Nutrition and reproductive condition of wild and cultured New Zealand scallops (*Pecten novaezelandiae*)

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Abstract

The New Zealand native scallop, *Pecten novaezelandiae*, is a species with ~~a~~ high economic value as a wild catch and has good potential for cultivation. As a mean to enhance the future of this growing shellfish industry, this thesis set out to investigate the nutritional requirements of *P. novaezelandiae* in relation to reproductive conditions, and determined the physical and biological factors that affect the condition of this scallop species in the wild and cultivated environments.

Adult scallops (*Pecten novaezelandiae*) were sampled from six populations in the Hauraki Gulf (Auckland, New Zealand) in the spawning season (October 2014), in order to evaluate the scallop reproductive condition and nutritional state across the population~~s~~. Results showed a spatial variation in reproduction condition (VGI and gonad index), with a higher number of mature scallops in populations closer to the shoreline, where higher food availability may be found. Conversely, nutrient content in scallop somatic tissues (adductor muscle carbohydrates and digestive gland lipids) did not vary across the population~~s~~, but was strongly associated with reproductive status of individual scallops (VGI).

Nutrient (carbohydrates, proteins and lipids) storage and utilization were investigated within scallops from two sites in the Hauraki Gulf, bi-monthly over a year (2012−2013). In addition, sediment samples were also taken to evaluate the potential for re-suspended nutrients as a food source for scallops. Water samples

were collected for seston and chlorophyll *a* analyses. Isotope analyses (carbon and nitrogen) and proximate analyses were conducted for the gonad, adductor muscle and digestive gland of wild *P. novaezelandiae*, sediment samples and the seston (1.2−5µm, >5µm). Isotope analyses revealed distinctly different signatures in suspended sediment and scallop tissues, indicating that re-suspended nutrients were unlikely to contribute to the diet of scallops. Nevertheless, seston (particularly the small fractions) signatures were closely related to scallop tissue samples, suggesting that it is likely to be the main food source for the wild *P. novaezelandiae*. Scallops from the two sampling sites exhibited similar reproductive cycles and utilization of nutrients. Gametogenesis started in winter, and took place at the expense of carbohydrates stored in adductor muscles. Spawning events were recorded in spring (October−November) and summer (January−March), and the energy demand required during spawning events was supported by digestive gland protein. Gonad re-maturation between spring and summer spawnings were supported by the utilization of digestive gland lipids.

The reproductive condition and nutrient content of scallops were then studied during the spawning season (October 2013) in wild populations and within experimental conditions (fed with a commercial microalgal diet; Shellfish Diet 1800®) in an aquaculture laboratory, in order to identify condition and nutrient requirements for scallop cultivation in New Zealand. Field scallops (feeding on natural food sources) spawned just before the end of the experiment, while experimental animals reached gonad maturity at the end of the experiment, but did not spawn. The trend in gonad maturation for field and experimental

animals indicates that there was a lag time of about 2 weeks, and that this lag is likely due to nutritional stress associated with the shift from natural food sources to the mixed microalgal formulated diet provided in the laboratory. Results indicate that experimental scallops had lower nutrient (carbohydrates, protein, lipids and total energy) reserves stored in adductor muscle tissues compared to wild animals, but both field and experimental animals utilized muscular reserves (especially carbohydrates and protein) to support reproductive activity. The fatty acid profiles revealed that polyunsaturated fatty acids (PUFA) were found in significantly lower quantities in gonad tissues of scallops from the laboratory compared to those in the field.

This thesis shows that *P. novaezelandiae* utilizes energy reserves from both adductor muscle and digestive gland to cover the full cost of gametogenesis. In addition, cultivation environments using microalgal diets are conducive to condition *P. novaezelandiae*, but the optimal nutrient requirements for an efficient aquaculture production of this species needs further investigation. It is recommended by this thesis that future investigation on the conditioning requirements for *P. novaezelandiae* will be the next step for New Zealand scallop fisheries.

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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."

Signed _____ Date_____

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Chapter 1 : General Introduction and Literature Review

Scallops biology and ecology

Scallops belong to the Family Pectinidae, with more than 30 genera and 400 scallop species from polar to tropical regions worldwide (Brand 2006). They are found in a wide range of sandy seabed habitats in intertidal and subtidal areas to water depths of 200m, although deep water species also can be encountered down to about 7000 m (Brand 2006). The regional distribution of scallops tends to be determined by habitat type (sandy to muddy) and food availability (Gosling 2008). However, hydrodynamic conditions (e.g., tidal movement) are also important, as water motion strongly affects feeding ability.

Scallops are characterised by having two calcareous shells or valves (one flat and one round valve), which enclose and protect the soft tissues. Shell growth is achieved by the mantle secretion of lamellae on the inside of the shell. The mantle itself is composed of sparsely-ciliated right and left lobes, which are fused dorsally along the cardinal plate and closely connected to internal organs, including adductor muscle, digestive glands and gills (Shumway & Parsons 2011). The adductor muscle allows for the opening and closing of the valves. Repeatedly clapping the valves of their shell together creates a form of jet propulsion that allows scallop to be non-sedentary bivalves.

The larval stage of scallops is pelagic. Larvae remain planktonic for over a month after fertilization (Stewart & Arnold 1994). Larval transport depends on the flow of currents in and around spawning areas. Swimming larvae then develop a foot and a byssus gland, which secretes threads that are used to attach to filamentous material (e.g., Zostera debris, algae, shell hash and hydroids). Upon attaining a size of 4–8mm, settled spat lose their byssal attachment and assume the role of free living adults (Shumway & Parsons 2011). Most adult scallops remain in one area for a prolonged period once they establish a position on the seabed; with a normal position of the round valve buried in the seabed, and sediment covering the flat top shell as camouflage. Predation on juvenile and adult sea scallops decreases with increasing shell height (Stokesbury & Himmelman 1995). The common predators of adult scallops include starfish, crabs, octopuses, fish and rays. When scallops sense the presence of a predator, they are able to escape by swimming. A coarse sediment substrate, such as shell or pebble, significantly reduces the predation rate on juvenile scallops, suggested to be due to the decrease in encounter rates between predator and juvenile scallops with increasing particle size of natural substrates (Wong and Barbeau 2003).

Global scallop industry

Scallop flesh is considered as luxury food in many counties. The main scallop producing countries are China, Japan, and Chile, for both fisheries and cultivation. Asia contributed more than 2,220,000 tonnes in 2014, nearly all of the total production of 2,660,000 tonnes worldwide (FAOSTAT 2016).

Scallop fisheries can be divided into wild harvests, enhanced fisheries and cultured stocks. Wild scallops are mainly harvested by dredging and bottom trawling, but hand-gathering by divers has become more common in the past years, since it is a more ecologically friendly practice (no seabed damage) (Lokkeborg 2005). Fishing for wild scallops is low cost, but worldwide declines in wild scallop populations have resulted in the growth of seeding and culturing (Hardy 2006). Scallop seeding is used to spread spat or juveniles (from wild or hatchery) in suitable habitats so that these areas can be harvested in the future (Hardy 2006). The two commonly used culturing practices are hanging and bottom culture. Enclosed culture systems have not yet been fully developed in a commercial context. However, it has advantages over other forms of aquaculture as tight control on feed and water parameters could be maintained (Hardy 2006).

Due to the high commercial value of scallops, they have been the subject of much scientific research. Despite the vast literature, detailed knowledge of the ecology is restricted to a few commercially important species, such as the queen scallop, *Aequipecten opercularis*, the bay scallop, *Argopecten purpuratus*, the Iceland scallop, *Chlamys islandica*, the commercial scallop, *Pecten fumatus*, and the great scallop, *Pecten maximus* (Shumway & Parsons 2011). However, there are still gaps in our understanding of various aspects, such as preference of organic *versus* inorganic matter in diets (Vahl 1980), factors affecting gametogenesis and broodstock conditioning (Martinez *et al*. 1992), nutritional requirements at different growing stage (Farias & Uriarte 2001), the role of un-vegetated *versus* vegetated habitats to scallop recruitment (Carroll *et al*. 2010), among others. Thus, it is important to understand the

natural requirements of scallops before favorable conditions can be provided for optimum growth in an aquaculture context. Hence, investigations on parameters that affect scallop nutrition and reproductive condition form a major part for this research.

Overview of New Zealand scallop fisheries

The New Zealand seafood industry consistently ranks as the fourth or fifth largest export earner for this country. The value of this industry ranges from $1.2 to $1.5 billion per annum, of which the aquaculture part contributes about $200m per annum. The three main cultured seafood species are Greenshell™ mussels, followed by King Salmon and Pacific Oysters. In 2007, Aquaculture New Zealand was charged with the implementation of a 10 point plan that paved the way to improve aquaculture revenues to $1 billion by 2025. In order to achieve the $1 billion goal, government support and scientific knowledge is needed to cultivate new high value species in New Zealand. Accordingly, a few additional species, such as the New Zealand native scallop, *Pecten novaezelandiae* are being researched or are in the pre-commercial stage.

Fisheries management practices in New Zealand have changed greatly over the years, and now the majority of the species with important commercial value are managed under the quota management system (QMS). The QMS was introduced in 1986, and to date, there are 97 species under the QMS, including the New Zealand scallop*, P. novaezelandiae*. Scallop fisheries in New Zealand occur in two main areas: the southern area (Challenger), including Tasman Bay, Golden Bay and the Marlborough Sounds, and the northern area, including Northland and Coromandel. The southern fishery started in 1959, and it experienced a dramatic decline in the late

1970s, with a closure for 2 years (1981–1982). A large-scale scallop enhancement programme, together with rotational fishing has made this privately-managed fishery highly successful (Annala *et al*. 2002). In northern New Zealand, commercial fishing for scallops began in the early 1970s, and initially resulted in relatively high and stable levels of production (Bull 1991). Recruitment to the fishery for this species throughout the North Island relies entirely on the natural settlement of spat.

P. novaezelandiae is a species that has undergone substantial, but poorly understood, declines in regional abundance. These declines may closely link with commercial and recreational fishing, but environmental drivers also have probably influenced their population dynamics (Williams 2005). By 2000, scallop biomass was the lowest recorded in surveys since the fisheries began (FAOSTAT 2016). Although harvested biomass has increased slightly since 2000, biomass levels are not back to the levels recorded in the 1980s. The reasons for the decline and sustained low abundances are unknown. As a result, the scallop fishery is considered to be at risk.

New Zealand scallop research and knowledge gaps

Eighteen different species of Pectinidae have been recorded from New Zealand waters (Powell 1997), and only two (*P. novaezelandiae* and *Chlamys delicatula*) are large enough or occur in sufficient densities to be suitable for any commercial exploitation. *P. novaezelandiae* is the most promising scallop species for cultivation in New Zealand.

Knowledge of the ecology of the New Zealand scallop, *P. novaezelandiae*, is relatively limited and is restricted to grey literature, mainly unpublished government reports, and graduate theses (Bull 1976, Nicholson 1978, Morrison 1999, Nesbit 1999, Lyon 2002, Williams 2005). Government reports have focused mainly on stock assessment for fisheries, and thesis studies have covered some baseline information on scallop ecology.

Bull (1976) provided the first comprehensive ecological study on this species. At that time, raft culture of scallops to marketable size was considered uneconomic in New Zealand, but partial culture and subsequent seeding of new scallop beds was worthwhile. Bull (1976) studied the early life history, growth, swimming behaviour and mortality of scallops in Pelorus Sound and nearby areas during 1973 and 1974. That work was conducted to contribute towards better management of the fishery for this species. His study provided the baseline information about this species. The results from Bull (1976) indicated that scallops of 15–45 mm in height have no particular preference for mud, sand or gravel substrates. This information supported the contention that the distribution of scallops after their early attached stage is caused by passive rather than active selection of substrates. Bull also suggested that adult scallops contributed little to the spawning pool until the end of their second year, and that they are more tolerant to salinity and temperature changes than spat. Predation, old age and indirect fishing were found to be the main causes of mortality. Lyon (2002) also studied scallops in the South Island, at Golden Bay. This study was prompted by the need to reseed in the Challenger area. Thus, Lyon (2002) investigated the health and handling techniques for scallop spat. She concluded that spat collection should

target those animals that were >20 mm in shell length, in order to seed the most robust scallops, and that the secondary harvest should also target larger juvenile scallops when transporting long distances (>8 hours).

Nesbit (1999) conducted the only thesis study of scallops in Coromandel. In his study, scallops in sand substrata had higher survival and growth rates than those found in shell gravel environments. Scallops became less vulnerable from benthic predators upon reaching shell lengths above 35 mm, highlighting the importance of seeding locations which can support optimum growth rates. He emphasised the importance of locating reseeding sites away from high sediment loading areas. He also reported that, as with other species of scallops, gonad size and maturity state of *P. novaezelandiae* were dependent on algal food rations and temperature.

The majority of graduate theses have been conducted in the Hauraki Gulf, Auckland, including Nicholson (1978), Morrison (1999) and Williams (2005). Nicholson (1978) studied the feeding and reproduction of *P. novaezelandiae*, and reported that scallops only utilize the bottom 3 cm of the water column when feeding. In this region, they ingest all biological particles that are less than 2µm, including phytoplankton, benthic diatoms and suspended detrital material. This study showed that even though *P. novaezelandiae* lack large latero-frontal cilia, they could still retain small particles (<7µm) efficiently. Nicholson (1978) also concluded that scallop reproductive cycles in the Auckland area were extremely variable. Morrison (1999) focused on the spatial distribution of *P. novaezelandiae* in the Hauraki Gulf and found that scallops aggregate at all spatial scales ranging from bay wide through to individual bed patchiness, down

to the scale of inter-animal distances. Furthermore, although scallops were found in three main habitats (muds, sands and gravels), muddy environments were the least favourable habitats for this species. *P. novaezelandiae* are sensitive to environmental conditions (e.g., sedimentation, freshwater run-off, reduced oxygen), but predation was the most important component of early mortality. Williams (2005) focused on reproductive success of scallops in the Hauraki Gulf. He reported the success of visual grading to determine maturity in this species. He also recorded gonad redevelopment between spawnings. Williams (2005)'s comprehensive survey on *P. novaezelandiae* reproduction in the Hauraki Gulf concluded that spawning timing of this species varies among sites. His results suggested that fertilization success was primarily dependent on sperm concentration, but also was influenced by gamete age and sperm-egg contact time. He also suggested that low scallop density populations may be susceptible to Allee effects and reproductive failure.

Previous studies on nutritional requirements of other scallop species have been documented. In general, scallops are capable of storing nutrient reserves in body tissues during periods of high food supplies, and subsequently mobilizing those reserves to be used during times of food shortage, decreased feeding rates, and/or high energy demands (Sastry 1968, Barber & Blake 2006). Inverse cycles of the biochemical components in the somatic tissue (adductor muscle and digestive gland) and gonad occur during the reproductive cycle of various scallop species, supporting the interpretation that materials are mobilized from the somatic tissue to support gonadal maturation. Such cycles have been observed for *Chlamys opercularis* (Taylor & Venn 1979), *Chlamys varia* (Shafee 1981), *Argopecten irradians concentricus* (Barber &

Blake 1981), *Argopecten irradians irradians* (Epp *et al*. 1988), *Placopecten magellanicus* (Couturier & Newkirk 1991), *Argopecten purpuratus* (Martinez 1991, Martinez & Mettifogo 1998), *Pecten maximus* (Pazos *et al*. 1997) and *Euvola* (*Pecten*) *ziczac* (Brea 1986, Boadas *et al*, 1997). Whereas nutritional preferences, storage of energy and timing of utilization for reproductive activity seem to vary among species and populations (Mathieu & Lubet 1993); hence the importance of this study to characterise the nutrition requirement and reproductive condition of *P. novaezelandiae*.

Bull (1976) reported on the biology of adult *P. novaezelandiae* in detail, feeding behaviour was studied by Nicholson (1978), distribution by Morrison (1999), spat biology by Nesbit (1999) and reproduction by Williams (2005). However, the relationship between nutrition and condition has not formed the main focus in any of these studies. Thus, the main focus of this study is to provide baseline information of scallop ecology and its relation to environmental conditions in New Zealand. Understanding of feeding, growth, development and behaviour of this bivalve in its natural environment will benefit the unpredictable and unsustainable scallop fishery and increase the potential of scallop aquaculture.

Scallop reproduction

Scallops can either be dioecious or hermaphroditic. In the case of *P. novaezelandiae*, its gonad is divided and separately contains the ovaries and testis. They are broadcast spawners that release sperm and eggs sequentially. While self-

fertilization can occur, successful reproduction relies mainly on cross-fertilization from neighbouring conspecifics. Once in the water column, external fertilization takes place, followed by embryological development (Williams 2005). Like other bivalves, all scallops have reproductive cycles that include periods of gamete formation, spawning, fertilization, larval development, settlement and metamorphosis, and growth to reproductive maturity (Barber & Blake 2006). The time to reach different life stages varies among species. *P. novaezelandiae* generally reaches sexual maturity after the first year, but it takes 2-3 years for them to become fully mature adults (100–120 mm shell length) (Morrison 1999). Fully matured adults may produce over 40 million eggs annually (Bull 1976). Various exogenous (e.g., temperature, food, depth) and endogenous (e.g., energetic demand, genetic) factors determine the timing and duration of spawning events for a particular species at a particular location and time. A good understanding of the effect of both exogenous and endogenous factors on reproductive behaviour is crucial for the development of optimized scallop aquaculture production. However, limited research on *P. novaezelandiae* has been undertaken in this area.

The anatomy of scallops is such that the gonad is easily visible and anatomically distinct from the rest of the visceral mass. This makes it relatively easy to access and hence determine the stage of maturity of each individual. As the gonad matures, macroscopic changes are readily visible. The gonad increases in weight and size and becomes thicker as gametes increase their size and become more numerous. After spawning and the release of gametes, gonads become smaller and flatter in cross section, and colourless and watery in appearance. Scallop gonads can be characterised

as either immature/spent, partially spawned, active, ripe (mature) based on external appearance (Table 1-1). Williams (2005) reported significant correlation of gonad appearance and histological gonad mass data in *P. novaezelandiae*. However, his study only provided a rough estimate on overall gonadal development, and no information on gamete development. By examining fresh gonadal smears microscopically (histology), more direct information can be obtained as to the development and viability of gametes.

Scallop nutrition

Nutrition is the process by which food particles are broken down into small pieces that can be digested to nourish and fuel the organism. Thus, nutrition involves ingestion and digestion of food, absorption of nutrients and elimination of waste. Scallops are filter feeders which feed on a variety of suspended material (seston) from the water column. Food particles are sieved by the gills and trapped by mucus. Then, cilia move the particles to a paired labial palp on either side of the mouth. The palps select 'edible' particles that move along ciliated tracts into the mouth while inedible matter is gathered into mucous blobs, and ejected from the mantle cavity as pseudofaeces. Food is then moved to the stomach, digested by digestive juices secreted by digestive glands. Digested molecules of food are absorbed and stored in different parts of the body. Waste is passed on through the intestine and exits via the anus.

Scallops are sestotrophic bivalve filter-feeders, which are capable of ingesting living and inert particles suspended in the water column (Lucas 1982). Even though phytoplankton is the main nutrient source for pectinids (Farias & Uriarte 2006), inert organic material in the seston and detritus were also reported to contribute to pectinid energetic input (Hunauld *et al*. 2005, Cranford & Grant 1990). Scallop feeding rates depends on a number of variable, including temperature, food concentration, nutritive value of the food, and water flow (Wildish *et al*. 1987, Bricelj & Shumway 1991). Absorption is controlled by the levels of digestive enzymes in their digestive tracts (Samain *et al*. 1992). Digestion in pectinids occurs partially in the stomach and partially in the digestive gland (Farias & Uriarte 2006). The digestive gland is rich in intracellular carbohydrases which breakdown carbohydrates into simple sugars, and peptidases which starts protein catabolism by hydrolysis (Henry *et al*. 1991).

Most feeding and nutritional studies have been carried out with microalgal cultures (Heasman *et al*. 1996, Martinez *et al*. 2000a, Velasco & Barros 2007), and although positive results have been obtained, there has been little explanation of how natural diets fulfil the nutritional needs of these bivalves. Also, little is known concerning how the pectinids responds to the complex mixtures of bacteria, phytoplankton, and chemical signals, which affect them in their natural environment. Variability in nutritional quality of seston may range from 5 to 80% in organic content, with a C:N ratio from ,4 to >26 (Bayne & Hawkins 1990). This spectrum may vary with the length of day, season of the year, or geographic locality, which make it difficult to define a “typical natural diet’, or reasonably define the ‘normal’ feeding behaviour of bivalve (Farias & Uriarte 2006.).

Pectinids are particularly suitable for the study of nutritional (= biochemical) composition and the relationships between different organs among marine bivalves, because their adductor muscles, digestive glands and gonads are clearly separate. The adductor muscle and digestive gland in scallops are the principle organs of energy storage (Ansell 1974, Comely 1974, Barber & Blake 1981, Pazos *et al*. 1997), to support gonad development and gametogenesis.

Scallops biochemical cycle

Seasonal cycles of energy storage and utilization (=biochemical cylcles) in marine bivalves are generally attributed to reproductive activity (Sastry 1979, Barber & Blake 1981, Beninger & Lucas 1984, Pazos *et al*. 1997, Perez-Camacho *et al*. 2003). Based on the relationship between gonad development and the accumulation and utilization of nutrients, animals can be categorised as conservative or opportunist (Bayne 1976). In conservative species, gametogenesis takes place using previously acquired reserves, while gametogenesis occurs when food abundance is high for opportunist. In general, scallop is likely to be a conservative species. As described for serval pectinids, energy is stored during periods of high food supplies, and subsequently mobilizing reserves to be used during times of food shortage, decreased feeding rates, and/or high energy demands (Sastry 1968, Barber & Blake 2006). Gametogenesis represents a period of particularly high energy demands. The cost of gamete synthesis must be met by high food supplies and/or stored reserves. In general, carbohydrates and/or lipids are stored prior to gametogenesis and they are

subsequently utilized in the production of gametes, when metabolic demand is high (Gabbott 1975, Bayne 1976).

In marine bivalves, carbohydrates are commonly recognised to be the primary energy source. Carbohydrates are important for forming gametes and maintaining adult condition during periods of nutritive stress. Variation in carbohydrate content shows an inverse relationship with the state of gonad maturity (Martinez *et al*. 1993, Perez-Camacho *et al*. 2003). According to Farias & Uriarte (2001), lipids form part of the reserves during periods of nutritional deficiency and are an important component of bivalves' oocytes (Holland 1978). Protein constitutes the largest fraction in the composition of oocytes and other soft tissues of bivalves, and is claimed to be the main energy source of *Argopecten irradians irradians*, due to its high contribution to total energy content during gametogensis (Epp *et al*. 1988). In addition to direct transfer and use of metabolic demand, inter-conversion of these substrates can also take place, e.g. lipogenesis of carbohydrates stored in scallop adductor muscle to support gonad development (Gabbott 1975).

