr><tr><td>Dec</td><td>15</td></tr><tr><td>Jan</td><td>17.5</td></tr><tr><td>Feb</td><td>18.5</td></tr><tr><td>Mar</td><td>16</td></tr><tr><td>Apr</td><td>15.5</td></tr><tr><td>May</td><td>13</td></tr><tr><td>Jun</td><td>11.5</td></tr><tr><td>July</td><td>11</td></tr></tbody></table></div>																						Month	Value	Aug	10	Sep	11	Oct	12	Nov	13.5	Dec	15	Jan	17.5	Feb	18.5	Mar	16	Apr	15.5	May	13	Jun	11.5	July	11
Month	Value																																														
Aug	10																																														
Sep	11																																														
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Feb	18.5																																														
Mar	16																																														
Apr	15.5																																														
May	13																																														
Jun	11.5																																														
July	11																																														
significance change		No		Yes (26.4%)	No	No	Yes (7%)	No	Yes (11.2%)		Yes (21.6%)	No		Yes (20%)						Yes (56%)																											
activities	Major spawning and Early stage begins		Late stage begins	Early development increases and major spawning reduces			Ripe stage begins		Late-to-ripe Ripe-to-partially spawning	Partially spawning increases, late disappears		Ripe-to-partially spawning	Partially spawning disappears and major spawning begins																																		
majority	Spent/ resting		Spent		Spent		Early/ late development/spent	late development / ripe	ripe /late development	Ripe/late ripe /late development		Ripe/ partially spawning	partially spawning		Ripe/ partially spawning		Partially spawning		Partially spawning																												

The condition index calculated monthly for the sample showed seasonal variation and interrelationships towards the reproductive cycles. At 95% confidence interval, a Kruskal-Wallis test showed significant seasonal variations in the condition index for the population that account for 28% variability in mean ranks of *Paphies donacina* in Cloudy. The highest values were for February and December, where spawning activities are the lowest for *Paphies donacina*. Thus, the presence of ripe individuals and absence of resting stage can contribute to high condition indices in summer. The lowest values were during June, July and August, where in NZ winter that the reproductive cycles were comprised of partial and major spawning activities. Therefore low condition indices can occur due to empty gonad. Therefore, the condition index can be used as an indication to recognize some of the activities in gametogenesis and gonadal development of *Paphies donacina* similar to *Spisula aequilatera* in Cloudy Bay. However, for both species, the condition index cannot provide comprehensive understanding about reproductive biology as described in sections 3.3.5.1 and 5.3.5.1. Thus, histological observations are the most appropriate method to evaluate reproductive biology in surf clams which is a similar conclusion made by Grant and Creese (1995). The behaviour of condition index is different to other two species such that there is a positive correlation of $r=0.252$ for the condition index *Paphies donacina* population with temperature. Thus, when temperatures increases their weight gain can be assumed due to low spawning activities.

6 Conclusion

This section compares the findings of this study for *Spisula aequilatera*, *Dosinia anus* and *Paphies donacina* in Cloudy Bay with particular emphasis towards the gametogenesis and gonadal development. This part of the study can be considered as the achievement of the fourth objective of this study.

In terms of sex ratios, both *Dosinia anus* and *Paphies donacina* have 1:1 distribution in Cloudy Bay. Only in *Spisula aequilatera*, male individuals are significantly predominant. Within each species, their reproductive cycles are mostly similar for males and females except little differences between the longevity of spawning activities.

Between the three species, the reproductive cycles of *Spisula aequilatera* and *Dosinia anus* have some similarities as well as differences to each other. In overall, reproductive behaviours of *Paphies donacina* are vastly different in Cloudy Bay environment compared to other two species.

Three species have significant seasonal variations in their reproductive cycles. The highest amount of seasonal variations can be seen in *Dosinia anus* for male and that of female is for *Spisula aequilatera*. In both cycles, the least, but significant, seasonal variability are for *Paphies donacina* among the three species.

Spawning period of *Spisula aequilatera* is the shortest among the three species. Their partially spawning activities stretch over late spring and early summer (Nov-Dec-Jan) and the required temperature for partially spawning is between 13.5 and 14.9 °C. Major spawning is limited for NZ summer and the least mean temperature for this period is 16 °C. This is the highest temperature threshold for major spawning among the three species. Major spawning activities for *Spisula aequilatera* are limited only for two months (Jan-Mar). In summer, the most rapid changes occur in the reproductive cycles for *Spisula aequilatera* among the three species. On the other hand, the least seasonal variations are for them.

Similar to *Spisula aequilatera*, *Dosinia anus* in Cloudy Bay is mostly active during NZ summer in terms of spawning activities. However, their spawning period (Aug-

Mar) is longer than *Spisula aequilatera*. In the latter part of winter (August), both partial and major spawning activities begin at temperatures as low as 10 °C, which is significantly lower than both threshold limits for major and partial spawning of *Spisula aequilatera*. As another similarity between both species, the beginning of a new series of reproductive cycles happens in NZ autumn.

Spawning activities are the longest for *Paphies donacina* with either partial or major spawning activities occurring in 11 out of 12 months. Compared to other two species, they have no particular resting period. When *Spisula aequilatera* and *Dosinia anus* are less active in NZ Autumn (March-April-May), *Paphies donacina* in Cloudy Bay gradually increases their partially spawning activities. Thus, their spawning is triggered at low temperatures. From May to November, major spawning activities can be observed where temperatures change between 10 °C and 13.5 °C. In NZ summer, no major spawning happens and the intensity of partially spawning is comparatively low for *Paphies donacina* in Cloudy Bay. The behaviour of reproductive cycles can be similarly explained using their correlations to temperature.

In terms the correlations between the reproductive cycles and temperature, both *Spisula aequilatera* and *Dosinia anus* have positive correlations. The highest sensitivity among the two species is for *Spisula aequilatera* in both male and female reproductive cycles. Reproductive biology shows opposite response to temperature for *Paphies donacina* in Cloudy Bay, where the correlation to temperature is negative. Thus, spawning activities increase for *Spisula aequilatera* and *Dosinia anus* while decreasing for *Paphies donacina* with increasing temperatures.

Seasonal variations are significant for the three species in their condition index. The highest seasonal variability is for *Spisula aequilatera* and other two species have similar amount of seasonal variations in the condition index. Similar to the correlations of the reproductive cycle, the behaviour between the condition indices and temperature for *Paphies donacina* is different from the other two species. While the condition indices of *Spisula aequilatera* and *Dosinia anus* decrease with rising temperatures, the condition index is increased for *Paphies donacina*.

As a similarity between *Spisula aequilatera* and *Paphies donacina*, some major changes in the reproductive cycles can be recognized from variations in condition index. These behaviours include the beginning of the early development stage and spawning activities, abundance of ripe individuals and the end of spawning period. The margin of partially spawning to major spawning, and beginning of the late development and ripe stage cannot be identified for *Spisula aequilatera* or *Paphies donacina* through the seasonal variations in the condition index. Interrelations between the reproductive cycles and the condition index cannot be described for *Dosinia anus* living in Cloudy Bay.

In addition to temperature, there can be other exogenous factors that govern seasonal variations of the reproductive cycles for these three species. Those factors are more important for *Paphies donacina* in Cloudy Bay since the findings of gametogenic and gonadal development show diverse patterns compared to other population of tautau studied in NZ. These factors may be salinity, availability of food or genetic variability. The current study suggests future investigations to fill this gap in the context of reproductive biology for *Paphies donacina* in Cloudy Bay.

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