Food quantity and quality both strongly influence scallop nutrition, and there is evidence to suggest that scallops are able to modulate ingestion of particles based on their quantity and quality (Delaunay *et. al*. 1993, Farias & Uriarte 2001, Farias & Uriarte 2006). Schneider *et al*. (1998) demonstrated that the ingestion efficiency of the bivalves *Dreissena polymorpha* responds to seston quality. Investigations on nutritive value of seston suggested that a protein to carbohydrate to lipid ratio of 4:3:1 meets the nutritional requirement of most molluscan filter feeders (Parsons *et al*. 1961).

Although lipid contributes the least amount to bivalve diets, numerous studies have highlighted the importance of high quality of lipids to bivalve nutrition and health, particularly polyunsaturated fatty acids (PUFA), (Delaunay *et al*. 1993, Soudant *et al*. 1996, Caers *et al*. 1998, 2000). Animals are unable to synthesis PUFA, and bivalves have limited ability to elongate shorter-chained dietary PUFSs into essential long chain PUFA, such as n-3 fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). These PUFA are commonly available in the marine environment, but how these essential fatty acids affect the condition of scallop is not well documented.

Nutritional preferences, storage of energy and timing of utilization for reproductive activity vary among species and populations (Giese 1967, Bayne 1976, Sastry 1979). Stored carbohydrates and proteins in adductor muscles have been shown to be used during gametogenesis in *Chlamys septemradiata* (Ansell 1974), *Pecten maximus* (Comely 1974, Faveris & Lubet 1991) and *Chlamys opercularis* (Taylor & Venn 1979); *Placopecten magellanicus* utilises carbohydrates and lipids stored in digestive glands (Robinson *et al*. 1981); *Lyropecten* (*Nodipecten*) *nodosus* utilised carbohydrates and protein in digestive gland (Lodeiros *et al*. 2001) for reproduction; and *Argopecten irradians irradians* (Epp *et al*. 1988) gametogenesis occurs mainly at the expense of adductor muscle protein and lipid. Nutritional requirements vary among species, depending on the growth, longevity and maximum size of each species, but environmental factors also appear to play an important role in marine bivalve nutrition (Mackie & Ansell 1993). In general, temperature and food availability are considered to be the most important environmental factors in bivalve nutrition. Broom & Mason (1978) reported that the growth of *Chlamys opercularis* significantly correlated with

temperature and concentration of chlorophyll *a*, while the seasonal growth patterns of C*. islandica* could be explained by concentrations of particulate organic matter (POM) and particulate inorganic matter (PIM) in the seston and water depth (Vahl 1980, Wallace & Reinsnes 1985). Thus, in order to investigate nutritional requirements and resource allocation (i.e., growth, maintenance, reproduction) in *P. novaezelandiae*, it is crucial to characterize its environment and quantify the carbohydrate, protein and lipid contents in its food source, as well as in different scallop tissues (i.e. muscle, digestive gland, gonad) throughout different seasons (e.g., reproductively active, reproductively inactive).

Potential for scallop aquaculture in New Zealand

New Zealand officially adopted the Japanese culture technique of bottom re-seeding in 1986, which was proven to be a success in Tasman Bay and Golden Bay, South Island. Scallops are cultivated by suspending spat-collecting bags in coastal waters during summer. Thousands of scallop larvae settle out of the plankton onto the fine feathery surface of the bag. The larvae are left to grow to suitable size before they are collected and released onto known natural scallop beds at densities of about six per square metre of seabed. They grow for one or two years until commercial size, and then the bed is harvested by dredging. This technique resurrected the Challenger scallop fishery, which was near collapse in the 1980s, to a level where 747 tonnes were harvested in 2004. However, spat survival has dropped since 2005. It was suggested

that change of habitat from gravel to silt in the last decade reduces the ability of juvenile scallops to survive (M. Campbell, per. comm.).

Although scallops have been a late entrant on the shellfish farming scene in New Zealand, as more countries have shown interest, culturing techniques have become more refined. Scallop farming is now a successful activity, generating significant revenue in many countries. The once successful bottom re-seeding in the South Island has proven the potential for scallop cultivation. In addition, *P. novazealandiae* is recognised as a suitable species for bottom culturing (Hardy 2006). However, for scallop cultivation to become commercially viable, a better understanding is needed of the physical and biological parameters that affect its nutrition and reproductive behaviour. The best place for such investigations is within the natural environments that sustain healthy and productive wild populations currently.

Significance of the Study

The New Zealand seafood industry is one of the top five export earners in the country. Seafood harvests are valued at $1.5 billion per annum, and aquaculture production contributes about $200 million per annum (MFish 2010). Recent efforts to improve the sustainability of New Zealand seafood production have resulted in government-targeted funding initiatives to support the development of this sector. In 2006, Aquaculture New Zealand was charged with the implementation of a 10 point plan to improve aquaculture revenues to $1 billion by 2025 (NZAS 2006). A focal point

of this national strategy is to fund research and development of new aquaculture species, such as scallops and clams.

The New Zealand native scallop, *P. novaezelandiae*, is a species that has undergone extensive harvesting pressure (commercial and recreational fishing), and has experienced significant population declines (Marsden & Bull 2006). Dramatic scallop biomass declines recorded in 2000 have put this fishery at risk (Williams 2005). In order to maintain and maximise the potential of the scallop fisheries in New Zealand, culturing scallops under local conditions may prove to be a successful practice for re-seeding wild populations and/or to develop a standalone aquaculture industry. The key to success in scallop aquaculture will rely on the understanding of feeding, growth, development and behaviour of this bivalve in land-based culturing operations. Hence, the investigation of environmental and biological parameters that affect scallop nutrition and reproductive condition form an integral part of the rationale for this research.

Scallops have gained much attention worldwide because of their high economic value, which encourages aquaculture research efforts by both academics and industrial researchers. Work has been undertaken to describe factors that may influence scallop production internationally (Carroll *et al*. 2010, Farias & Uriarte 2006, Heasman *et al*. 1996, Lodeiros & Himmelman 1994, Samain *et al*. 1992). Nutritional requirements vary among species, depending on growth rates, longevity and maximum size of each species. However, in general, temperature and nutrition (food quality and quantity) have been found to be important factors that affect scallop production (Broom &

Mason 1978, Farias & Uriarte 2001, Martinez *et al*. 2000b, Pazos *et al*. 1997, Rheault & Rice 1996, Vahl 1980, Wallace & Reinsnes 1985, Wilson 1987). In addition, a good understanding of reproductive behaviour and broodstock condition are essential to establish a reliably hatchery production.

Knowledge of the ecology of the New Zealand scallop, *P. novaezelandiae*, is limited and restricted to grey literature, mainly unpublished government reports, and graduate theses (Bull 1976, Nicholson 1978, Morrison 1999, Nesbit 1999, Lyon 2002, Williams 2005). A few studies have hinted at the importance of environmental factors in *P. novaezelandiae* mortality (e.g., sensitive to sedimentation, freshwater run-off, reduced oxygen) (Morrison 1999). However, these studies have not clearly identified the role of different environmental parameters on scallop nutrition and reproductive condition, which are crucial to develop a scallop aquaculture industry. Part of the reason for this knowledge gap is that scallop ecology has been addressed either from a commercial or general biology perspective, and there is no fully integrated view on aquaculture production potential. New Zealand government reports have focused mainly on stock assessment for fisheries, with some baseline information on scallop ecology.

Scallop growth and recruitment are highly variable, and this variability leads to an unpredictable and unsustainable fishery. A common sustainable practice is to use hatchery produced and/or scallop juveniles (3mm in shell size) to re-seed wild populations. This practice has successfully rescued the scallop industry from collapsing in many areas worldwide, including the top of the South Island, New Zealand.

However, commercial re-seeding has not been undertaken in the North Island, but Morrison (1999) suggested that this is highly feasible in the Leigh region. Thus, it is clear that the future of scallop production in New Zealand will have to rely on aquaculture practices, which are likely to improve the international revenues with this species. Cultivation of this species might not only alleviate fishing pressures on wild stock, but could be lead to commercial production of cultivated scallops for local markets and exports, thus helping us meet the government's 1 billion dollar export target.

Aims

The overall impetus for this thesis is to investigate the nutritional requirements and the physical and biological factors that influence condition of wild and cultivated New Zealand scallops (*Pecten novaezelandiae*), in order to maintain and maximize the potential of the scallop fisheries in New Zealand. The following aims were investigated:

1) To evaluate the variation reproductive and nutrition state among scallop populations in the Hauraki Gulf
2) To identify the nutritional requirements of wild scallops over an annual cycle.
3) To determine the reproductive condition of wild and cultivated scallops.

Table 1-1 Visual grading system for measuring gonad condition in *Pecten novaezelandiae*, modified from Bull (1976) & Heasman *et al*. (1996) by Williams (2005).

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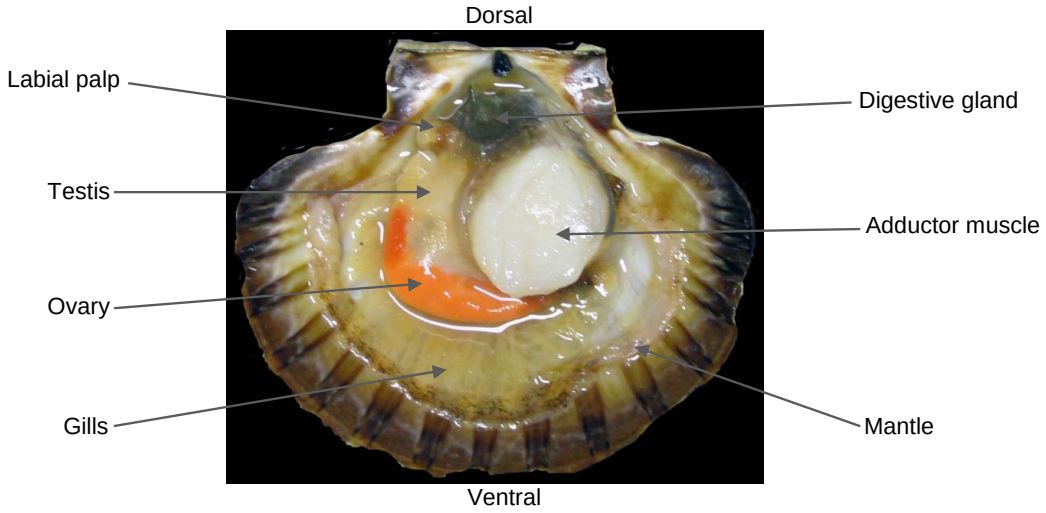

Figure 1-1: *Pecten novaezelandiae*: Left valve removed to show internal organs.

Chapter 2 : Reproductive and nutritional state of *P. novaezelandiae* in six populations within the Hauraki Gulf, New Zealand—A one-off survey

Introduction

Gametogenesis is a period of particularly high energy demands, and the cost of gamete synthesis must be met by high food supplies and/or stored reserves. Therefore, reports on energy storage and utilization in marine bivalves are generally attributed to reproductive activity (Sastry 1979, Barber & Blake 1981, Beninger & Lucas 1984, Pazos *et al*. 1997, Perez -Camacho *et al*. 2003). In general, carbohydrates and/or lipids are stored prior to gametogenesis and they are subsequently utilized in the production of gametes when the metabolic demand is high (Gabbott 1975, Bayne 1976).

In *P. novaezelandiae*, reproductive conditions were previously investigated by histological analysis (Bull 1976, Nicholson 1978, Williams 2005), gonad condition index (Bull 1976, Nicholson 1978, Morrison 1999, Williams 2005) and most recently visual gonad index (Williams 2005), but gonad biochemical composition was not investigated in any of these studies. Furthermore, energy storage and utilization of somatic tissue was not previously reported for this species. Therefore, the aim of this study was to evaluate the variation of reproductive and nutritional state of *P. novaezelandiae*

among different population in the Hauraki Gulf, and to provide baseline information of the biochemical composition of this species for future study.

Methods and Materials

Study sites and sample collection

Six populations (sites) of *P. novaezelandiae* around the Hauraki Gulf were sampled on 24th October 2014 (Fig. 2-1). Reproduction condition of *P. novaezelandia*e from 3 of these sites were previously reported; Motuketekete Island by Morrison (1999), and Tiritiri Mantangi and Pembles Island by William (2005). In this study, the reproductive condition of each scallop was assessed by condition index, visual gonad index and gonad lipids content. The nutritional state of the animal were also measured, represented by somatic tissue condition indices, adductor muscle carbohydrates and digestive gland lipid content. All sites were <10 meters in water depth. Five to eight scallops were collected by SCUBA divers from each site. Scallops were cleaned thoroughly, and their shell heights and widths were measured to the nearest 0.01 mm with Vernier callipers. Then, each scallop was dissected. The gonad, adductor muscle and digestive gland were removed from each individual and weighed separately. Tissues were then freeze-dried and weighed. Condition indices of gonad (GI), adductor muscle (AMI) and digestive gland (DGI) were determined according to Barber & Blake (1981):

Condition index= (tissue DW/total tissue DW) *100

where, DW is the dried weight.

Reproductive condition

Visual gonad index (VGI) was determined for all animals just prior to dissection. Visual grades 1—8 were assigned to each gonad, based on their appearance and following the classification by Williams & Bobcock (2004). These grades included spent (grade 1), partially spawned (grade 2, 3), active (grade 4−6) and ripe (grade 7, 8), and were based on morphological appearance of the gonad to the naked eye. Lipid contents within gonads of each scallop were determined by the charring method according to Marsh & Weinstein (1966). Lipids were extracted in a 2:1 chloroform-methanol mixture (Bligh & Dyer 1959). A serial of lipid standard solution was prepared using tripalmitin (Sigma Aldrich) in chloroform. Extracted lipids and standard solutions were then dried under a flow of nitrogen and the amount of total lipid was determined by charring with concentrated sulphuric acid for 15 min at 200°C (Marsh & Weinstein 1966), and are reported as tripalmitin equivalents. The optical density of the solution was read at 375nm.

Nutritional state

Carbohydrate contents in the adductor muscle and lipid levels in the digestive glands were determined for each scallop. Carbohydrates were determine according to Hedge & Hofreiter (1962) and reported as glucose equivalents. Samples were

hydrolyzed into simple sugars using dilute hydrochloric acid (2.5N). Glucose powder (Sigma Aldrich) with distilled water was used to prepare a serial of glucose standard solution. Hydrolysed samples and standard solutions were then mixed with four parts of Anthrone solution. Samples were then incubated for 8 min at 100°C, and the optical density of the green coloured solution was read at 630nm on a spectrophotometer. Total lipids of the digestive gland were determined in the same manner as for gonad samples (see above).

Statistical analyses

Results were analysed statistically using one-way ANOVA and Gabriel post hoc tests to identify the difference between sites, using SPSS statistics package. Then, data were further evaluated using Principal Components Analysis (PCA) using Plymouth Routines in Multivariate Ecological Research (PRIMER) on log-transformed data, to determine the correlation among the variables that were measured in this study (reproductive condition: gonad index, gonad lipids level; nutritional state: somatic condition indices, adductor muscle carbohydrate levels, and digestive gland lipid levels).

Results

Reproductive condition

Visual gonad index data analyses resulted in significant differences among sites (Table 2-1), and Gabriel *post-hoc* tests revealed three main groups between the sites (Fig. 2-2). Site 1 and Site 2 were grouped in Group A, Motuketkete Island and Pembles Island were grouped in Group AB, and Tiritiri Matangi and Otata Island were grouped in Group B. Immature/spent gonads (grade 1) were not found in any animal within the study area, while partially spawned gonads (grade 2) were found in scallops in group B only. Active gonads (grade 3-5) were found in animals from all sites, while animals with ripe gonads (grade 6-8) were dominant in Group A, and present in all sites of group AB.

Scallop gonad condition indices (GI) were not significantly different between sites (Fig. 2-3, Table 2-1). The lowest mean GI value was recorded in site 1 (11.2±1.4%), while the highest value was found in Site 2 (15.7±1.6%). In contrast, lipid contents in gonads were significantly different among sites. The lowest lipids content was recorded at Tiritiri Matangi (116.1±12.3mg g^{-1}), while the highest average lipid content in gonad tissues was found at site 1 (174.5±11.3 mg g^{-1}). Gabriel *post-hoc* tests revealed three main groups of gonad lipid contents among sites (Fig. 2-3).

Nutritional state

Scallop adductor muscle indices (AMI) and digestive gland indices (DGI) were both significantly different among sites (Fig. 2-3, Table 2-1). The lowest AMI

(39.7±1.1%) was recorded at site 1, while the highest AMI (48.3±2.1%) was found at Otata Island. DGI values were lowest at Pembles Island (8.8±1.1%) and highest at site 1 (13.4±0.2%).

Analysis of adductor muscle tissues revealed no significant difference in carbohydrate contents between sites throughout the study area, ranging from 90.5±13.7 mg g^{-1} at Pembles Island to 122.6±25.8 mg g^{-1} at Site 2 (Fig. 2-3). Lipid levels in the digestive gland showed no significant difference among sites. Lipid contents were lowest at Otata Island (144.8±17.2 mg g^{-1}), while Site 2 had the highest lipid values (217.0±12.9 mg g^{-1}) (Fig. 2-3).

Multivariate analyses (PCA)

Figure 2-4 shows the principal component analysis (PCA) of the variables measured in this study (i.e., condition index and tissue biochemistry). PC1 and PC2 represented 72.3% of the total variance. PC1 accounted for 45.2% of data variance, and had high loadings for adductor muscle carbohydrate levels and gonad lipid levels. The second component accounted for 27.1% of data variance, with high positive loadings for lipid contents of digestive glands, lipids content in gonads, and gonad indices (GI). There was no identifiable spatial pattern among variables and sites, but a notable pattern was found between variables and visual gonad index (VGI), which showed a strong trend of increasing gonad maturation level (VGI values) along the PC1 (left to right), indicating that gonad maturity was highly associated with adductor muscle carbohydrate and gonad lipid levels.

Discussion

In the present study, analyses on visual gonad index (VGI) and gonad lipid levels revealed clear differences in scallop reproductive condition among the sampled sites in the Hauraki Gulf. Among the six sites, Site 1 and Site 2 having the most mature gonads, while scallops from Tiritiri Matangi and Otata Island were least mature, and Motuketekete Island and Pembles Island had a mixture of maturity level. The reproductive cycle of scallop from Motuketekete Island and Prospect Bay (Site 1 and Site 2) were studied in 1991−1992 (Morrison 1999) and 2012−2013 (Chapter 3) respectively, and the studies indicated that spawning time at these sites were around late October. In the present study, samples from these sites showed a high number of mature scallops in October 2014, suggesting that the reproductive maturation time might be relatively similar between years for these scallop beds. On the other hand, gonad condition of populations from Pembles Island and Tiritiri Matangi Island tended to have multiple peaks starting from September through to June which varies among years, with spawning occuring earlier in Tiritiri Matangi than Pembles Island (Williams 2005). Results from the present study showed higher maturity of scallop from Pembles Island than Tiritiri Matangi, which was different to the observation of Williams (2005), or it is also possible that scallops in Tiritiri Matangi have spawned by the time they were sampled. The data indicates that reproductive state of *P. novaezelandiae* is spatially variable within the Hauraki Gulf.

Spatial variations in the reproduction of *P. novaezelandiae* observed in the present study were previously reported in the Hauraki Gulf (Nicholson 1978, Williams

2005). For example, Williams (2005) recorded a trend that *P. novaezelandiae* from shallow water (Tiritiri Matangi Island, 5 m depth) tend to have high fecundity than scallops from deeper water (Jones Bay, 18 m). Similarly, shallow water populations of the giant scallop, *Placopecten magellanicus*, in the northwestern Atlantic have been shown to have greater gonad production (MacDonald & Thompson 1985), faster rates of gametogenic development and maturation (MacDonald & Thompson 1986), and higher fecundities (Barber *et al*. 1988) than those from deeper water. Multiple studies have suggested that reproductive variation among scallop populations tend to reflect differences in environmental conditions, such as water depth, temperature and food availability (Sastry 1968, Mackie & Ansell 1993, Heasman *et al*. 1996). Scallop beds with similar water depth were chosen in this study, and sea water temperature were similar between sites, thus, variation in gonad maturation/spawning time between populations recorded here was unlikely influenced by these two parameters. Because of the high variability of nutritional quality of seston (due to changes in length of day, season of the year), it is often difficult to define the 'natural diet' for wild scallop populations. However, Zeldis and Smith (1999) reported that ~65% of the nutrient flux to the Hauraki Gulf was from the continental shelf, ~30% from rivers, and ~5% from municipal sources. Thus, sampling sites that are closer to the shelf (Site 1 and Site 2) are likely to have higher nutrient inputs, which subsequently support phytoplankton growth and increase food availability for the scallop population. Therefore, it is possible that Site 1 and Site 2 in the present study might have higher food availability than other sites; hence it is likely that food availability was one of the environmental factors that affected *P. novaezelandiae* reproduction in the Hauraki Gulf.

The findings of this study indicated that adductor muscle and digestive gland condition varies among populations, but biochemical analysis revealed similar nutrient content for the two types of tissues between populations. This indicated that there might be a spatial difference in somatic mass between sites, but nutrient storages were similar. Furthermore, multivariate analysis revealed no pattern between scallop nutrients and population, but the associations between nutrient content (adductor muscle carbohydrate level in particular) and reproductive state (VGI values) were strong.

In conclusion, spatial variation in reproductive condition of *P. novaezelandiae* was reflected in this one-off survey, suggesting gonad maturation time varies in the Hauraki Gulf. Somatic tissue on the other hand indicated differences in tissue mass, but nutrient content were similar among scallop population.

Table 2-1 Statistical analyses of reproductive condition and nutrition state of scallops among 6 sites in the Hauraki Gulf. Bold numbers indicate significant differences, at $p < 0.05$.

Source	*df*	VGI	
		F	p
Between group	5	12.88	**<0.01**
Within group	31		
Total	36		

Condition Index

Source	*df*	Gonad Index		Adductor muscle Index		Digestive gland Index	
		F	p	*F*	p	*F*	p
Between group	5	1.56	0.20	3.73	**0.01**	3.77	**0.01**
Within group	31						
Total	36						

Nutrient content

Source	*df*	Gonad lipids		Adductor muscle carbohydrates		Digestive gland lipids	
		F	p	*F*	p	*F*	p
Between group	5	5.00	**0.00**	0.20	0.96	1.38	0.26
Within group	31						
Total	36						

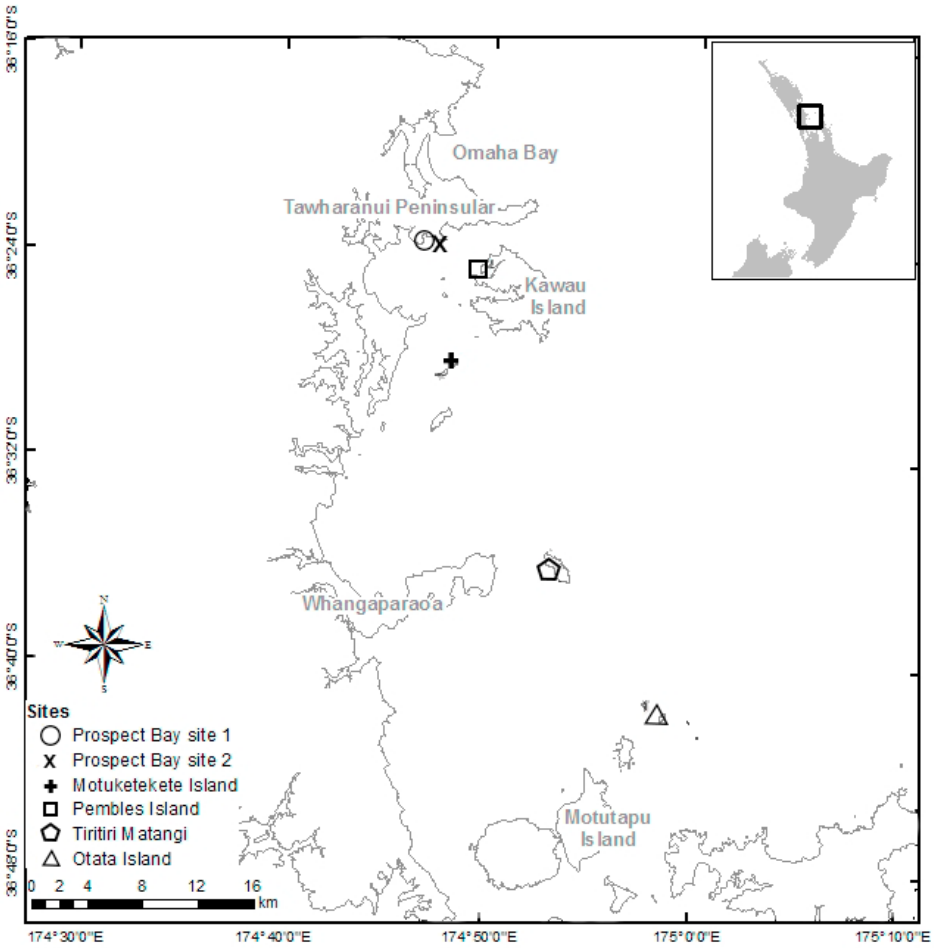

Figure 2-1 Study sites in the Hauraki Gulf, New Zealand. Prospect Bay site 1, Prospect bay site 2, Motuketekete Island, Pembles Island, Tiritiri Matangi and Otata Island.

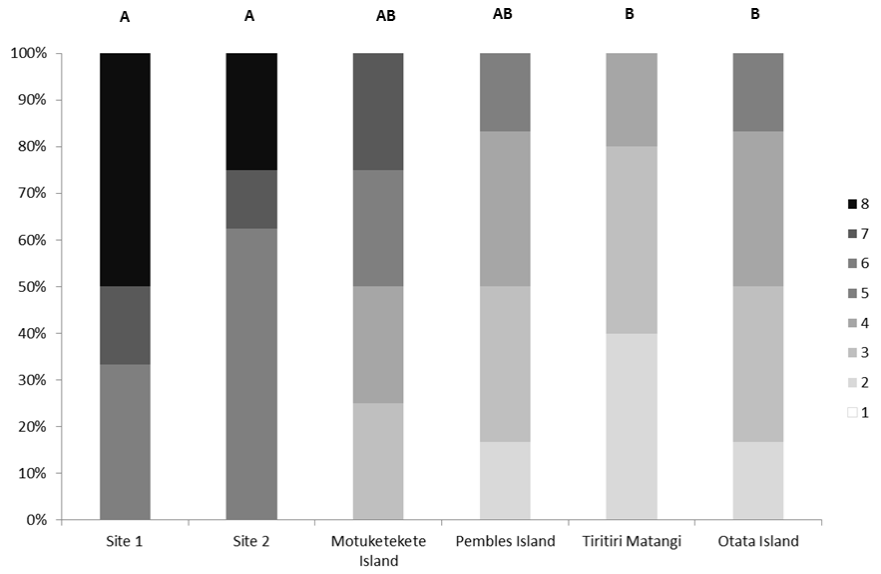

Figure 2-2 Frequency of visual gonad index (VGI), classes (1–8) for Site 1, Site 2, Motuketekete Island, Pembles Island, Tiritiri Matangi and Otata Island, 24th October 2014

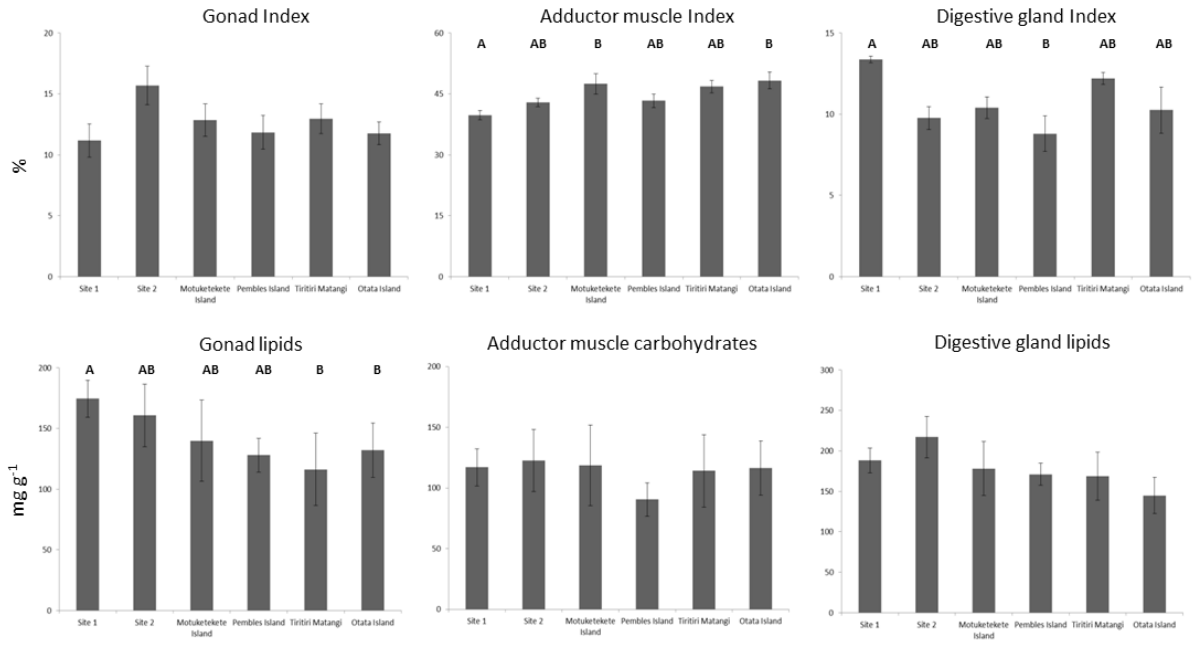

Figure 2-3 Condition indices and biochemical composition of gonad, adductor muscles and digestive glands of scallops from 6 sites in the Hauraki Gulf on 24th October 2014. Mean values ± standard error.

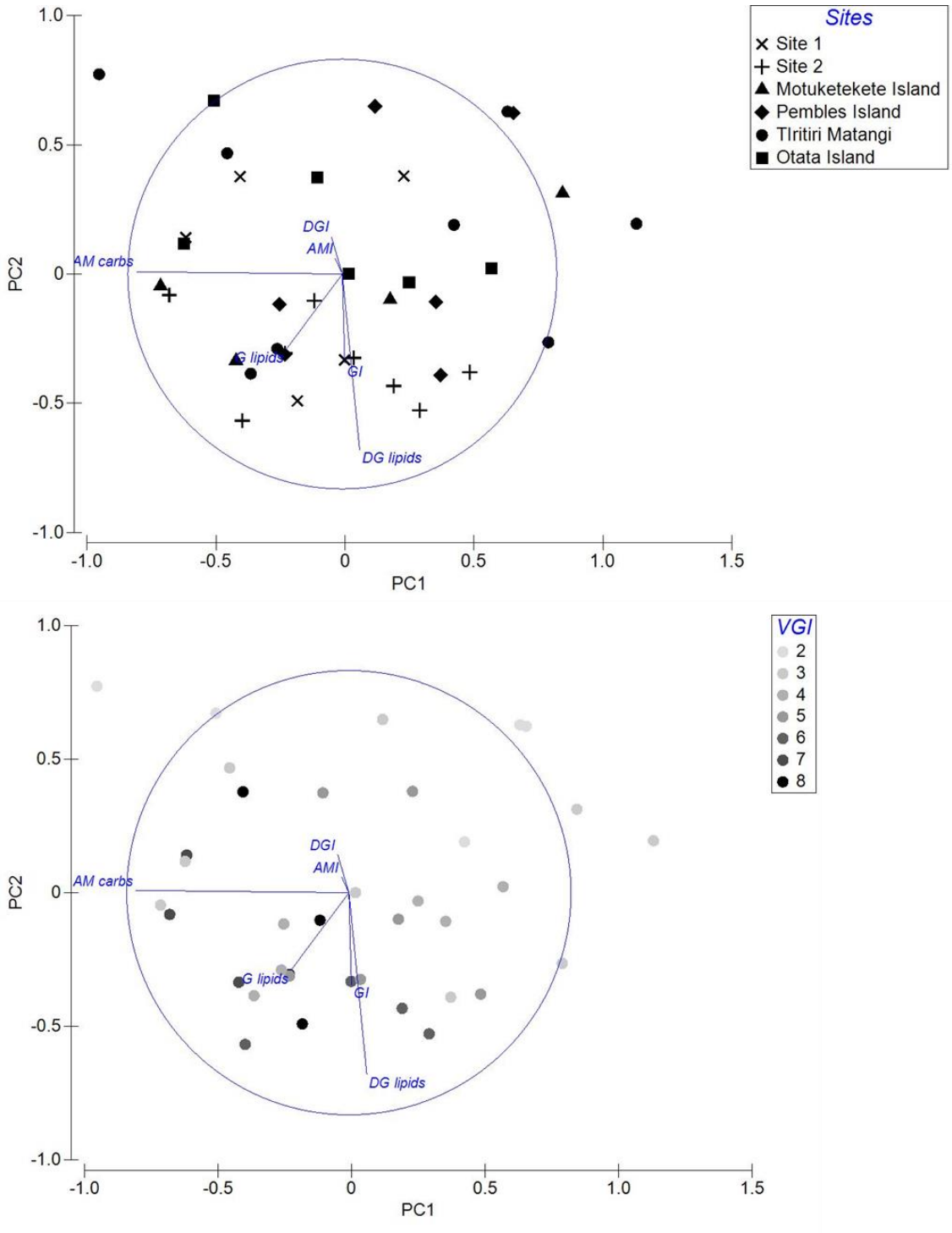

Figure 2-4 PCA plots of biochemical composition of gonad, adductor muscles and digestive glands of scallops from the Hauraki Gulf on 24th October 2014. The resemblance matric was created using Euclidean distances.

Chapter 3 : Nutrient storage and utilization in relation to reproductive condition of Pecten novaezelandiae

Introduction

Most marine bivalves have well-defined annual reproductive cycles that take into account environmental conditions, such as water temperature and food availability. The ultimate purpose of these cycles is to produce gametes during optimal periods to ensure successful fertilization and larval development. Usually, gonad maturation and spawning take place once or twice a year in spring and summer (Mathieu & Lubet 1993). Since gonad maturation and/or gametogenesis represent periods of particularly high energy demands, the cost of gamete synthesis must be met by high food supplies and/or stored reserves. Two reproductive strategies commonly used by bivalves are conservative and opportunistic strategies, which are based on the relationship between gonad development and the accumulation and utilization of nutrients (Bayne 1976). In conservative species, gametogenesis takes place using previously acquired reserves, while for opportunistic species gametogenesis occurs when food availability is high. As described for many scallop species, energy is stored during periods of high food supplies, and subsequently these reserves are mobilized for use during times of food shortage, decreased feeding rates, and/or high energy demands (Sastry 1968, Zandee *et al*. 1980, Martinez 1991, Barber & Blake 2006). Storage of energy in bivalves, such as scallops, undergoes various biochemical

processes that convert assimilated nutrients into storage compounds that can be re-mobilized when needed.

Seasonal changes in body tissue weights (condition index) and biochemical composition provide information regarding the ways in which nutritional components (i.e., carbohydrates, lipids, and protein) are used in gamete production. In general, carbohydrates are used as primary energy sources in bivalves, and are important for gamete development and maintenance of adult condition during periods of nutritive stress (Bayne 1976, Barber & Blake 1981, Berthelin *et al*. 2000). Indeed, a direct relationship has been established between gonad index and somatic carbohydrate content in many bivalve species (Beninger & Lucas 1984, Perez-Camacho *et al*. 2003). Lipids also represent an important component in energy reserves during periods of nutritional deficiency (Farias & Uriarte 2001), and are accumulated within oocytes during development (Holland 1978). Proteins constitute the largest fraction in the composition of oocytes and other soft tissues within bivalves, and somatic protein loss during winter has been observed in numerous bivalve studies (Bayne 1976, Beninger & Lucas 1984, Epp *et al*. 1988). In addition to direct use of nutritional components for metabolic demands, inter-conversion of these components, such as stored carbohydrates to lipids (lipogenesis) to support gonad development can also take place (Gabott 1975).

Due to the high commercial value of scallops, considerable research has been generated with regards to reproductive physiology (Saout *et al*. 1999, Racotta *et al.* 2003, Guerra *et al.* 2012), broodstock conditioning (Martinez *et al*. 1992, Pazos *et al*.

1996, Pernet *et al.* 2003), energy storage and utilization (Epp *et al*. 1988, Pazos *et al*. 1997, Lodeiros *et al.* 2001) of a number of species. Based on such studies, it is well established that Pectinids filter food particles from their surrounding environment. Phytoplankton and detrital material may be consumed from the water column (seston) and/or from re-suspended material from the seabed. In addition, scallops are known to have the ability to discriminate microalgae from other particles and reject particles of poor nutrition as pseudofaeces (Nicholson 1978, Cranford & Gordon 1992). However, nutritional preferences, storage of energy and timing of utilization for reproductive activity seem to vary greatly among scallop species and populations (Giese 1967, Bayne 1976, Sastry 1979). While it is generally accepted that stored nutrients are mobilized from somatic tissues (adductor muscle and digestive gland) to support yolk synthesis for gonadal maturation (Barber & Blake 2006), there are some notable differences regarding the type of nutrients utilized in this process. For example, maturation of gametes is fuelled by carbohydrate and protein reserves stored in the adductor muscle in *Chlamys opercularis* (Taylor & Venn 1979), *Pecten maximus* (Faveris & Lubet 1991) and *Argopecten purpuratus* (Martinez 1991). However, in *Argopecten irradians irradians*, gametogenesis occurs mainly at the expense of adductor muscle protein and lipid reserves (Epp *et al*. 1988). Furthermore, *Lyropecten* (*Nodipecten*) *nodosus* utilizes carbohydrates and proteins from the digestive gland (Lodeiros *et al*. 2001) for reproduction. Thus, it is clear that more specific research is needed to identify the nutritional requirements and biochemical processes involved in reproductive allocation and development for each species in question.

The New Zealand native scallop, *Pecten novaezelandiae*, is a species that has undergone extensive harvesting pressure (commercial and recreational fishing), and has experienced significant population declines in the last 17 years (Marsden & Bull 2006). Dramatic scallop biomass declines recorded in 2000 have put this fishery at risk (Williams 2005). Knowledge of the ecology of *P. novaezelandiae* is limited and restricted to grey literature, mainly unpublished government reports, and graduate theses (Bull 1976, Nicholson 1978, Morrison 1999, Nesbit 1999, Lyon 2002, Williams 2005). A few studies have hinted at the importance of environmental factors (e.g., sedimentation, freshwater run-off, habitat complexity) and food availability in *P. novaezelandiae* growth, reproductive condition, and survivability (Morrison 1999, Morrison & Cryer 2003, Talman *et al*. 2004). However, these studies have not clearly identified the role of different environmental parameters and food supplies on scallop nutrition and reproductive condition. Thus, the aims of this study are to investigate the source (sediment and water column) of nutrients consumed by wild scallop (*P. novaezelandiae*) populations, and the storage and utilization of nutrients (carbohydrates, proteins and lipids) within scallop tissues (gonad, adductor muscle, digestive gland) during the reproductive cycle.

Methods and Materials

Study sites

Two study sites were selected in the Southern Tawharanui Peninsula, Hauraki Gulf, New Zealand (Fig. 3-1). The subtidal sites were ~800m apart along the peninsula and approximately ~100m away from land. One of the characters of these two sampling sites was that the location of these scallop beds were not reported previously; therefore they are less prone to be affected by anthropogenic disturbance. An initial survey of the general area was also conducted with no sign of dredging, which indicated that these sites contained extensive scallop populations that were subjected to minimal fishing pressure. Thus *P. novaezelandiae* collected for this study was considered to come from its natural habitat in the Hauraki Gulf. The first site (36°23'13''S, 174°46'44''E) is located at 5-8 metres water depth and is composed of mostly sand with sparse seagrass beds, which provided a distinctive three-dimensional protective structure habitat. The second site (36°23'20''S, 174°47'09''E) is a muddy-sand habitat without benthic vegetation, and is also located at 5-8 metres water depth. Pictures of the two sites were included in (Appendix I).

Sample collections

Seven field sampling events were carried out at proximately bi-monthly intervals over a year to collect sediment, water and scallop samples from each of the two sites, by SCUBA. The sampling period extended across a reproductive season from

May 2012 to March 2013, which included the 'typical' pre-Christmas spawning in November 2012 and one further spawning in March 2013. Bottom water temperatures were recorded at the same time when scallop were collected.

Sediment samples:

Two replicate sediment samples were collected during each sampling event at slack tide to minimize collection variability. The samples were collected by SCUBA divers with a core sampler (130 cm diameter and 3 cm depth). Each sample was double bagged and transported to the laboratory in a cold box. Once at the laboratory, the sediment samples were centrifuged to remove excess water and freeze-dried until further analysis. Particulate organic matter (POM) quantification of each sediment sample was conducted by combustion at 490°C. The loss on ignition (LOI) was then calculated according to the following equation:

$$LOI_{490} = ((DW_{\text{freeze dried}} - DW_{490}) / DW_{\text{freeze dried}}) * 100$$

where, DW is the dry weight. One-off sediment grain-size analyses were conducted at the start of the study by wet sieving to characterize the habitats within each site. Sediment grain-size characteristics are presented as median particle diameter (MPD) and classified according to Gray and Elliott (2009).

Water samples:

To quantify seston concentrations in the water column, two replicate water samples of 20 litres each were collected from both the surface (0.5m below the water surface) and bottom (0.1m above the seabed) waters at each site during each sampling event (Appendix II). The water samples were screened through a 250 µm mesh net to remove large particles. Water was transported to the laboratory in dark containers cooled with cold packs. Once at the laboratory, the samples were kept at 4°C and processed within 24 hr. First, the samples were filtered through 5 µm polypropylene membranes to collect the large seston fraction, and then through 1.2 µm (GF/C) pre-combusted glass fibre filters to obtain the small seston fraction. The filters containing seston were then washed twice with 5ml of 0.9% ammonium formate and freeze-dried for further biochemical analysis. These seston size fractions were selected following Cranford *et al*. (1998), who suggested that scallops consume mostly particles larger than 5µm, while other authors (MacDonald & Thompson 1985, Pazos *et al*. 1997, Lorrain *et al*. 2002) report the consumption of smaller-sized fractions. In addition to these potential food particle size classes, chlorophyll *a* concentrations were determined using 1–3 litre aliquots from each replicate water sample. These sub-samples were concentrated on Whatman®GF/C filter discs, and the chlorophyll was extracted with 90% methanol (24 hr at 4°C). The absorbance of each sample was measured at 652, 665 and 750nm (Porra 2002). Chlorophyll *a* concentrations were calculated according to the following equation:

$$\text{Chl } a \text{ } (\mu g \text{ } ml^{-1}) = 16.29 \text{ } A^{665-750} - 8.54 \text{ } A^{652-750}$$

where, A is the absorbance.

Scallop samples:

Thirteen to nineteen scallops (>80 mm shell height) were collected by SCUBA divers at each site during each sampling event. Due to the design of the experiment required regular sampling, number of scallops collected from the sampling sites were chosen carefully to avoid significant impact to its natural population. Scallops were cleaned thoroughly, and theirs shell heights and widths were measured to the nearest 0.01 mm with Vernier callipers. Then, each scallop was dissected within 6 hours post-collection. The adductor muscle, digestive gland and gonad were removed from each individual and weighed separately. When digestive gland was prepared, food particles were extracted from lumen to minimise contamination of digesting food. Tissues were then freeze-dried and weighed. Condition indices of gonad (GI), adductor muscle (AMI) and digestive gland (DGI) were determined according to Barber & Blake (1981):

Condition index= (tissue DW/total tissue DW) *100

where, DW is the dried weight.

Reproductive condition

Visual gonad index (VGI) was determined for all animals just prior to dissections. Visual grades 1—8 were assigned to each gonad, based on their appearance and following the classification by Williams & Babcock (2004) (Table 1-1). These grades included spent (grade 1), partially spawned (grade 2, 3), active (grade 4−6) and ripe (grade 7, 8), and were based on morphological appearance of the gonad

to the naked eye. For gonads that were >3 g wet weight, tissue samples of approximately 15x10x5 mm were taken from the centre of each gonad at the interface of the testis and ovary, and stored separately in 10% buffered formalin for histological analysis. For sampling events when all gonads were too small (i.e., all gonad were <3g), the entire gonad from 5–10 animals were used for histology, and separate animals from the same sampling event were used for biochemical analyses (see below).

Histological sections of gonad tissue were observed to identify scallop reproductive stages. Fresh gonad tissues were fixed in 10% buffered formalin for a minimum of 48 hours. Tissues were dehydrated in A graded ethanol series, cleared in xylene and embedded in paraffin wax. Blocks were sectioned to 5 µm thickness, mounted on slides, and stained with haematoxylin and eosin. Each histological section was observed to determine the state of follicle and gamete development, and to assign a developmental stage (1-5 according to Bull 1976). The diameters of 30 randomly selected nucleated oocytes were measured per scallop to provide quantitative data on reproductive condition of each individual.

Nutritional condition

Biochemical composition of three main tissues (adductor muscle, digestive gland and gonad) were determined for each scallop from each of the sampling sites and sampling events. For gonad samples, female and male gonads were analyzed separately only in October 2012, November 2012 and January 2013, when enough material was available. The biochemical analyses included total carbohydrates, total

protein, total lipids and fatty acid profiles. In addition, enough material was available from the >5µm seston samples to conduct fatty acid profile analyses. Stable isotope (^{13}C and ^{15}N) signatures were obtained for scallop tissues, seston and sediment, as discussed below.

Biochemical composition:

Carbohydrates were determined using the Anthrone method according to Hedge & Hofreiter (1962) and reported as glucose equivalents. Carbohydrates were hydrolyzed into simple sugars using dilute hydrochloric acid (2.5N). A serial of glucose standard solution was prepared using glucose powder (Sigma Aldrich) in distilled water. Then hydrolyzed samples and standard solutions were mixed with four parts of anthrone solution. Samples were then incubated for 8 min at 100°C, and the optical density of the green coloured solution was read at 630nm on a spectrophotometer. Protein was determined using a commercial kit (The Pierce BCA Assay by Thermo Scientific™) and reported as bovine serum albumin (BSA) equivalents. Samples were digested with 0.5N NaOH for 30min at 56°C for this analysis. A serial of protein standard solution was prepared using the Albumin Standard (BCA kit) in 0.5N NaOH. The supernatant and standard solutions were then incubated with BCA solution for 2 hours at 37°C. The optical density of the solution was read at 562nm. Total lipid contents were measured according to Bligh & Dyer (1959) and Marsh & Weinstein (1966), and are reported as tripalmitin equivalents. Lipids were extracted in a 2:1 chloroform-methanol mixture. A serial of lipid standard solution was prepared using tripalmitin (Sigma Aldrich) in chloroform. Extracted lipids and standard solutions were

then dried under a flow of nitrogen and the amount of total lipid was determined by charring with concentrated sulphuric acid for 15 min at 200°C. The optical density of the solution was read at 375nm on a spectrophotometer. All biochemical components reported in this chapter was standardised to dry weight.

Energy content:

Energy conversion factors were used to obtain energy contents (Saout *et al.* 1999). These factors were 17.2, 23.9 and 33kJ g^{-1} for carbohydrates, proteins and lipids, respectively. The energy content of each tissue type was calculated for individual scallops in order to determine the energy contribution of each organ to the animal's energetics over the reproductive cycle.

Fatty acid profiles:

Fatty acids were prepared by a gas chromatographic one-step methylation extraction method, which was adopted from a method by Garcia *et al.* (2000). An aliquot (10-20mg) of homogenized freeze-dried sample was added to 0.49 ml of toluene, 0.75 ml freshly prepared 5% methanolic HCl (0.75 ml) and 10µl of 2g l^{-1} tridecanoic acid (internal standard). Tubes with the sample solutions were closed under nitrogen to avoid lipid oxidation and heated at 70°C in a water bath for 2 hours. Then, 1 ml of 6% aqueous K_2CO_3 and 0.5 ml of toluene were added and centrifuged at

1100 g for 5 min. The organic phase on the top layer, which included FAMEs, was transferred into a beaker while the water was precipitated by anhydrous Na_2SO_4.

The separation and quantification of FAMEs were conducted with a 2010 Shimadzu GC-2010, which was integrated with a split-splitless injector and flame ionization detector (FID). The individual FAMEs were separated and identified by a Zebron ZB-Wax capillary column (30 m x 0.25 mm x 0.25 µm) from Phemomenex. The temperature of the oven was from 140 to 245°C. The temperature increased from 5°C min^{-1} to 245°C and held for 15 minutes. One cycle took a total of 50 min. The gas carrier was nitrogen with a flow-rate of 60 ml min^{-1} and the flow of hydrogen was 40 ml min^{-1}. The average linear velocity was set to 20 cm sec^{-1}, with a head pressure of 8.7 psi (1 psi = 6894.76 Pa). The temperature of the detector was 250°C and the volume of injection was 1 µl.

FAMEs were identified by comparing the retention times with a standard (Supelco 37 Component FAME Mix, 18919-1AMP) and FAMEs from vegetable oils, fish oils and New Zealand green-lipped mussels (*Perna canaliculus*). The response factor of the internal standard was used for quantification.

Stable isotopes:

Two samples of sediment, seston (from the 2 size classes), and isolated scallop tissues (adductor muscle, digestive gland and gonad), all from each sampling site and event were used for stable isotope analyses. Isotope analyses were processed by the

Waikato Stable Isotope Unit, The University of Waikato, Hamilton, New Zealand. Isotopic analyses were carried out on a fully automated Europa Scientifi c 20/20 isotope analyzer. Samples were combusted and the resulting gases were separated by gas chromatography, and then analyzed by continuous flow-mass spectrometry. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ = (R sample / R standard - 1) × 1000,

where, R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. δ ^{13}C was measured to a precision of ±0.5%. All samples were referenced to pre-calibrated C_4 sucrose, which was cross-referenced to the PeeDee Belemnite standard. $\delta^{15}N$ was measured to a precision of ±1%. All samples were referenced to a urea standard that is traceable to atmospheric nitrogen.

Statistical analyses

Two-way analyses of variance (ANOVA) was used to identify differences in scallop reproductive and nutrition condition between months and sites, performed using the SPSS statistic package. Multidimensional scaling (MDS) was used to identify patterns in fatty acid composition among different scallop tissues throughout the sampling period. Centroids by tissue type and by sampling month were plotted. Similarity of percentage (SIMPER) was used to identify the fatty acids that primarily provided the discrimination between groups (i.e., tissue types). Plymouth Routines in Multivariate Ecological Research (PRIMER), was used to perform the MDS and SIMPER

analyses on untransformed fatty acid data (organ-specific fatty acid compositions in animals always consist of arrangements of the same fatty acids present in different proportions (Napolitano *et al*. 1997), hence data were not transformed to avoid giving more weight to fatty acid present in small quantities), with a nonparametric Bray-Curtis similarity matrix, following methods by Parrish *et al*. (2015).

Results

Sediment samples

The sediment data from the two sites revealed slight differences in sediment composition. Site 1 had medium to fine sand of 1.3 ϕ median particle diameter (MPD), while site 2 had fine sand of 2 ϕ MPD.

Analysis of particulate organic matter (POM) in sediment samples collected throughout the year revealed significant differences between the two sites and among months, with a significant interaction (Table 3-1). While the POM values in site 1 were relatively stable between 4.1 and 5.8% DW over the year with a small peak in November, site 2 had a wide range of values from 3.2 to 6.8% DW with a large peak in October (Fig. 3-2).

Water samples

The water temperature was the same at both sites, with high values in summer and early autumn (maximum of 22°C in January, 2013) and low in winter (minimum of 12°C in August, 2012) (Fig. 3-3).

Analysis of water samples indicated that seston abundances were relatively similar between sites with no significant differences (Table 3-1) found throughout the sampling period (Fig. 3-4). Surface water samples from both sites consistently had low seston concentrations (< 5 mg l^{-1}) for both size classes (1.2−5 and >5 µm), except for a peak in concentration of about 13 mg l^{-1} for the 1.2−5 µm particle fraction in October 2012. For bottom water samples, seston concentrations varied greatly from 3 to 22 and 0.7 to 16 mg l^{-1} for small (1.2−5 µm) and large (> 5 µm) size classes, respectively at site 1, while seston concentrations at site 2 ranged from 3 to 24 and 2.2 to 27 mg l^{-1} for small and large size classes, respectively. The temporal pattern of seston concentrations at the bottom revealed a major peak at both sites in August 2012.

Surface water samples from the 2 sites were not significantly different (Table 3-1), consistently had low chlorophyll concentrations (< 2 µg l^{-1}), except for a peak in concentration of about 6 µg l^{-1} for site 2 in August 2012. Bottom chlorophyll concentrations were significantly difference between the 2 sites (Table 3-1), with a major peak between July and November at site 2, while a smaller peak was observed in October at site 1. There was no agreement between the patterns in bottom chlorophyll concentration and seston abundance at site 1. At site 2, chlorophyll concentrations overlapped well with seston abundances for both size classes.

Reproductive condition

Visual gonad index (VGI):

Visual gonad index (VGI) analyses showed seasonal variations for scallops at both sites over the sampling period (Fig. 3-5), but VGI were not significantly different between site 1 and site 2, and there was no significant interaction (Table 3-2). Immature/spent gonads (grade 1) were most frequent in March 2013 followed by May 2012 at both site, while partially spawned gonads (grade 2) were most frequent in May 2012 for site 1 and July 2012 for site 2. Active gonads (grade 3-5) were recorded most often in August and November 2012 at both sites, while ripe gonads (grade 6-8) were dominant in October 2012 and January 2013 at both sites.

Histology:

Analyses of histological sections of scallop gonads indicated that all 5 developmental stages used in the gonadal development classification by Bull (1976) could be observed over the sampling period (Fig. 3-5 & 3-6). Gonadal development was not significantly different between the 2 sites (Table 3-2). Spent gonads (stage I) were most frequent in May 2012 for site 1 and March 2013 for site 2 , while gonads in the early development stage (stage II) were most frequent in May 2012 at both sites. Gonads within the active developmental stage (stage III) were recorded in high percentages in July, August, November 2012 and January 2013 at both sites, while ripe

gonads (stage IV) were dominant between August 2012 and January 2013 for site 1, and August 2012 to March 2013 (expect November 2012) at site 2. Partly spent gonads (stage V) were recorded most frequently between October 2012 and January 2013 at both sites. Oocyte diameters measured from photomicrographs of gonadal sections showed similar overall patterns at both sites with two periods of maximum oocyte diameters in October 2012 and January 2013 (Fig. 3-5). These two peaks were followed by sharp declines indicating likely spawning. Oocyte diameters ranged from 25 – 48 and 25 –51 for site 1 and site 2, respectively, over the entire sampling period. The seasonal variation of oocyte diameters overlapped well with the VGI data, indicating scallop maturity can be reflected by both parameters.

Scallop gonad condition indices (GI) were not significantly (Table 3-2) different between the 2 sites (Fig. 3-7). At both sites, notable increases in GI were observed from May 2012 and reached the highest values in October 2012, with 13.7% for site 1 and 15.1% for site 2. GI values dropped in November 2012, and reached a second peak of 12.7% (site 1) and 13.6% (site 2) in January 2013. The seasonal variation of GI values was found to be similar to that of VGI values and oocyte diameters over the sampling period.

Biochemical analyses:

Biochemical composition of gonad tissues is presented in Figure 3-8. Analyses of carbohydrates, proteins and lipids within gonads resulted in relatively similar trends for scallops at both sites, but some noteworthy differences were observed: gonad protein

and lipid levels were significant different between the two sites (Table 3-3). The fluctuation in carbohydrate contents within gonad samples was low, but a notable association with gonad maturation was observed, with a major increase in October 2012 to a maximum of 66±8 mg g^{-1} for females and 33±5mg g^{-1} for males at site 1, and 76±9 mg g^{-1} for females and 64±12mg g^{-1} for males at site 1 and 2 (Fig. 3-8). At site 1, protein content in gonads reached two peak levels in October 2012 and January 2013, each followed by a serious decline in the next sampling month. Female and male gonad protein levels dropped from 384±13mg g^{-1} to 282±14mg g^{-1} and 327±14mg g^{-1} to 240±12mg g^{-1}, respectively, between October and November, while protein levels dropped from 356±103mg g^{-1} (female) and 288±16mg g^{-1} (male) to 213±1mg g^{-1} (combined), between January and March. On the other hand, gonad protein levels of scallops from site 2 did not reach a maximum until January 2013 (females: 356±10mg g^{-1}; males: 288±16mg g^{-1}), and a minimum level was recorded in July 2012. The average protein value was 201±13mg g^{-1}. The changes in lipid contents in gonads were similar to those of carbohydrates and gonad maturation. Lipid contents increased gradually from May 2012 to October 2012, when the first peak was reached (site 1: 179±8mg g^{-1} for females and 95±8mg g^{-1} for males; site 2: 157±7mg g^{-1} for females and 76±4mg g^{-1} for males). Subsequent declines likely coincided with spawning events. Lipid levels reached a second peak in January (site 1: 202±8mg g^{-1} for females and 104±10 mg g^{-1} for males; site 2: 152±11mg g^{-1} for females and 70±5 mg g^{-1} for males), followed by a major decline and potential second spawning in March 2013.

Nutritional condition

Adductor Muscle:

Adductor muscles had the largest condition indices, ranging from 44–53% and 44–54% for sites 1 and 2, respectively. Scallop adductor muscle condition indices (AMI) were generally negatively correlated with gonad condition indices (GI) over the annual cycle. The AMI values were generally higher in winter (July and August 2012) compare to summer for both sites. The maximum value was reached in July 2012 and the minimum in October 2012 for both sites (Fig. 3-7). AMI was not significantly different between the 2 sites (Table 3-3).

Analysis of adductor muscle tissues revealed similar overall patterns in biochemistry for scallops from both sites over the sampling period, although some differences were observed, but muscular protein and lipid contents were significantly different between sites, and also there were significant interaction terms (Table 3-4, Fig. 3-8). The fluctuations of carbohydrate contents within scallop muscles from both sites were similar, with a clear maximum in November of 134±10 mg g^{-1} and 112±12 mg g^{-1} for animals from site 1 and 2, respectively. Conversely, protein content in the adductor muscle of scallops from both sites was lowest in November. Protein values ranged from 110±3 mg g^{-1} in November to 234±12 mg g^{-1} in January for site 1 and from 125±9 mg g^{-1} in November to 257±4 mg g^{-1} in July for site 2. The drop in protein content in the adductor muscle tissues in November coincided well with the drop in scallop condition index in October, suggesting a lag time of about one month between these physiological parameters (Fig. 3-8). Lipid levels in adductor muscle tissues were

low (26±1.6 – 55±3.1 mg g^{-1} and 38±1.4 – 81±4.3 mg g^{-1} for site 1 and site 2, respectively) throughout the year with a slight increase to 55±3.1 mg g^{-1} in October at site 1 and to 81±4.3 mg g^{-1} in November at site 2 (Fig. 3-8). This slight increase in lipid muscular lipid content appears to be associated with post-spawning.

Digestive gland:

Analysis of digestive gland tissues revealed no significant difference (Table 3-3) between the 2 sites over the sampling period, but there was a significant interaction term. Digestive gland condition indices (DGI) of scallops from both sites did not fluctuate much over the seasonal cycle (Fig. 3-7). The variation in DGI was from 7.3 to 9.1% and 7.6 to 8.7% for sites 1 and 2, respectively.

The biochemical analyses of digestive gland revealed no significant difference between the 2 sites, but there were significant interaction terms (Table 3-4). The carbohydrate contents within digestive glands were relatively similar for scallops from both sites, between 56±8 to 116±10 mg g^{-1} at site 1 and 54±9 to 129±15 mg g^{-1} at site 2 throughout the year (Fig. 3-8). Protein contents in digestive glands were similar for animals from both sites (Fig. 3-8). Protein values followed a constant increase from the lowest values in May (291±9 mg g^{-1} at site 1 and 358±12 mg g^{-1} at site 2) to the highest values in October (535±19 mg g^{-1} at site 1 and 546±17 mg g^{-1} at site 2). Then, there was a small decrease in protein content to about 450 mg g^{-1} that was maintained in scallops at both sites until March 2013. Lipid contents in digestive glands were relatively constant throughout the year, ranging from 105±5 to 221±15 mg g^{-1} for scallops at site

1 to 106±4to 179±11 mg g^{-1} for scallops at site 2 (Fig. 3-8). While a distinct November peak in lipid content was observed in digestive glands of scallops at site 1, no distinct peak was evident for individuals at site 2, hence the significant interaction term in Table 2-4.

Energy content:

The temporal pattern of average energy content within gonads, muscles and digestive glands showed a relatively similar trend for scallops from site 1 and 2 throughout the year (Fig. 3-9), but statistical analyses revealed that the adductor muscle energy was significantly different between the 2 sites and there was also a significant interaction (Table 3-5). For scallops from both sites, the tissue with the highest energy content was the adductor muscle (site 1: 4.7−15.8 kJ; site2: 7.4−19.5 kJ), followed by the digestive gland (site1: 2.8−8.7kJ; site2: 4.8−7.8kJ) and gonad (site1: 1.1−10 kJ; site 2: 1.9−8.7kJ). The pattern in gonadal energy content was consistent with that of the gonad index (GI), which was lowest in May 2012 and highest in October 2012 and January 2013. The energy content in scallop adductor muscles was lowest in March 2013 (after the presumed January spawning), while the energy content in digestive glands was low in the colder season, at approximately 5kJ for both sites, but contribution to the overall energy pool increased during spawning and/or summer (up to 9kJ).

The highest values in calorific content per gram were found in digestive glands due to protein accumulation. Thus, digestive glands exhibited a maximum energy of

20.3kJ g^{-1} (site 1) and 19.8kJ g^{-1} (site 2) in November 2012 and August, respectively, and a minimum of 12.4kJ g^{-1}(site 1) and 14.1kJ g^{-1} (site 2) in May 2012. In the adductor muscle, there was no clear variation in caloric content, ranging between 4.5−7.6 kJ g^{-1} and 6.9−8.9 kJ g^{-1} for sites 1 and 2, respectively. The seasonal variation of gonad energy content was found to be similar to that of gonadal maturation (i.e., GI, VGI values, oocyte diameter), with a maximum energy content of 13.7 (site 1) and 12.1 (site2) in October 2012, and minimum of 7.2 in March 2013 for site 1 and 7.7 in July 2012 for site 2.

Fatty acid profiles

MDS plots show clear clusters of fatty acids from seston and scallop tissues (gonad, adductor muscle and digestive gland) for each site. The gonad samples clustered between adductor muscle and digestive gland samples, while adductor muscle samples clustered tightly and separated well from the more dispersed group of digestive gland samples (Fig. 3-10). These spatial patterns were quantified by SIMPER, having similarities of >82% for all groups, with seston, female gonad, male gonad, adductor muscle and digestive gland having similarities of 85, 85, 88, 92 and 83%, respectively, for site 1, and 82, 92, 93, 95 and 82%, respectively, for site 2 (Table 3-6). As identified by SIMPER, C16:0, C16:1, C20:2 were the main contributors for the seston grouping, while C16:0, C20:5n3 and C22:6n3 were the top contributors for clustering of all three types of scallop tissues (gonad, adductor muscle and digestive gland), with the

addition of C18:0 for digestive gland. Fatty acid compositions for all samples are shown in Appendix IV.

Stable isotopes

Clear separation and clustering in stable isotope ($\delta^{15}N$ and $\delta^{13}C$) signatures were observed for all scallop tissues, sediment and seston samples from both sites over the sampling period (Fig. 3-11). Scallop tissues (gonad, adductor muscle and digestive gland) had tight and separate clusters with $\delta^{13}C$ signatures between -18.1 to -20.4, -17.7 to -18 and -19.4 to -22‰ for each tissue respectively, and $\delta^{15}N$ signatures between 8.5 to 9.9, 9.6 to 10.3 and 8.0 to 9.3‰ for each tissue respectively, at both sites. Sediment samples also separated well from seston and scallop samples with a narrow range of $\delta^{13}C$ signatures from -9.3 to -11.6 ‰, and a wide spread of $\delta^{15}N$ values from 4.7 to 9.3‰ (sites combined). Carbon isotopes for seston samples within the >5µm size class clustered between -18.1 to -24.1‰, while seston samples within the 1.2−5µm size class were slightly more enriched -20.7 to -26.3 ‰ for both sites. $\delta^{15}N$ values for the >5µm seston fraction ranged from 5.3 to 6.4 ‰ , while those of the 1.2−5µm size fraction ranged from 5.1 to 7.1‰ for both sites.

Temporal differences were found in $\delta^{13}C$ and $\delta^{15}N$ isotopes among tissues (Fig. 3-12). In samples from both sites, $\delta^{13}C$ values for adductor muscle varied little over time. Greater and comparable temporal variations were found for gonad and digestive gland tissues, with a general increase in $\delta^{13}C$ values from August 2012 to January 2013. Nitrogen values for gonad tissues exhibited a temporal shift in relation to somatic

tissues. Gonads and adductor muscles revealed similar signatures in winter (May – October 2012), then gonad signatures became closely related to those of digestive glands during November 2012.

Discussion

Environmental parameters

The two sites sampled in this study had similar sandy grain size composition, and higher seston abundances and chl *a* concentrations in the bottom water column compared to the surface. There were also comparable temporal patterns of low nutrient composition in the water column in winter followed by a spring bloom. However, the presence of seagrasses in site 1 provided a distinctive three-dimensional protective structure with potential higher nutritional content for scallops compared to site 2, which is composed of a flat sandy substrate. These physical site characteristics are correlated with the higher variation of POM values, seston level and chl *a* concentrations in site 2 compared to site 1 throughout the sampling period. Although chl *a* and sediment POM were the only environmental parameters that were significantly different between the two sites, the year-long monitoring on the environmental parameters showed a more stable environment in the seagrass site (site 1) than the sandy site (site 2).

The seston and chl *a* levels recorded at the two sites fall within the range of values previously recorded in the Hauraki Gulf, and share the same seasonal cycle of

minimum concentrations in winter followed by a spring bloom reported previously (Booth & Sondergaard 1989, Rhodes *et al*. 1993, Chang *et al*. 2003). The seston and chl *a* concentrations were consistently high in August and October 2012, indicating that phytoplankton was most abundant in spring in both surface and bottom waters, although bottom waters always had higher concentrations. The overall lower chl *a* concentrations in bottom waters at site 1 may to indicate limited benthic diatom growth on the seabed due to shading from the seagrass communities (Hemminga & Duarte 2000). Indeed, yellow-green biofilms (commonly containing diatoms) were observed on the seabed at site 2, but were absent at site 1. This material may be re-suspended by strong water currents and/or during storm events, thus providing a potentially important food supply for scallops. While the two sites may provide slightly different food types to scallops, it is difficult to associate these differences to specific patterns in nutritional and reproductive condition, especially when scallops are a mobile species that may use seagrass beds for other reasons, such as a protective environment against predators (Bologna & Heck 1999).

Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures for seston, sediment and scallop samples indicate that the small seston fraction (1.2−5 µm) is likely to be the main source of food for *P. novaezelandiae* (especially at site 2), while the large seston fraction (>5 µm) contributed to a lesser extent. As can be seen in Figure 3-11, sediment samples had considerably greater carbon values than those of scallops. Contrary to the present study, Cranford & Grant (1990) reported that the optimal retention efficiency of *Placopecten magellanicus* included food particles of 5−40 µm. However Ward & Shumway (2004) reviewed particle selection in suspension feeding

bivalves, and concluded that retention efficiency is species-specific, especially for particles < 6 µm. These species-specific differences have been suggested to be related to the morphology of feeding parts (Ward & Shumway 2004) and food availability (Zhang *et al*. 2010). While *P. magellanicus* (Cranford & Grant 1990) and *Monia squama* (Jorgensen *et al.* 1984) primarily consume seston particles that are > 5 µm, high retention efficiency of 2−5µm seston has been shown for *Mytilus edulis* (Vahl 1972), *Argopecten irradians* (Palmer & Williams 1980), and *Petricola pholadiformis* (Jorgensen *et al.* 1984). In addition, Pectinids have the ability to control the quantity (Cahalan *et al*. 1989) and quality (organic *versus* inorganic) of particle consumption (Brillant & MacDonald 2002). Nicholson (1978) documented that selectivity in *P. novaezealandiae* is achieved by using their lips and palps to control the ingestion of seston <5 µm, and these seston contributed as much as 50% of the total diet in this species.

It is difficult to classify wild seston into phytoplankton groups based on the size difference , due to the seasonal variation in phytoplankton combination (flagellates and cryptophytes dominate in spring and winter, whereas diatoms are abundant in summer) (Bănaru *et al*. 2014), and the variation in cell size among populations (i.e. cell size decreases when population size increases) (Cassie 1959). In the present study, isotopic analyses revealed different carbon signatures between the two seston fractions collected, with large seston being more enriched (-20.9±0.9‰ in site 1 and -19.5±0.4‰ in site 2) than small seston (-23.2±0.9‰ in site 1 and -22.8±1‰ in site 2). Typically, suspended organic matters (mainly detrital material) have a more depleted ^{13}C than phytoplankton (Harmelin-Vivien *et al*. 2008). In addition, small phytoplankton might also be reflected by lower carbon values. Few studies have reported larger

microphytoplankton particles being markedly ^{13}C enriched compared with small cells (< 6 µm) (Rau *et al*. 1990, Rolff 2000). Although carbon isotope signatures of diatoms were more enriched than other phytoplanktons when pure net plankton samples were analysed (Fry & Wainright 1991), the authors also reported insignificant correlation between carbon signature and taxonomic composition of phytoplankton when samples were classified by dominant phytoplankton (Wainright & Fry 1994). Hence, isotopic signature cannot provide a straight answer to determine wild seston composition. Therefore, in the present study, the isotope signatures and environmental parameters suggested that *P. novaezelandiae* consumes primarily small seston fraction (likely to be detritus and small phytoplankton), with larger phytoplankton as a secondary food source at the study sites. Indeed detrital material has been shown to be consumed by scallops *P. novaezelandiae* (Nicholson 1978) and *P. magellanicus* (Cranford & Grant 1990), while phytoplankton has been reported to be a food item of *P. maximums* (Chauvaud *et al.* 2000) and *Zygochlamys patagonica* (Botto *et al*. 2006).

Reproduction

Despite the potentially different nutritional environments found at the 2 sampling sites, the gametogenic cycle of *P. novaezelandiae* did not differ between sites. The reproductive cycle was characterized by two major spawning periods, reflected by a sharp decrease in visual gonad index (VGI), oocyte diameter, gonad condition index (GI) and lipid content. Spawning events occurred in November 2012 (referred to as spring spawning) when the temperature was about 18°C and a second

spawning in March 2013 (referred to as summer spawning) when the temperature reached 22°C. Nicholson (1978) and Morrison (1999) recorded a similar spawning pattern for *P. novaezelandiae* in the Hauraki Gulf. Nicholson (1978) surveyed populations in Takatu and Ti Point in the Hauraki Gulf (~1.5 km east and ~8.5km northeast from the sampling sites in this study, respectively) to investigate the reproductive cycle of *P. novaezelandiae.* He measured gonad index and macroscopic visual classification between 1976 and 1977, and concluded that *P. novaezelandiae* was limited to one pre-Christmas spawning with a further potential spawning in February and March, should environmental conditions prove suitable. Fourteen year later, Morrison (1999) collected reproductive data again from Takatu, south of Ti Point (~7 km northeast of the sampling sites here) and Motuketekete Island (~9.5 km southeast of the sampling sites here), and confirmed a clear biannual spawning pattern for *P. novaezelandiae* in the Hauraki Gulf. Although Nicholson (1978), Morrison (1999) and the present study recorded two spawning events per year, spawning of *P. novaezealandiae* is not necessarily predictable. Williams (2005) recorded 3 spawning events between 2000 and 2001 near Plembles Island (~4.5 km southeast of the sampling sites here), with the addition of partially spawned individuals recorded throughout the year. He concluded that the spawning timing of this species is variable among populations, but the largest spawning events tend to occur mostly in December (which is referred to as spring spawning in this study). Such spawning variations may be a result of size structure variations among populations and/or environmental conditions, such as temperature and food availability. Indeed, Sastry (1968) found that temperature was a strong determinant on the rate of gamete maturation, whereas

fecundity and size of gonad were primarily determined by food availability. Experiments using *P. fumatus* (Heasman *et al*. 1996) and *P. novaezealandiae* (Nebsit 1999) provide further evidence that gonad size is strongly affected by diet quantity, whereas gonad condition varies more with temperature. Although laboratory experiments have shown that low temperature (12°C) may slow down the rate of gametogenesis (Nebsit 1999), spawning has been recorded in the Hauraki Gulf during winter when surface water temperatures have been 14°C (bottom water temperatures would have been lower) (Williams 2005). Thus, it is possible to conclude that gametogenesis may be slowed down but not prevented by winter temperatures at the study site. In addition, food availability may be a primary factor responsible for variations in spawning timing and number of spawning events.

In the present study, the biochemical composition of gonad samples generally supported the VGI and histological data; carbohydrates, protein and especially lipid showing an increase in nutrition levels with gonad maturation. Energy content in gonad started to increase in August 2012, indicating the beginning of gametogenesis, and reached a maximum in October, followed by a pronounced drop in November, representing the spring spawning. Carbohydrates, proteins and lipids have been shown to increase in gonads during gametogenesis, leading to spawning in other Pectinid species (Racotta *et al.* 1998, Saout *et al*. 1999, Barber & Blake 2006). For example, higher carbohydrate contents were found to be associated with gonad development during broodstock conditioning of *Argopecten purpuratus* (Martinez *et al.* 1992) and *A. ventricosus* (Racotta *et al.* 1998), and it has been suggested that carbohydrates accumulated in gonads of molluscs can be used directly as energy for spawning

(Racotta *et al.* 1998, Ruiz-Verdugo *et al.* 2001). Protein is the fundamental component of all tissues, as it provides structural material to the cell. Accumulation of proteins in gonads during maturation is commonly reported in scallop species in association with gonad growth, (Barber & Blake 1981, Epp *et al.* 1988, Couturier & Newkirk 1991, Pazos *et al*. 1996, Racotta *et al*. 1998, Martinez *et al*. 2000b, Ruiz-Verdugo *et al.* 2001). Also, accumulation of lipids in gonads during gametogenesis is an important and well-documented process (Vassallo 1973, Barber & Blake 2006, Couturier & Newkirk 1991, Martinez 1991, Pazos *et al*. 1997), and lipids are essential for equipping eggs with the needed resources to fuel their energetic embryo and larval development processes (Holland 1978). Indeed, Devauchelle & Mingant (1991) demonstrated that the hatching success rate of *P. maximus* is highly dependent on egg lipid reserves. In the present study, spat were not collected to assess the hatching success rate of *P. novaezelandiae* in the sampling area. However, polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA; 20: 5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are essential for bivalve larval survival (Helm *et al*. 1997, Uitting & Millican 1997), and these were the main fatty acids in gonad samples tested. In addition, lipid contents in female gonads decreased dramatically during the two spawning events, indicating that lipids were accumulated in developing eggs, which were then released to the ocean.

Nutrition

The high energy requirements of gametogenesis usually require storage of nutrients where they can be easily accessed when needed (Barber & Blake 2006). In

the present study, energy gain in scallop gonads was more than the loss of energy recorded in the adductor muscle and/or digestive gland, indicating that energy demands for gametogenesis in *P. novaezelandiae* were supported by both ingested food and nutrient reserve. The biochemical data suggested that energy reserves used during gametogenesis were mainly obtained from carbohydrates and proteins in the adductor muscle and lipids in the digestive gland.

Bivalves have no localized nutrient storage components or sites, such as the liver in vertebrates, which is responsible to the storage of glycogen (Barbara & Blake 1981). However, specific cells involved in storage reserves have been described for some bivalve species. For example, adipogranular cells store glycogen, protein and lipids, and vesicular cells store glycogen in Mytilidae (Pipe 1987). In Pectinids, it has been documented that the large adductor muscle provides energy for reproduction, but storage cells have not yet been described (Mathieu & Lubet, 1993). In the present study, the energy level in the adductor muscle progressively decreases from July 2012 to March 2013, with a more pronounced decrease post spring spawning. This indicates that energy from adductor muscles is likely to fuel gametogenesis and satisfy the high energy demand during spawning periods. In addition, carbohydrate levels decrease during August to October, November to March in adductor muscles, indicating that these carbohydrates are likely to be used for oogenic development and gonad maturation. Once in the gonads, carbohydrates could be converted into lipids and stored in the ripening gametes, as reported for many marine bivalves (Gabott 1975, Berthelin *et al*. 2000). The role of carbohydrates (mainly glycogen) is predominant in most bivalves in term of nutrient storage to support gametogenesis (Barber & Blake

1981, Martinez *et al*. 2000b, Lodeiros *et al*. 2001). On the other hand, proteins are the major structural components in cells and have a high contribution to total energy levels in the adductor muscle. The decrease in protein in the adductor muscle from July (when food availability and temperature are lowest) through to the spring spawning in November, indicates that protein is likely to be used to meet the maintenance requirements during winter and the high energy demands of spawning in spring. Indeed, the breakdown of adductor muscle protein to provide maintenance energy has been recorded in *A. irradians concentricus* (Barber & Blake 1981), *A. irradian irrdians* (Epp *et al*. 1988), and *Euvola zizac* (Brokordt *et al*. 2000), and other bivalve species, such as *Tapes philippinarum* (Beninger & Lucas 1984) and *Mytilus edulis* (Bayne *et al*. 1982).

The digestive gland is known to play an important role in regulating the distribution of assimilated nutrients in body tissues (Vassallo 1973). Depending on seasonal food supplies and reproductive state, the digestive gland can also serve as a storage organ (Thompson *et al*. 1974). In the present study, the fatty acid profiles support the notion that the digestive gland was involved in the initial assimilation and subsequent transfer of nutrients from the ingested food (seston) to the gonad during the sampling period. Indeed, the MDS plots of fatty acid signatures show a closer association between digestive gland and seston samples than gonad or adductor muscle samples and seston. This is likely to indicate that fatty acids originating from seston were first incorporated into the digestive gland and then re-allocated to the gonad, while the adductor muscle has no or little involvement in storage of fatty acids from seston. Caers *et al*. (2003) also reported dietary lipids being stored in the

digestive gland and subsequently transferred to the developing female gonad in *A. purpuratus*. In addition, adductor muscle tissues are unlikely to store large quantities of lipids, and lipid components within this organ are normally used for cellular structure (Napolitano *et al*. 1997).

In the present study, energy levels in digestive gland samples continuously increased from winter to spring at both sampling sites, and energy utilization started from January (post spring spawning) onwards, suggesting that the energy from the digestive gland was being used for gonad recovery. Consistently low carbohydrate levels in the digestive gland were recorded over the course of the study, but an inverse relationship between carbohydrates in the digestive gland and gonad maturation was observed. This relationship may indicate the use of digestive gland carbohydrates for gametogenesis. However, previous studies have found no evidence for the use of digestive gland carbohydrates for reproductive purposes in Pectinids (Barber & Blake 1981, Pazos *et al*. 1997, Racotta *et al*. 2003). Comparatively high protein contents were observed in digestive gland tissues, with an increasing trend during gametogenesis, peaking in October (prior to spring spawning), followed by a slight drop during spring spawning. This pattern suggests that digestive gland proteins are not required for gametogenesis, but accumulated in digestive gland tissues and used during gonad spawning. Lipid contents within digestive gland tissue increased with increasing gonad maturation in scallops prior to spring spawning, but appeared to be depleted by January. This pattern suggests that digestive gland lipids are not required to prepare for spring spawning, but stored lipids contribute to gonad re-development after this spawning event. Although the digestive gland lipids appeared to fluctuate more in

samples from site 1 than site 2, the general pattern at both sites was similar over the recorded period. Lipid reserves in digestive glands have been reported to be important energy reserves for gametogenesis in *Argopecten irradians concentricus* (Barber & Blake 1981), *Pecten maximus* (Comely 1974, Pazos *et al*. 1997, Saout *et al*. 1999) and *Placopecten magellanicus* (Robinson *et al*. 1981).

Energy reserves and utilization in *P. novaezelandiae* and their relationship to the reproductive cycle represent complex physiological interactions between gonad, adductor muscle and digestive gland. Often, gamete production needs to compete for energy resources with somatic growth and/or maintenance expenses (Barber & Blake 1985). For the most part, the utilization and storage of energy can be identified through investigation of biochemical and/or metabolic processes within and among different tissues. For example, Lorrain *et al*. (2002) suggested that $\delta^{15}N$ signatures can be used to indicate metabolic fluxes (energy transfer) among tissues in the scallop *Pecten maximus* (i.e., variations in $\delta^{15}N$ are consistent with energy transfers). As can be seen in Figure 3-12, nitrogen signatures in gonad samples experienced temporal changes in relation to somatic tissue in the present study: gonad samples shared a similar signature to adductor muscle samples during winter, then gonad signatures became comparable with digestive gland signatures during and after spring spawning (November). This pattern suggests that energy was transferred from adductor muscle to gonad to support the energy demand for gametogenesis during winter, and digestive gland energy was allocated to gonad from November for spawning and gonad recovery.

Overall, the nutritional condition of *P. novaezealandiae* revealed by $\delta^{15}N$ signatures and biochemical analyses of tissues suggested that this scallop species exhibits temporal changes in energy transfer. These are indicative of the use of energy (mostly carbohydrates) from the adductor muscle to fuel gametogenesis (gonad maturation) during winter, and a shift to energy derived from the digestive gland for use during spring spawning (mostly protein) and gonad recovery (mostly lipids) during summer (Fig. 3-13).

Summary

The findings of this study show that energy allocation between gonad and somatic tissues in *P. novaezealandiae* exhibits a temporal (seasonal) trend. When food availability is low in winter, adductor muscle energy reserves are utilized to initiate gametogenesis. In spring/summer, when food resources are abundant, assimilated food is directly transferred from the digestive gland to the gonad for spawning and gonad recovery. During autumn, when food availability decreases, assimilated food within the digestive gland is used for maintenance expenses, and excess energy is transferred to the adductor muscle for storage. Thus, *P. novaezelandiae* requires both long-term energy reserves from the adductor muscle and energy mobilized from ingested food through the digestive gland to achieve its reproductive cycle. Carbohydrates in the adductor muscle provide energy to initiate gametogenesis in winter, protein from the digestive gland provides the threshold levels of energy for

spawning in spring/summer, and lipids from the digest gland provide energy for gonad recovery after spring spawning.

Table 3-1 Statistical analysis of particular organic matter (POM) of sediment, surface seston concentration, bottom seston concentration, surface chl *a* and bottom chl *a* for site 1 and site 2. Bold numbers indicate significant differences, at $p < 0.05$.

Sediments

Source	*df*	POM	
		F	p
Site	1	6.58	**0.02**
Month	6	5.60	**<0.01**
Site x Month	6	10.39	**<0.01**
Error	14		

Surface seston

Source	*df*	1.2–5µm		>5 µm	
		F	p	*F*	p
Site	1	1.86	0.19	0.12	0.74
Month	6	1.94	0.14	0.55	0.76
Site x Month	6	0.05	0.69	0.73	0.64
Error	14				

Bottom seston

Source	*df*	1.2–5µm		>5 µm	
		F	p	*F*	p
Site	1	1.06	0.32	0.01	0.95
Month	6	4.04	**0.02**	2.26	0.10
Site x Month	6	0.49	0.81	0.48	0.81
Error	14				

Chlorophyll *a*

Source	*df*	Surface		Bottom	
		F	p	*F*	p
Site	1	1.06	0.32	32.66	**<0.01**
Month	6	2.01	0.13	19.35	**<0.01**
Site x Month	6	0.86	0.55	11.50	**<0.01**
Error	14				

Table 3-2 Statistical analyses of reproductive condition of scallops from site 1 and site 2. Bold numbers indicate significant differences, at $p < 0.05$.

Reproductive condition

Source	*df*	VGI	
		F	p
Site	1	0.01	0.94
Month	6	64.67	**<0.01**
Site x Month	6	1.49	0.18
Error	208		

Histology

Source	*df*	Gonad developmental stage		Oocytes diameter	
		F	p	*F*	p
Site	1	1.88	0.17	0.01	0.94
Month	6	11.95	**<0.01**	16.87	**<0.01**
Site x Month	6	1.48	0.21	0.96	0.46
Error	56				

Table 3-3 Statistical analyses of condition index for scallop tissues from site 1 and site 2. Bold numbers indicate significant differences, at $p < 0.05$.

Condition Index

Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p
Site	1	2.67	0.10	0.17	0.68	1.34	0.25
Month	6	51.26	**<0.01**	19.06	**<0.01**	4.87	**<0.01**
Site x Month	6	1.20	0.31	1.53	0.17	3.31	**<0.01**
Error	208						

Table 3-4 Statistical analyses of biochemical composition for scallip tissues from site 1 and site 2. Bold numbers indicate significant differences, at $p < 0.05$.

Carbohydrates

Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p
Site	1	1.35	0.25	0.29	0.59	0.01	0.94
Month	6	29.35	**<0.01**	18.73	**<0.01**	19.59	**<0.01**
Site x Month	6	5.80	**<0.01**	1.56	0.16	2.77	**0.01**
Error	208						

Protein

Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	P	*F*	P	*F*	P
Site	1	13.68	**<0.01**	5.90	**0.02**	3.64	0.06
Month	6	14.48	**<0.01**	15.58	**<0.01**	28.79	**<0.01**
Site x Month	6	9.46	**<0.01**	4.70	**<0.01**	2.33	**0.03**
Error	208						

Lipids

Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	P	*F*	P	*F*	P
Site	1	21.50	**<0.01**	41.52	**<0.01**	1.18	0.28
Month	6	179.54	**<0.01**	44.19	**<0.01**	8.69	**<0.01**
Site x Month	6	27.68	**<0.01**	5.14	**<0.01**	6.34	**<0.01**
Error	208						

Table 3-5 Statistical analyses of energy content per scallop tissues from site 1 and site 2. Bold numbers indicate significant differences, at $p < 0.05$.

Energy							
Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p
Site	1	2.97	0.09	12.14	**<0.01**	0.36	0.55
Month	6	42.50	**<0.01**	5.98	**<0.01**	18.35	**<0.01**
Site x Month	6	0.881	0.51	4.315	**<0.01**	2.77	**0.01**
Error	208						

Table 3-6 Average similarities (SIMPER) by fatty acid proportions (%) of >5µm seston and *P. novaezelandiae* tissues (female gonad, male gonad, adductor muscle, digestive gland) for sites 1 and 2, from May 2012 – March 2013. Similarity, the cut-off is shown at 80%.

Site 1		Site 2	
Seston			
Average similarity: 84.59		Average similarity: 81.39	
Fatty Acids	Cum.%	Fatty Acids	Cum.%
C16:0	41.61	C16:0	44.98
C16:1	68.01	C16:1	73.65
C20:2	77.24	C20:2	84.03
C20:4n6	86.21		
Female gonad			
Average similarity: 85.28		Average similarity: 92.07	
Fatty Acids	Cum.%	Fatty Acids	Cum.%
C16:0	30.26	C20:5n3	25.3
C20:5n3	56.69	C16:0	50.25
C22:6n3	69.79	C22:6n3	68.45
C16:1	78.39	C16:1	76.86
C18:0	83.39	C18:0	84.28
Male gonad			
Average similarity: 88.31		Average similarity: 92.78	
Fatty Acids	Cum.%	Species	Cum.%
C16:0	25.52	C20:5n3	24.96
C20:5n3	50.99	C22:6n3	49.19
C22:6n3	72.13	C16:0	71.48
C18:0	81.57	C18:0	81.78
Adductor muscle			
Average similarity: 92.44		Average similarity: 94.84	
Species	Cum.%	Species	Cum.%
C22:6n3	38.46	C22:6n3	40.22
C16:0	57.71	C16:0	59.8
C20:5n3	76.67	C20:5n3	78.11
C18:0	87.2	C18:0	88.26
Digestive gland			
Average similarity: 83.05		Average similarity: 81.81	
Species	Cum.%	Species	Cum.%
C16:0	30.18	C16:0	30.52
C18:0	45.48	C18:0	45.98
C20:5n3	60.09	C22:6n3	60.77
C22:6n3	73.5	C20:5n3	74.61
C16:1	80.51	C16:1	80.54

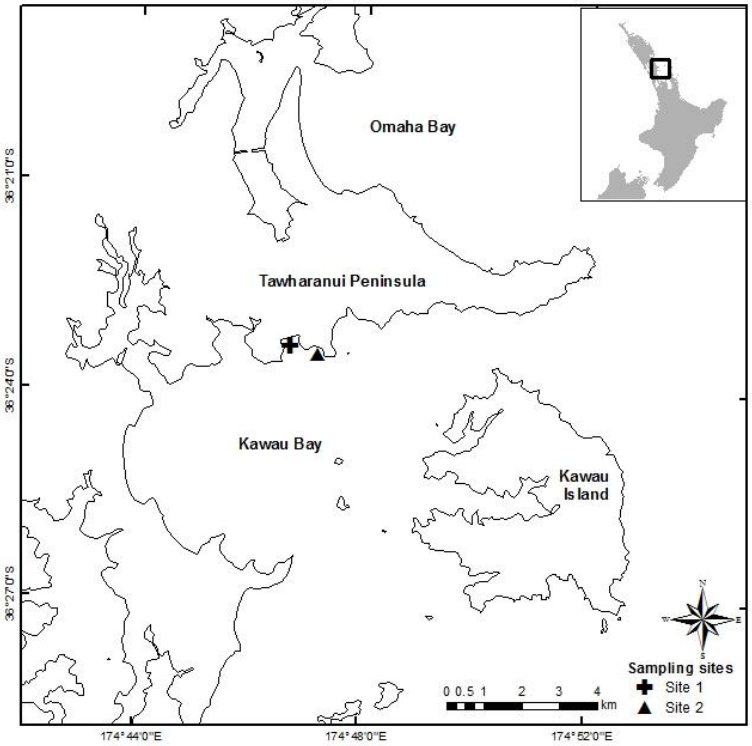

Figure 3-1 Study sites, southern Tawharanui Peninsula, Hauraki Gulf, New Zealand. Site 1 is denoted with a cross (+) and site 2 is denoted with a triangle (Δ).

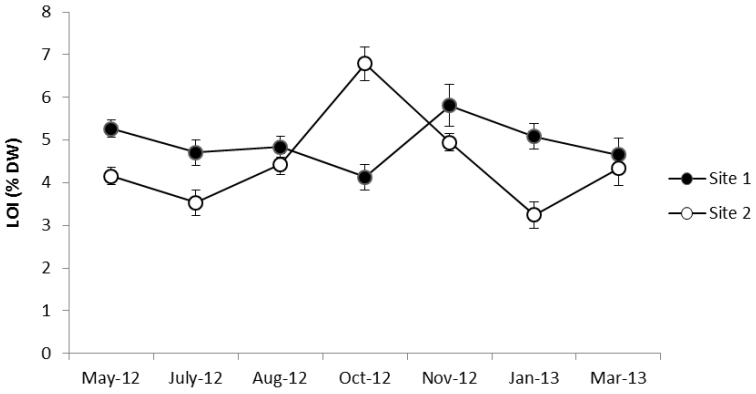

Figure 3-2 Particular organic matter (POM) composition in sediment samples from Site 1 and site 2, May 2012 – March 2013. Mean values ± standard error.

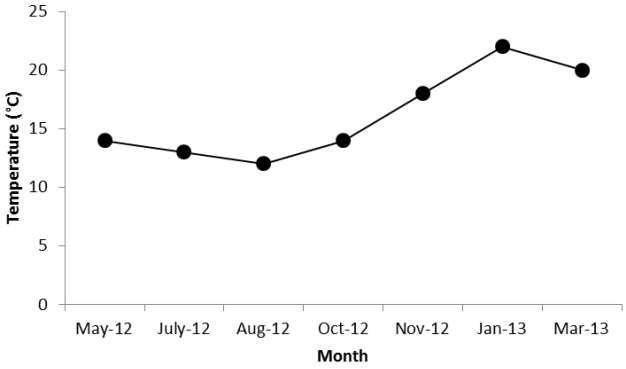

Figure 3-3 Bottom water temperature (°C) in the sampling area (site 1 and site 2), May 2012 – March 2013.

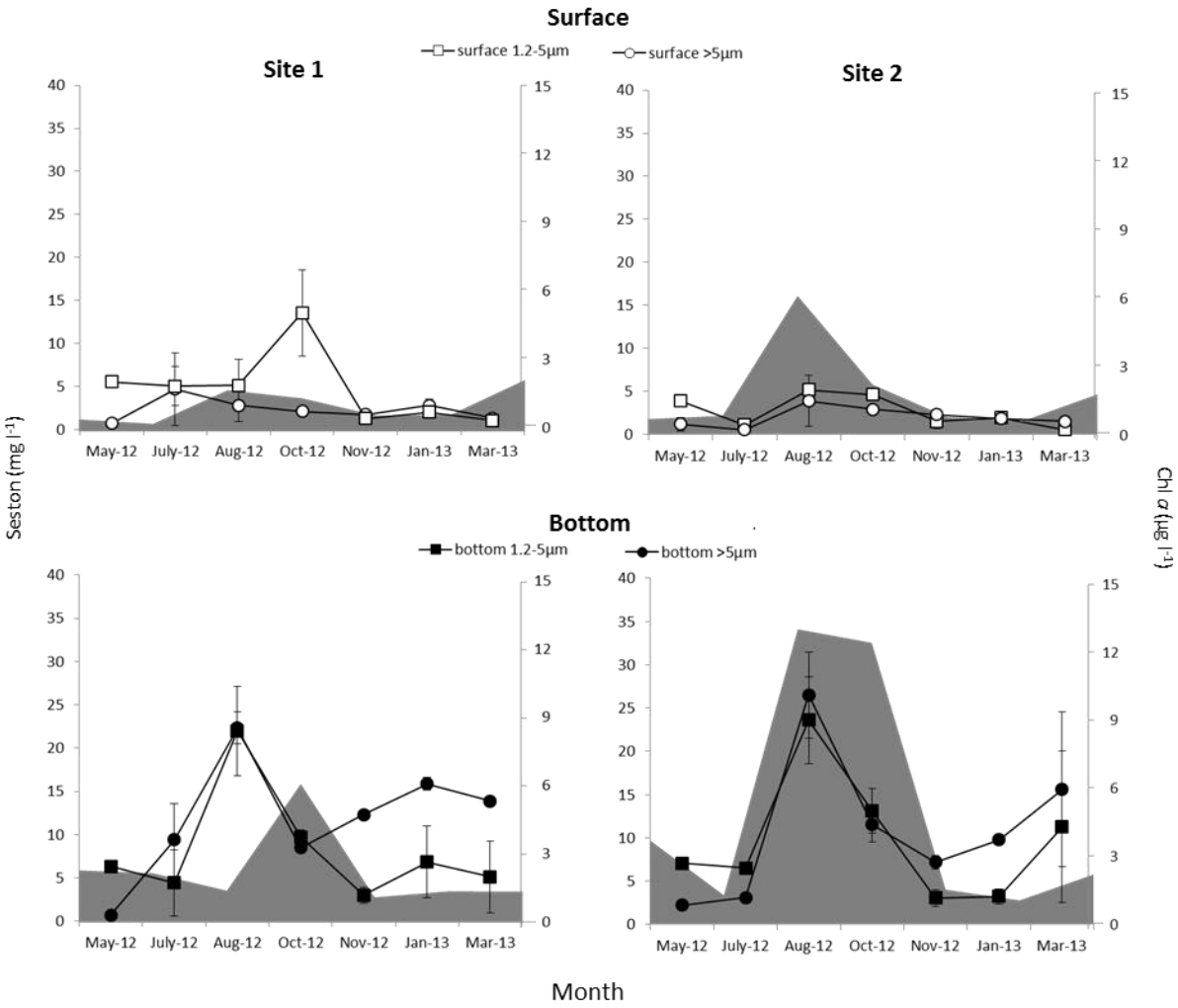

Figure 3-4 Surface and bottom seston abundances (mean values ± standard error) of 2 size classes (1.2−5 and >5µm), and chlorophyll a concentration (grey areas). Site 1 (left) and site 2 (right), May 2012 – March 2013.

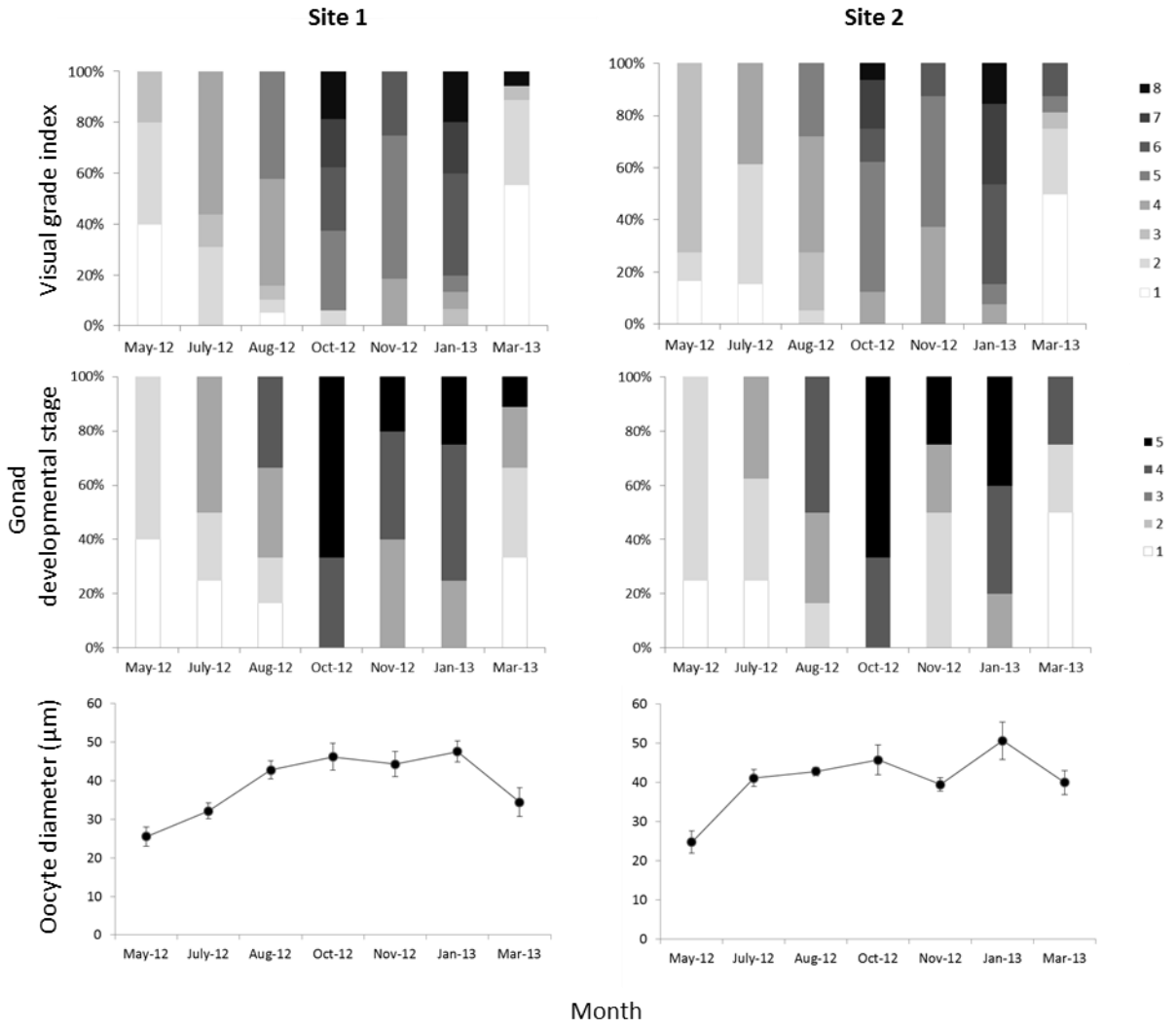

Figure 3-5 Frequency of visual gonad index (VGI) classes (1−8) (top graphs), frequency of gonad developmental stages (1−5) (middle graph), and mean oocyte diameter (± SE) of scallops (bottom graphs), for sites 1 and 2, from May 2012 – March 2013.

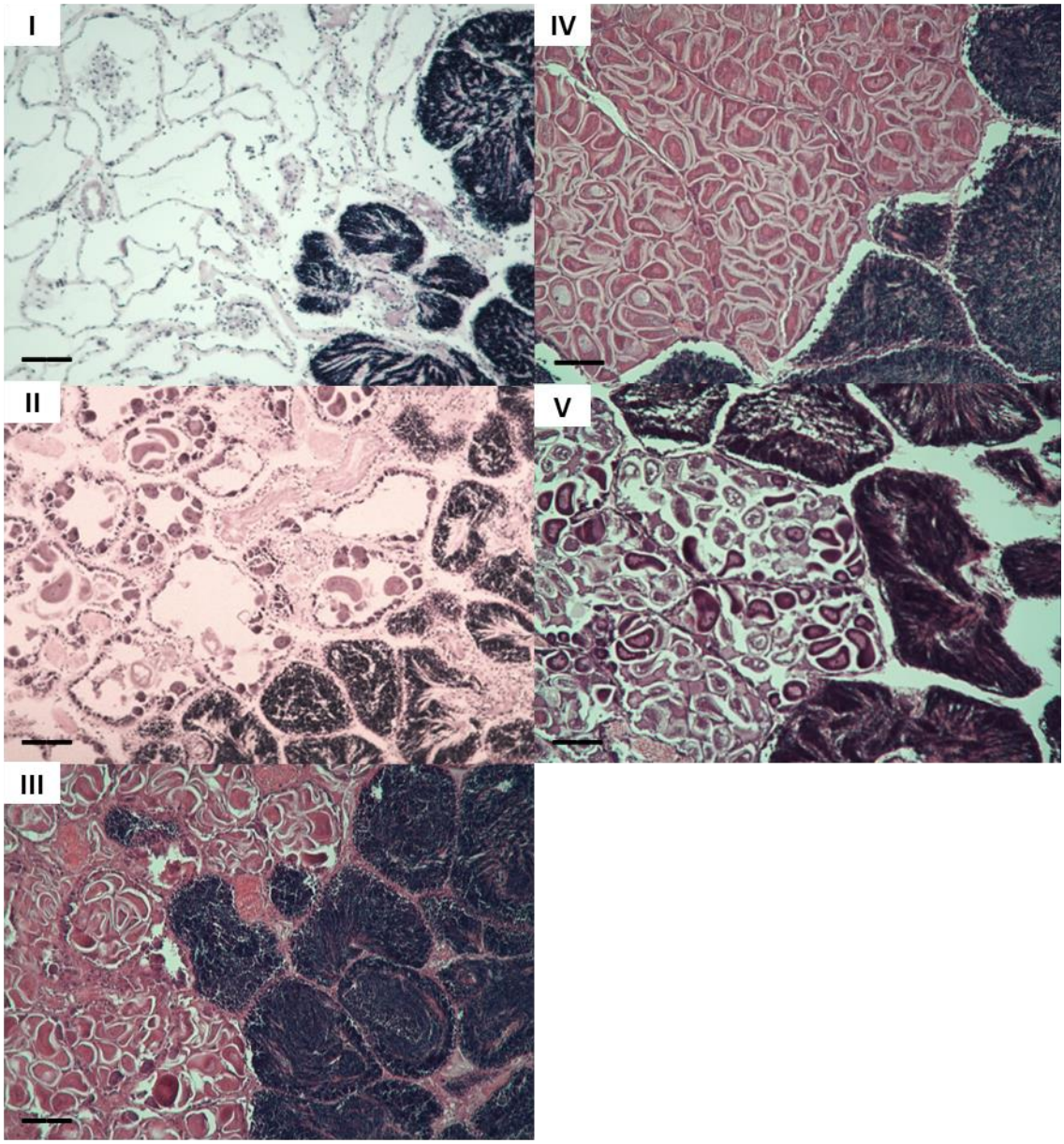

Figure 3-6 Photomicrographs of gonad thin sections. Stage I: spent, stage II: early development, stage III: active development, stage IV: ripe, stage V: partly spent; based on Bull (1976) classification. Scale bar: 100µm. Gonad appearance, visual grade index (VGI), gonad index (GI), gonad wet weight, scallop shell length, collection site and date are detailed in Appendix III.

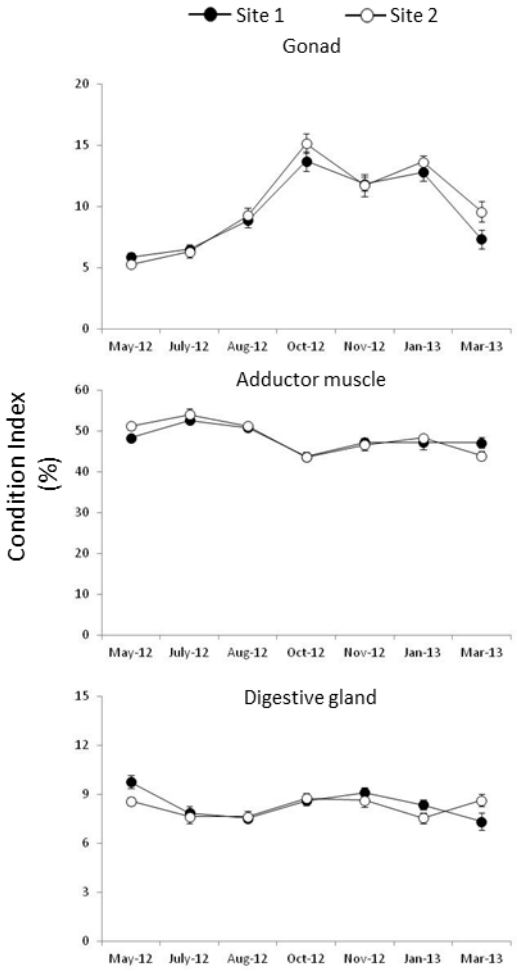

Figure 3-7 Condition indices for gonad, muscle and digestive glands, of scallops from site 1 and site 2 from May 2012 —March 2013. Mean values ± standard error.

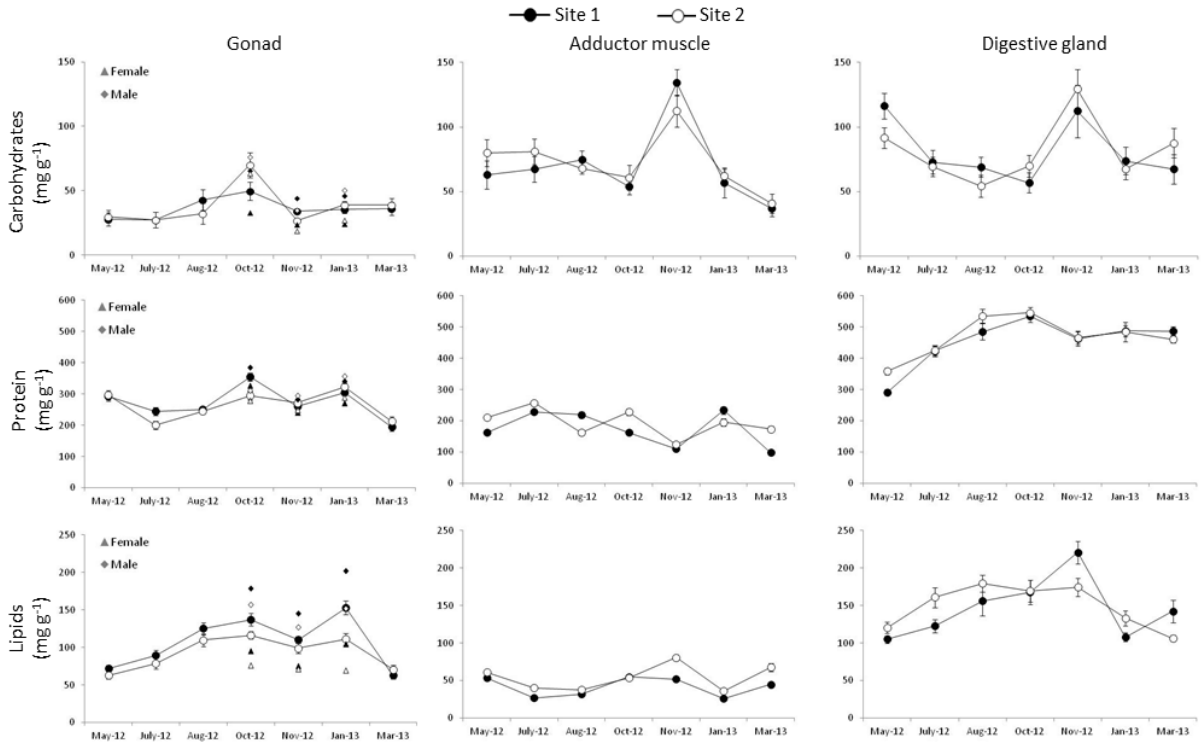

Figure 3-8 Biochemical composition (proteins, lipids and carbohydrates) of gonad, adductor muscles and digestive glands of scallops from site 1 and site 2 from May 2012 —March 2013. Separate values for female and male gonad for October, November 2012 and January 2012. Mean values ± standard error.

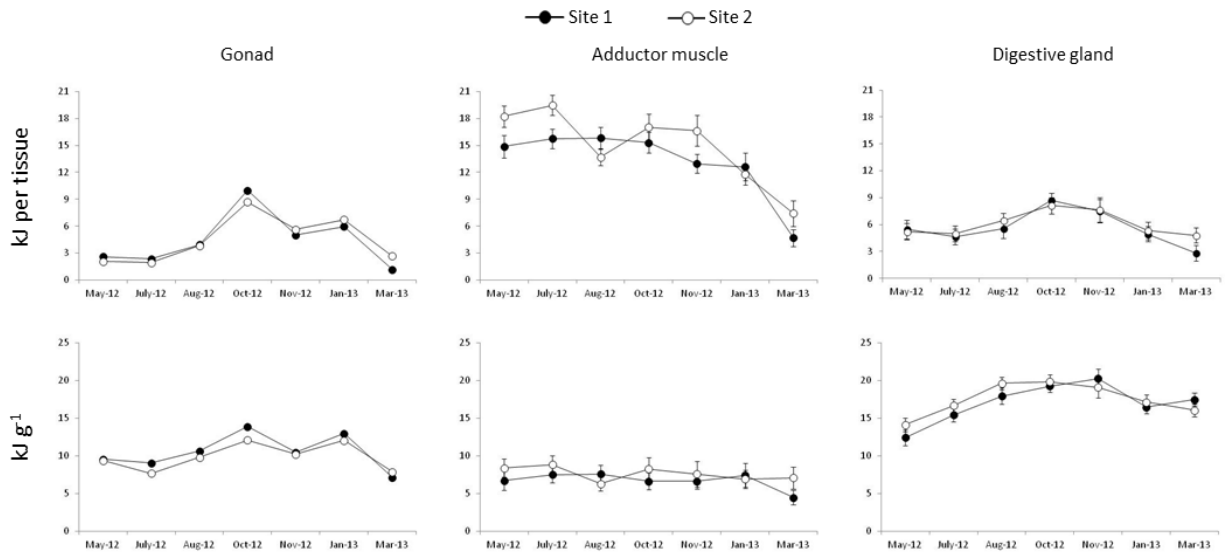

Figure 3-9 Mean energy content (±SE) within gonad, adductor muscle and digestive gland expressed in kJ per tissue (top graph) and in kJ g^{-1} (bottom graph) for site 1 and site 2, between May 2012 – March 2013.

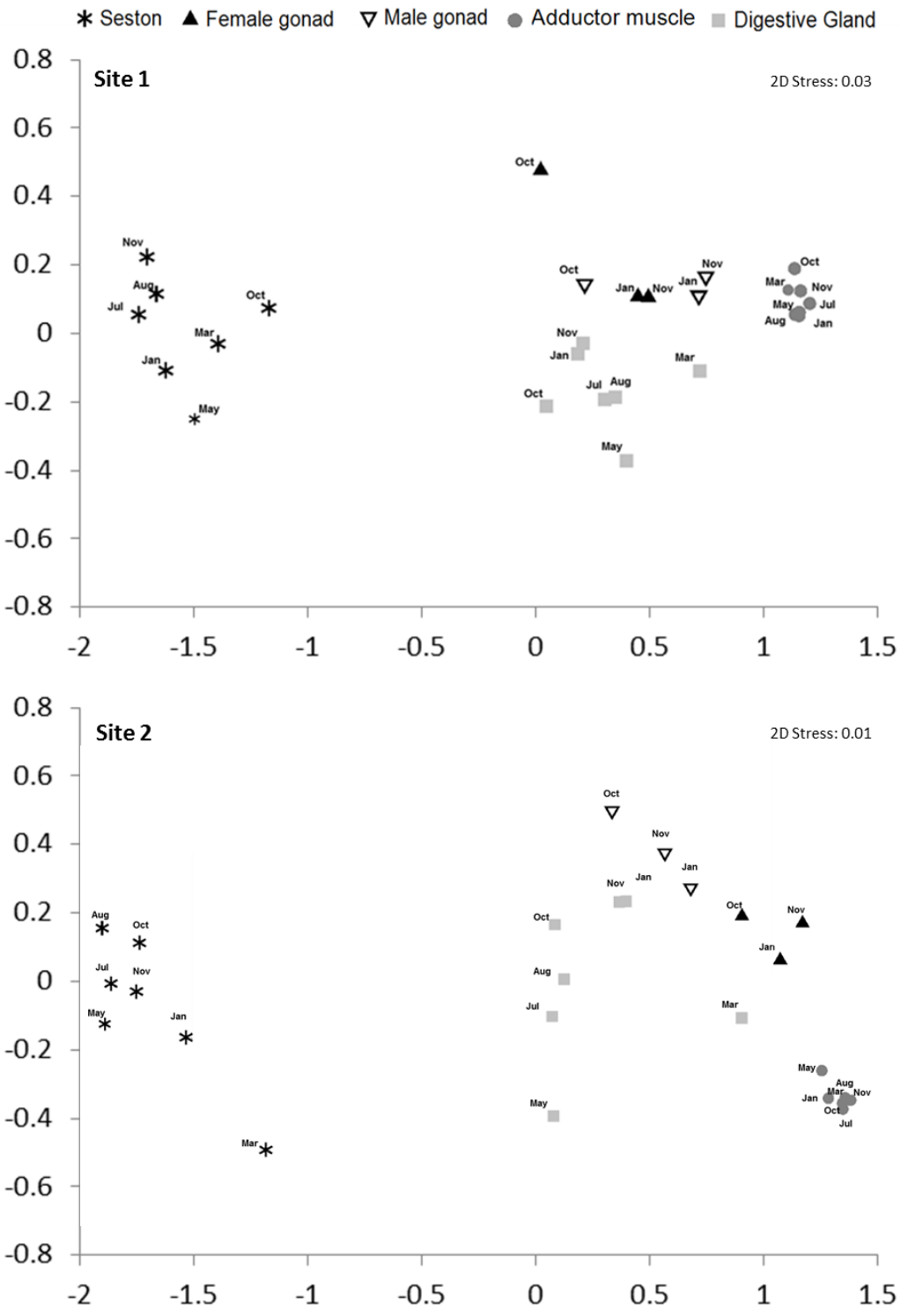

Figure 3-10 MDS plots of fatty acid proportions (%) of >5µm seston and *P. novaezelandiae* tissues (female gonad, male gonad, adductor muscle, digestive gland) for sites 1 and 2, from May 2012 – March 2013. The resemblance metric was created using Bray-Curtis similarity coefficients.

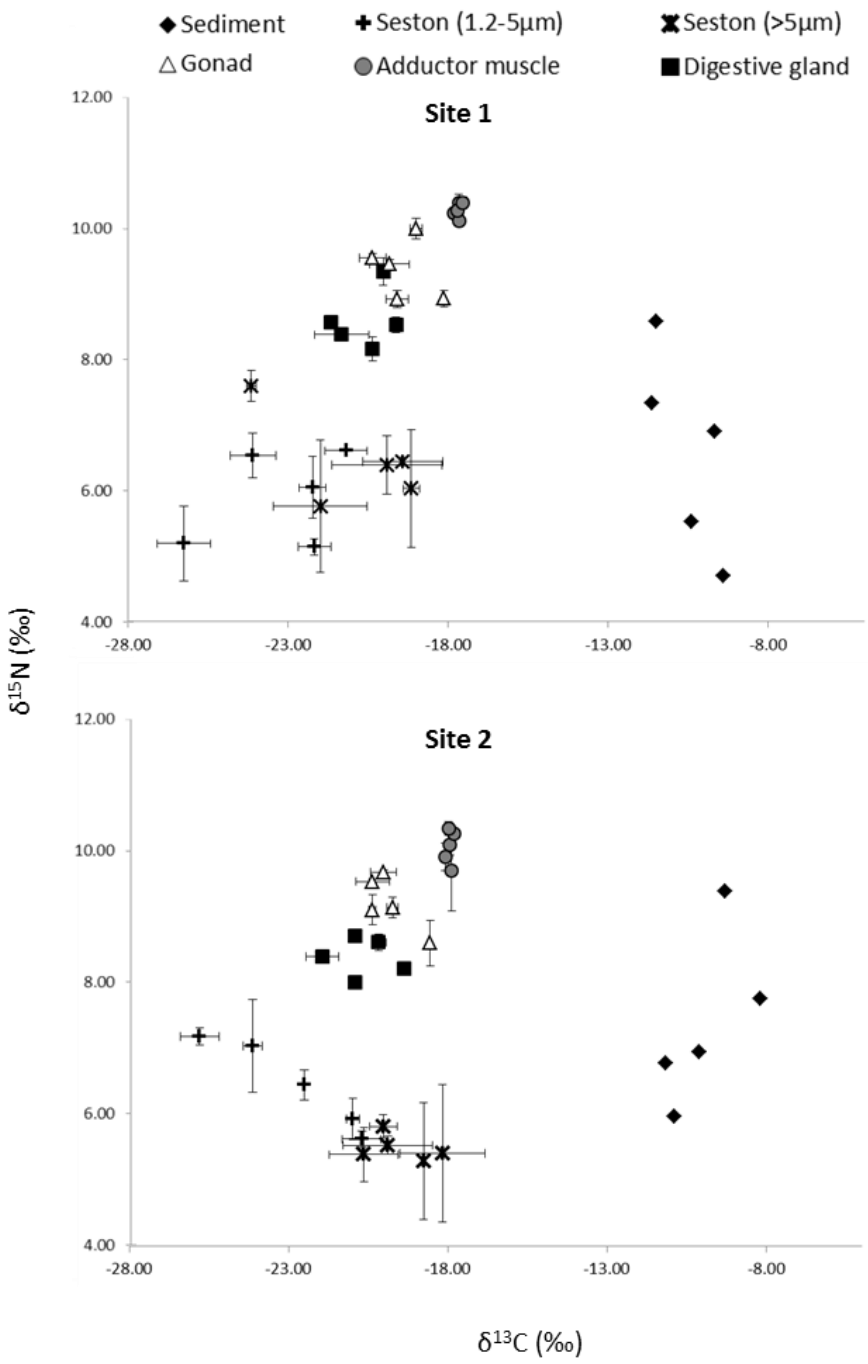

Figure 3-11 Stable isotope ($\delta^{15}N$ *vs*. $\delta^{13}C$) signatures of seston (1.2−5µm and >5 µm size classes), sediment, and scallop tissues (gonad, adductor muscle and digestive gland) from *P. noveazelandiae*. Values for seston and scallop tissues are means (±SE) of 2 replicates, while only one replicate was analyzed for sediment samples.

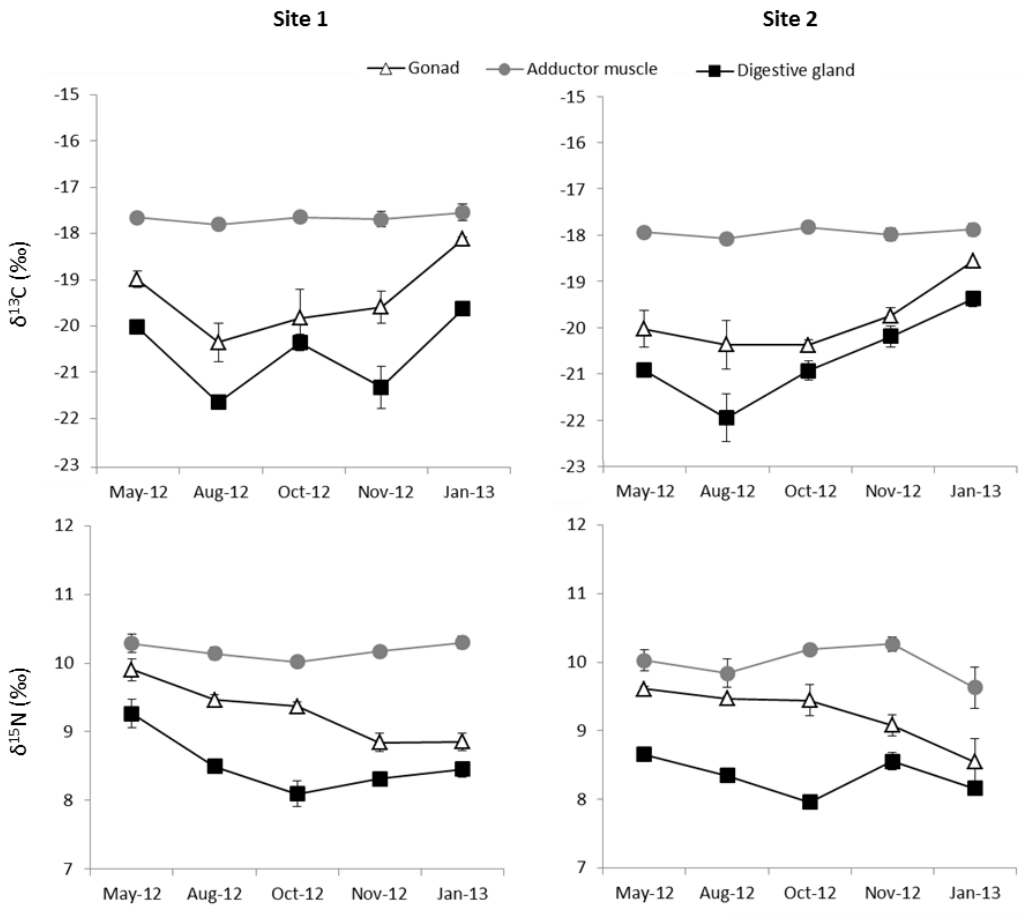

Figure 3-12 Mean (±SD) temporal isotopic variation in gonad, adductor muscle and digestive gland of *P. novaezelandiae*.

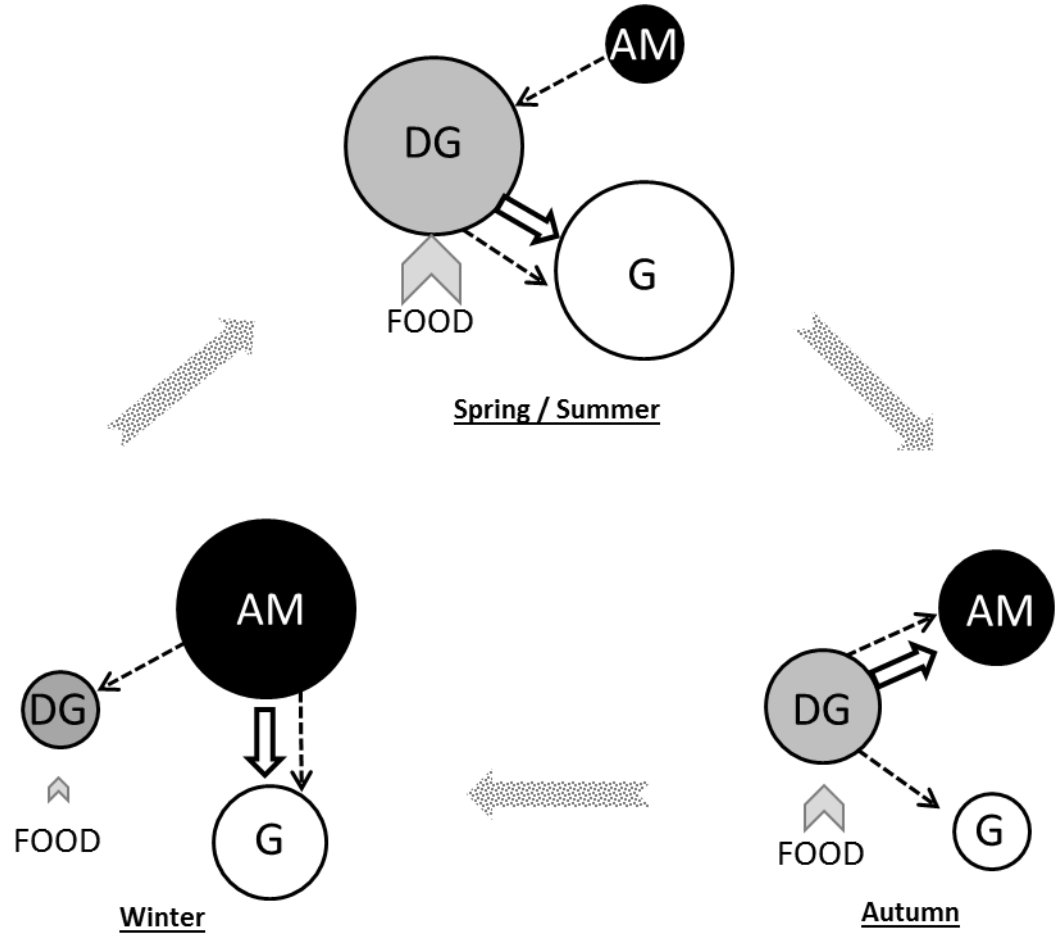

Figure 3-13 Simplified scheme of energy distribution in *P. novaezelandiae*. Circle size corresponds to relative energy level in gonad (G), adductor muscle (AM) and digestive gland (DG). Primary (⇨) and secondary/maintenance (-->) energy transfer among gonad, adductor muscle and digestive gland.

Chapter 4 : Reproductive condition and nutrient composition of scallops (*Pecten novaezelandiae*) within field and laboratory settings.

Introduction

One of the principal aspects of shellfish aquaculture production is the conditioning of broodstock to supply high quality seed for mass culture of the targeted species. Whether the broodstock are collected from the wild or grown within aquaculture conditions, a comprehensive understanding of the reproductive biology is necessary to meet optimal requirements for conditioning. For some species, such as the Pacific oyster (*Crassostrea gigas*) and the hard clam (*Mercenaria mercenaria*), this information is relatively well established, and reliable cultivation practices can be achieved for global markets (Muranaka & Lannan 1984, Gallager & Mann 1986, Dillon & Manzi 1987, Utting & Millican 1997, Soudant *et al*. 1999). For newer species, a detailed understanding of broodstock conditioning and larval rearing will need to be established in order to produce a sustainable aquaculture venture. Scallop aquaculture is a relatively late entrant on the shellfish culturing scene. Some studies on reproductive conditioning have been carried out on scallops of commercial interest, such as *Argopecten purpuratus* (Martinez *et al.* 2000a), *A. ventricosus* (Guerra *et al.* 2012), *Lyropecten* (*Nodipecten*) *nodosus* (Lodeiros *et al*. 2001), *Pecten fumatus*

(Heasman *et al*. 1996), *Pecten maximus* (Devauchelle & Mingant 1991, Utting & Millican 1998) and *Placopecten magellanicus* (Wildish *et al*. 1987, Wang & Croll 2004). However, techniques for broodstock conditioning are still far from ideal (Millican 1997, Utting & Millican 1997 and Robert & Gérard 1999). For example, wild post-spawned adult scallops have been shown to be consistently better at achieving re-maturation compared to hatchery-reared scallops, such as *Argopecten purpuratus* (Martinez *et al.* 1992) and *Argopecten ventricosus* (Guerra *et al*. 2012). These findings have been associated with the more stressful conditions encountered within culturing environments, which often fail to replicate the natural environment that may support optimal growth. Even when growth conditions are satisfactory, there are a range of behavioural responses that may be attributed to sub-optimal conditions and/or stress factors that are difficult to identify. Indeed, Lafrance *et al*. (2003) reported that wild scallops (*Placopecten magellanicus*) often have stronger shells and perform a more intense escape response compared with cultured individuals, which results in vulnerable juveniles when hatchery spat is released to the wild.

Conditioning of scallops is based on knowledge of the natural reproductive cycle, which is often affected by exogenous factors (i.e., temperature, salinity, light, food) and species-specific responses to these variables (Utting & Millican 1998, Louro *et al*. 2003 and Barber & Blake 2006). For example, high temperatures (20°C) tend to increase metabolic demands, leading to decrease in gonad nutrient, as has been reported for *A. purpuratus* (Martinez *et al.* 2000a), while higher temperatures (20−25°C) may induce rapid gonad maturation, as seen in *A. irradains* (Sastry 1968). In addition, mixed microalgal diets with lipid supplements have been shown to encourage

gonad maturation rates in *A. purpuratus* (Navarro *et al*. 2000). On the other hand, a mono-algal diet of *Isochrysis galbana* has been shown to promote growth in *A. nucleus* and *N. nodosus* (Velasco & Barros 2007). Such species-to-species variation highlights the need to identify the physiological responses to various exogenous parameters, in order to optimize not only growth and maturity of cultured scallops, but to minimize stress, provide an optimally efficient diet, and improve condition timing for the cultured animals. One of the most common ways to understand physiological responses of scallops is through the investigation of biochemical processes. In some scallop species, gonads reach maturation by using nutrients stored previously in various tissues (e.g., digestive gland, adductor muscle). In *A. purpuratus*, nutrients have been shown to first accumulate in the adductor muscle during broodstock conditioning as carbohydrate, and later are transferred to gonad for gametogenesis (Martinez *et al*. 2000a). Such studies highlight the complex dynamics of nutrient acquisition and storage for reproductive purposes and the need to understand how nutrients are utilized throughout the reproductive cycle to provide the optimal diet during conditioning, and a baseline for gonad maturation timing.

The New Zealand scallop, *Pecten novaezelandiae*, is the focus of a well-established fishery that has experienced dramatic declines in wild stocks in recent years (Williams 2005). Indeed, scallop biomass was recorded to have been lowest between 1999 and 2000, since the fisheries began in the early 1960s. These wild stock declines and the realization that harvest volumes to meet consumer demands are not sustainable has prompted interest in developing a scallop aquaculture industry in New Zealand. Thus, it is envisaged that future cultivation of scallops will not only supply

local and export markets, but could also produce seed to enhance and/or re-establish natural scallop populations. A considerable advantage of this new aquaculture species for New Zealand is that scallops have a well-established global mark. However, the successful implementation and growth of this industry will necessitate a comprehensive understanding of the general biology and ecology (e.g., broodstock conditioning, larval development, nutrition, growth and health) of this species to optimize its cultivation potential.

P. novaezelandiae is a functional hermaphrodite that exhibits a temporal gamogenetic cycle. The reproductive cycle of *P. novaezelandiae* has been documented by several authors (Nicholson 1978, Morrison 1999 and Nesbit 1999). Nicholson (1978) and Morrison (1999) measured gonad indices and established a macroscopic visual classification. These studies concluded that *P. novaezelandiae* is limited to one major spawning event during late spring/early summer in the Hauraki Gulf, North Island, while further secondary spawnings are dependent on environmental suitability. Nesbit (1999) identified the optimal temperature (15−18°C) for broodstock conditioning of *P. novaezelandiae* based on gonad visual index assessment. Williams (2005) assessed scallop size at maturity and developed a gonad visual index that could be used reliably to identify reproductive maturity. However, these studies were only focused to identify gonad condition through indices and histological sections, but did not provide detailed information regarding the biochemical process by which nutrients are stored and utilized within different tissues throughout the reproductive cycle. With the biochemical changes in relate to scallop gametogenic cycle being investigated in Chapter 3, the aim of this study is to understand the acute responses of *P.*

novaezelandiae around final gonad conditioning and spawning, by parallel comparing the biochemical condition of wild and laboratory reared scallops during its spawning season.

Methods

Wild scallops

Mature adult scallops (> 80 mm) from Prospect Bay, Southern Tawharanui Peninsula (36°23'13''S, 174°46'44''E) (Fig. 4-1) were collected periodically during the spawning season (October) in 2013 for analysis of their nutrient composition and reproductive state. At approximately weekly intervals, 12−20 scallops (82−104mm shell height) were collected by SCUBA divers from Prospect Bay between 27th September and 29th October 2013.These scallops were cleaned thoroughly, and theirs shell heights and widths were measured to the nearest 0.01 mm with Vernier callipers. Then, each animal was opened and its gonad maturity was graded with a visual gonad index (VGI) (see below). All animals were dissected to remove gonad, muscle and digestive gland tissues for further analyses on reproductive condition and nutrient content (see below).

Feeding experiments

On 27th September 2013, 47 scallops (84−103mm shell height) were collected from Prospect Bay, Southern Tawharanui Peninsula, by SCUBA divers and transported back to the Auckland University of Technology (AUT) Aquaculture Laboratory. Shell heights and widths of individual scallops were recorded to the nearest 0.01 mm (with Vernier callipers), and cleaned thoroughly before transferring to seawater containers. Scallops were kept in 4L black containers individually supplied with air and 1µm filtered seawater (salinity ~35 ppt) from a re-circulating water system at the aquaculture facility (Appendix V). The containers were cleaned with fresh seawater twice a week to remove any biofilms growing on the walls of the containers. All scallops were starved for 48 hours before feeding with a commercial shellfish diet (Shellfish Diet 1800®, containing 3 flagellates: *Isochrysis*, 40%; *Pavlova*, 15%; *Tetraselmis*, 25%; and 1 diatom: *Thalassiosira weissflogii*, 20%) at the ratio of 4% scallop soft tissue dry weight per day. This ratio was chosen based on manufacturer recommendation and actual feeding trail to *P. novaezelandiae* in the Aquaculture Laboratory. The re-circulating system was switched off during feeding periods that lasted 2 hours each, at the same time every day. Scallops were maintained under a 12:12 hour light/dark cycle throughout the experimental period. Water temperature in the facility started at 15°C and increased gradually to 18°C at the end of the experiment, mimicking field water temperature conditions.

About 12−20 of these experimental animals were randomly selected every 1−2 weeks (on the same days that the wild scallops were collected) and sacrificed to assess the reproductive and nutrient state (see below). Gonad maturation, tissue biochemical composition (carbohydrates, proteins and lipids) and tissue fatty acid profiles were assessed on individuals from the laboratory and compared to those from the wild animals (Fig. 4-2).

Water samples

To quantify seston concentrations in the water column at the field site, water samples were collected at Prospect Bay at the same time that scallop collections took place. Two replicates of 20 litres were collected from the surface and bottom during each sampling event. The water samples were screened through a 250 µm mesh to remove large particles. Then, the water samples were filtered through 5 µm polypropylene membranes to collect the large seston fraction, followed by a 1.2 µm (GF/C) pre-combusted glass fibre filters to obtain the small seston fraction. The filters containing seston were washed twice with 5ml of 0.9% ammonium formate and freeze dried for further analysis. In addition, chlorophyll *a* concentrations were determined using 1–3 litre aliquots from each replicate of bottom water sample. These sub-samples of water were filtered through 1.2 µm (GF/C) filter discs. Then, chlorophyll *a* was extracted from the filters using 90% methanol for 24 hr at 4°C. The absorbance of each sample was measured at 652, 665 and 750nm on a spectrophotometer (Porra 2002). Chlorophyll *a* concentrations were calculated according to equation:

Chl *a* (µg ml^{-1}) = 16.29 $A^{665-750}$ – 8.54 $A^{652-750}$

where, A is the absorbance.

Scallop condition

Wild and experimental scallops were analyzed to determine the reproductive and nutrition state of each individual. Gonad, adductor muscle and digestive gland tissues were removed from each scallop, weighed separately, and freeze-dried for further analyses. Condition indices of gonad (GI), adductor muscle (AMI) and digestive gland (DGI) samples were determined according to Barber & Blake (1981):

Condition index= (tissue DW/total tissue DW) *100

where, DW is the dried weight.

Reproductive condition

A visual gonad index (VGI) was recorded for all animals just prior to dissection. Visual grades were assigned to each gonad based on morphological appearance of the gonad to the naked eye, each gonad being classified between grade 1 (spent) and grade 8 (ripe) (referring to Williams & Bobcock, 2004). Histological sections of the centre of each gonad at the interface of the testis and ovary were made. The tissues were first dehydrated using a series (70% to 100% concentration progressively) of A

graded ethanol, then xylene was used to clear the samples. Then, samples were embedded in paraffin wax. Blocks of embedded gonad samples were sectioned to 5 µm thickness, mounted on slides, and stained with haematoxylin and eosin. The diameter of 30 randomly selected nucleated oocytes was measured per scallop to provide quantitative data on the reproductive condition of each individual.

Nutrient content

Biochemical composition:

Biochemical compositions of three scallop tissues (gonad, adductor muscle and digestive gland) were determined for all wild and experimental animals (Fig. 4-2). For gonad samples, female and male gonads were analyzed separately. The biochemical analyses included total carbohydrates, total protein, and total lipids. Total carbohydrates were determined using the Anthrone method according to Hedge & Hofreiter (1962). Carbohydrate contents in the samples were hydrolyzed into simple sugars with dilute hydrochloric acid (2.5 N) for 3 hours in a boiling bath. Carbohydrates were hydrolyzed into simple sugars using dilute hydrochloric acid (2.5N). A serial of glucose standard solution was prepared using glucose powder (Sigma Aldrich) in distilled water. Four parts of anthrone solution were then added to the supernatant and standard solutions, then incubated for another 8 min at 100°C. The optical density of the green colored solution was read at 630nm, and reported as glucose equivalents. Protein was determined using a commercial kit (The Pierce BCA Assay by Thermo Scientific™). Samples were first digested using 0.5 N NaOH (56°C, 30 min). Protein

standard solution was prepared using the Albumin Standard (BCA kit) in 0.5N NaOH. The supernatants and standard solutions were incubated with BCA solution (37°C, 2 hr). The optical density of the solution was read at 562nm, and reported as bovine serum albumin (BSA) equivalents. The lipids within each sample were extracted according to Bligh & Dyer (1959), using a 2:1 chloroform-methanol mixture. Tripalmitin (Sigma Aldrich) in chloroform was used to prepare a serial of lipid standard solution. Extracted lipids were dried under a flow of nitrogen and the amount of total lipid was determined by charring with concentrated sulphuric acid (200°C, 15 min), according to Marsh & Weinstein (1966). The optical density of the solution was read at 375nm, and reported as tripalmitin equivalents. All biochemical components reported in this chapter was standardised to dry weight.

Energy content:

The total energy content of each scallop tissue (gonad, adductor muscle and digestive gland) was estimated from its carbohydrate, protein and lipid composition, assuming conversion factors of 17.2, 23.9 and 33 kJ g^{-1}, respectively (Saout *et al*. 1999).

Fatty acid profiles:

Fatty acid profiles were obtained for scallop tissues (female gonad, male gonad, adductor muscle and digestive gland), seston and the commercial shellfish diet used to feed the experimental scallops. Fatty acids were prepared by a gas chromatographic

"one-step methylation extraction method", according to Garcia *et al.* (2000). Samples were incubated with toluene and freshly prepared methanolic HCl for 2 hours. Then, K_2CO_3 and toluene were added to the mixture, and phase separation was accomplished by centrifugation. The organic phase on the top layer, which included fatty acid methyl esters (FAMEs), was dried with anhydrous Na_2SO_4.

The separation and quantification of FAMEs were conducted using a 2010 Shimadzu GC-2010, with a split-splitless injector and flame ionization detector. The temperature of the detector was 250°C and the volume of injection was 1 µl. Individual FAMEs were separated by a Zebron ZB-Wax capillary column (30 m x 0.25 mm x 0.25 µm) from Phemomenex. Each separation cycle took 50 min, starting at 140°C, increasing by 5°C min^{-1} until reaching 245°C and held for 15 min. The gas carrier was nitrogen with a flow-rate of 60 ml min^{-1} and the flow of hydrogen was 40 ml min^{-1}. The average linear velocity was set to 20 cm sec^{-1}, with a head pressure of 8.7 psi (1 psi = 6894.76 Pa).

Statistical analyses

All reported average values in this study are mean ± standard error (±SE). Two-way analyses of variance (ANOVA) were used to identify significant differences between growing environments and sampling time (sampling event). These analyses were performed using a SPSS statistics package. Multidimensional scaling (MDS) was used to identify differences in fatty acid composition among scallop tissues (female

gonad, male gonad, adductor muscle and digestive gland) between environments (field and laboratory). Similarity of percentage (SIMPER) was used to identify the difference in fatty acid composition of seston from the field water samples and the commercial diet, and wild and cultured scallop tissues, represented in the average dissimilarity that primarily provided the discrimination between wild and experimental conditions. Plymouth Routines in Multivariate Ecological Research (PRIMER) was used to perform the MDS and SIMPER on log transformed percentage fatty acid data with a nonparametric Bray-Curtis similarity matrix.

Results

Seston and chlorophyll *a*

Analysis of water samples resulted in consistently low seston concentrations (< 2 mg l^{-1}) and chlorophyll *a* concentrations (<0.4 µg l^{-1}) in surface water, while seston concentrations varied greatly from 1.9−31.0 mg l^{-1} to 2.1−46.2 mg l^{-1} for small (1.2−5 µm) and large (> 5 µm) size classes for bottom water samples (Fig. 4-3). The temporal changes of seston concentrations at the bottom revealed a major peak on 27th September 2013. Bottom seston concentrations did not appear to be directly related to chlorophyll *a* levels, and the highest chlorophyll *a* concentration was recorded on 23rd October 2013 (Fig. 4-3).

Reproductive condition

Visual gonad index (VGI):

Visual gonad index (VGI) results revealed a non-significant difference in gonad maturation between wild and experimental scallops. Wild scallops showed a notable variation in VGI, while VGI scores across sampling periods were more evenly distributed in experimental scallops, except for a relatively higher number of 'ripe' gonads recorded on 29th October (Fig. 4-4). 'Immature/spent' gonads (grade 1) were recorded most frequently on 27th September and 13th October for wild scallops, while grade 1 scallops were encountered throughout the conditioning period for experimental scallops. 'Partially spawned' gonads (grade 2) were encountered only in the first (27th September) and last (29th October) sampling events for wild scallops, while the occurrence of gonads with grade 2 VGI was similar across the experimental period in culturing conditions, except for a relatively low occurrence on 29th October. 'Active' gonads (grade 3-5) contributed more than 60% of wild scallops on 23rd October, while the contribution was highest on 29th October for experimental scallops. 'Ripe' gonads (grade 6-8) contributed 17-40% for wild scallops and 18-42% for experimental scallops throughout the experiment, with the highest values recorded on 23rd October and 29th October, respectively.

Histology:

Analyses of histological sections of gonads from wild and experimental scallops are presented in Figure 4-5. The oocyte diameters measured from photomicrographs of gonadal sections were statistically different between wild and experimental scallops, also with a significant interaction. Mean (±SE) were 44±1.5µm for wild scallops and 42±3µm for experimental scallops. The diameter ranges in wild and experimental scallops were from 33−59µm and 28−61µm, respectively (Fig. 4-6).

Condition indices:

Gonad indices (GI) were not statistically different between wild and experimental scallops (Fig. 4-7; Table 4-1). GI values ranged from 8 to 37% and 7 to 26% for wild and experimental scallops, respectively. There was a general increase in mean (± SE) GI from 12 ±1.2% to 18±1.6% from 27th September to 23rd October, followed by a sharp decrease to 13±0.5% in October, in wild scallops. For experimental scallop, there was an increase in mean (± SE) GI from 12 ±1.2% at the start of the experiment to 16±0.9% on 29th October.

Biochemical composition:

The statistical analyses of gonad biochemical analyses are presented in Table 4-2. The carbohydrate contents in female gonads were significantly difference over time, while male gonads had a significant interaction. The variation in carbohydrate contents within wild gonad samples was low, ranging between 63–158 mg g^{-1} (female) and

36–107 mg g^{-1} (male), while those for experimental scallops ranged from 36–164 mg g^{-1} (female) and 35–99 mg g^{-1} (male). The average carbohydrate levels were maximum on 29th October (female: 130±6.1 mg g^{-1}, male: 73±6.7 mg g^{-1}) for wild scallops and 13th October (female: 114±16.8 mg g^{-1}, male: 67±6.5 mg g^{-1}) for experimental scallops (Fig. 4-8).

Protein contents in female gonads had significant interaction, while male gonads were significantly different between wild and experimental animals with significant interaction. For wild scallops, mean (± SE) gonad protein levels reached a peak of 394±12.3 mg g^{-1} (female) and 284±9.6 mg g^{-1} (male) on 23rd October, followed by a major drop (female: 347±8.3 mg g^{-1}, male: 236±12.8 mg g^{-1}) on 29th October. Conversely, mean (± SE) protein levels within the experimental scallops remained similar at 309±8.3 to 322±11.4 mg g^{-1} (female) and 166 ± 11 to 249 ±18.1 mg g^{-1} (male) between 27th September and 23rd October, with maximum levels of 420±14.3 mg g^{-1} (female) and 288±15.1 mg g^{-1} (male) on 29th October (Fig. 3-8). Overall protein levels ranged between 233−488 mg g^{-1} for wild scallops and 238−481 mg g^{-1} for experimental scallops, based on female gonads, and 120−345 mg g^{-1} and 94−335 mg g^{-1} based on male gonads for wild and experimental animals, respectively.

The lipid contents in both female and male gonads were significantly difference over time, with significant interaction in female gonads only. The gonad lipid levels ranged between 110−303 mg g^{-1} (female) and 60−245 (male) in wild scallops, while experimental animals had 116−240 mg g^{-1} in female gonads and 83−183 mg g^{-1} in male gonads. Average lipid levels (mean ± SE) in wild scallops increased gradually from

169.9±11 mg g^{-1} (female) and 110.0±6.2 mg g^{-1} (male) on 27th September and reached maximum levels on 23rd October (female: 213±4.7 mg g^{-1}, male: 141±2.6 mg g^{-1}), followed by a major decrease to 169±5.8 mg g^{-1} and 126±9.1 mg g^{-1} on 29th October for female and male gonad, respectively. The average lipid level (mean ± SE) in experimental scallops was highest at the start of the experiment (27th September), and then decreased to a minimum on 13th October (female: 146±7.8 mg g^{-1}, male: 103±5.4 mg g^{-1}), but gradually increased to 164±5.6 mg g^{-1} (female) and 122±4.5 mg g^{-1} (male) at the end of the experiment (29th October) (Fig. 4-8).

Nutrition condition

Adductor muscle:

Adductor muscle indices (AMI) were significantly different between wild and experimental scallops, with a significant interaction (Fig. 4-7, Table 4-1). Adductor muscles had the highest mean (± SE) AMI at the start of the experiment (27th September) for both wild and experimental scallops of 49±1.3%, followed by a depletion to its lowest recorded value of 41±0.9 % on 13th October and 643±1.8% on 23rd October for wild animals and experimental animals, respectively . Overall, AMI values ranged between 32−56% for wild scallops, and 27−53% for experimental scallops through the course of the study period.

Analysis of adductor muscle tissues revealed variations in biochemical composition between wild and experimental scallops over the sampling period. All

three biochemical components (carbohydrates, protein and lipids) were significantly different between wild and experimental scallops, with a significant interaction (Fig. 4-8, Table 4-2). Generally, muscular carbohydrate levels were higher in wild scallops than in experimental scallops (range of 32–278mg g^{-1} and 13–132 mg g^{-1}). Mean adductor muscle carbohydrate levels decrease from 126±27.4 mg g^{-1} on 27th September to its minimum of 75±7.7 mg g^{-1} on 13th October, followed by a rebound of carbohydrate content of 143±24.7 mg g^{-1} on 23rd October for wild scallops, while experimental animals decreased to its minimum of 34±10.8 mg g^{-1} on 23rd October.

Protein levels in adductor muscles ranged from 32 to 332 mg g^{-1} and 28 to 268 mg g^{-1} in wild and experimental scallops, respectively. In wild scallops, there was a decrease in protein level (mean ±SE) from 173±8.6 mg g^{-1} (27th September) to 62±5.3 mg g^{-1} (13th October), followed by a pronounced increase to 242±9.4 mg g^{-1} on 23rd October. In experimental scallops, mean levels decreased from the start of the experiment (27th September) to 23rd October (73±7.5 mg g^{-1}), followed by an increased to its maximum levels of 176±7.8 mg g^{-1} on 29th October.

The muscular lipid level was low in general, and ranged from 35 to 84 mg g^{-1} and 27 to 63 mg g^{-1} for wild and experimental scallops, respectively. A notable increase of mean lipid level from 40±1.2 to 75±5.6 mg g^{-1}, occurred from 13th October to 29th October in wild scallops. In experimental scallops, the fluctuation of mean lipid levels between sampling events was small, with the minimum level of 36±2.0 mg g^{-1}recorded on 13th October and maximum level of 50±3.8 mg g^{-1} recorded on 23rd October.

Digestive gland:

Digestive gland condition index (DGI) showed little fluctuation over the sampling period for scallops from both environments; expect for a notable increase in wild scallop DGI at the end of the experiment (Fig. 4-7). DGI ranged between 5−15 % and 4−11 % for wild and experimental scallops, respectively. DGI values of wild scallops were significantly different to those of experimental scallops, with a significant interaction (Table 4-1). Mean (± SE) DGI values of 7±1.3 % were recorded at the start of the experiment (27th September), and there were minimal changes for both wild and experimental scallops, expect for a dramatic increased between 23rd and 29th October, and a maximum peak of 11±0.7 % (wild) and 8±0.4% (experimental).

Carbohydrate contents were low in digestive glands, but significantly different between wild and experimental scallops (Table 4-2). Carbohydrate levels ranged from 35 to 170 mg g^{-1} and 24 to 142 mg g^{-1} in wild and experimental scallops, respectively. In wild scallops, mean digestive gland carbohydrate levels were unchanged between 27th September (78±20.1 mg g^{-1}) and 13th October (83±10.8 mg g^{-1}), and followed by a dramatic depletion on 23rd October (51±9.2 mg g^{-1}), and bouncing back to the initial levels of 84±22.3 mg g^{-1}. Conversely, carbohydrate levels of experimental scallops increased steadily from 27th September and reached a peak of 115±26.7 mg g^{-1} on 23rd October, followed by a decrease to 74±25.5 mg g^{-1} on 29th October (Fig. 4-8).

There were no significant differences in the protein content in digestive glands between wild and experimental scallops (Fig. 4-8, Table 4-2). Protein levels ranged from 208−645 mg g^{-1} and 280−620 mg g^{-1} in wild and experimental scallops,

respectively. The variation in mean protein levels was low for both wild and experimental scallops, expect for a major increase from 372±13.5 to 508 mg g^{-1} between 13th October and 23rd October for wild scallops. In experimental scallops, mean levels steadily increased from 387±30.1 mg g^{-1} on 27th September to a maximum level of 420±10.5 mg g^{-1} on 23rd October, followed by a slight decrease to 403±23.3 mg g^{-1} at the end of the experiment (29th October).

The fluctuation in lipid contends within digestive glands was larger in wild than experimental scallops, ranging from 64−392 mg g^{-1} and 81−198 mg g^{-1}, respectively. In wild scallops, mean lipid levels dropped from 159±9.6 mg g^{-1} in 27th September to 93±9.7 mg g^{-1} in 13th October, followed by a steady increase to 271±11 mg g^{-1} at the end of the experiment (29th October). In contrast, mean (± SE) lipid levels in digestive glands of experimental scallops varied little with a minimum of 127±9.7 mg g^{-1} recorded on 23rd October and a maximum value of 168±8.1 mg g^{-1} on 29th October (Fig. 4-8).

Energy content

The energy content in adductor muscle and digestive gland tissues were significantly different between wild and experimental scallops and a significant interaction, while there was no statistical difference in gonad tissues from these two scallop groups (Fig. 4-9, Table 4-3). Adductor muscles had the highest energy contents, ranging between 4.7−13.4 kJ in wild and 4.6−7.3 kJ in experimental scallops, followed

by gonad (wild: 4.4−6.7 kJ, experimental: 4.6−6.0 kJ) and digestive gland (wild: 2.6−6.5kJ, experimental: 2.9−3.3 kJ).

The mean (± SE) energy content in gonad tissues increased gradually from 27th September (5±0.3 kJ) and reached a peak level of 7±0.2 kJ on 23rd October and 6±0.3 kJ on 29th October, for wild and experimental scallops, respectively. Adductor muscle energy content (mean ± SE) of wild scallops exhibited a major decrease between 27th September and 13th October, from 12±1.1 kJ to 5±0.5 kJ, followed by a rebound of energy on 23rd October (13±1.1 kJ) and maintenance at a similar level until the end of experimental period (29th October). In experimental scallops, mean adductor muscle energy levels decreased from 27th September (12±1.1 kJ) to 13th October (4±0.8 kJ), followed by a slight increase to 7±0.3 kJ on 29th October. Digestive gland tissues from wild scallops exhibited notable changes in mean energy content within the study period, while there was little variation in the experimental animals. In wild scallops, the digestive gland energy level (mean ±SE) decrease slightly from 27th September to 13th October (4±0.3 kJ to 3±0.2 kJ), and then gradually increased to 6±0.3 kJ at the end of the experiment (29th October).

Fatty acid profiles

Multivariate analyses (SIMPER) revealed differences in fatty acid composition within the two size classes of natural seston and commercial microalgal diet (Fig. 4-10, Table 4-4). Dissimilarity between the two seston size classes was high (53%), while the dissimilarity between the commercial diet and the large seston class was 49%, and 75%

between commercial diet and small seston class. SIMPER revealed C 16:0, C16:1and C18:0 were the main fatty acids responsible for the differences between small and large wild seston, and between small seston and microalgal diet, while C14:0, C14:1, C18:0 and C18:3n3 were the main differences between large seston and microalgal diet (Table 4-4).

Fatty acid composition of wild and experimental gonads showed clear clusters in MDS, and the dissimilarity revealed by SIMPER between wild and experimental scallops was 18% and 13% for female and male gonad, respectively (Figure 4-11). Adductor muscles had the most similar fatty acid compositions between wild and experimental scallops, with an average dissimilarity of 9%. Digestive gland tissues had the most pronounced dissimilarity in fatty acid composition, with an average dissimilarity of 40%. SIMPER revealed that C 16:0, C20:5n3 (EPA) and C22:6n3 (DHA) were the main fatty acids responsible for the differences in gonad (female and male) and adductor muscle tissues between wild and experimental scallops, while the main differences in fatty acid composition in digestive glands were C16:0, C16:1 and C18:0 (Table 4-5).

EPA and DHA levels were generally higher in wild gonads than experimental ones (Fig. 4-12). Mean EPA levels in gonad tissues increased gradually from 27th September (female: 18±2.6%, male: 15±3.1%) to the highest level of 24±1.6% (female) and 20±1.5% (male) on 23rd October for wild scallops. In contrast, mean EPA levels in gonads of experimental animals decreased progressively from 27th September to a minimum level of 12±3.3% (female) and 7±1.9% (male) at the end of the experiment

(29th October). The mean DHA levels had a similar pattern between wild and experimental gonads. Mean DHA levels in female gonads decreased steadily from 13±2% on 27th September to 10±0.5% (wild) and 7±2.4% (experimental) at the end of the experiment (29th October), while male gonads increased slightly from 8.3±3% to 12±0.1% (wild) and 10±0.4% (experimental) between 27th September and 13th October, followed by a depletion on 23rd October until 29th October). In somatic tissue (adductor muscle and digestive gland), there was no clear variation pattern of EPA and DHA throughout the course of the study (Fig. 4-12).

Discussion

Reproductive

Although the reproductive condition of wild (feeding naturally) and experimental (fed a commercial diet) scallops were similar through the sampling period, some differences were observed that are attributed to gonad performance. In general, wild scallops had higher gonad indices and lipid contents than the experimental animals. It is possible that the quantity and/or quality of the microalgae supplied in the laboratory were not as sufficient (compared to wild seston) for scallop optimal reproductive development. Martinez *et al*. (1992) also recorded a lower gonad index for the Chilean scallop, *Argopecten purpuratus*, after the animals were transferred from the wild to a hatchery environment, while gonad lipid contents were not significantly different between animals in the two growing environments.

Furthermore, peak gonad index of *A. ventricosus* were significantly higher in animals from the wild compared to those in animals held in aquaria with simulated field temperatures (Guerra *et al*. 2012). The data suggest that the laboratory environment was conducive to condition *P. novaezelandiae*, but the optimal condition for culturing this species needs further optimization.

Although all the scallops were originally sourced from the same study site, wild scallops spawned within the study period, while the experimental animals continued to mature until the end of the experiment (no evidence of spawning). The difference in maturation timing was reflected in the wild scallops with a sharp decrease in visual gonad index (VGI), oocyte diameter, gonad index (GI), gonad (both female and male) protein content and lipid content, between 23rd and 29th October. Conversely, the experimental scallops reached their maximum visual gonad index (VGI), oocyte diameter, gonad protein content and gonad lipid content at the end of the experiment (29th October) with no signs of a decrease in these parameters. Variations in the timing of bivalve gonad development have been shown to be due to both environmental and genetic components (Lannan 1980). For example, Martinez *et al*. (1992) investigated the reproductive condition of the scallop *Argopecten purpuratus* held in natural and hatchery environments, and recorded a slower maturation rate in scallops kept in the hatchery. The authors suggested that the delay in gonad maturation was due to nutritional stress that resulted in some resorption of ripe gametes in the hatchery-reared scallops. Resorption of previously formed gametes under stressful condition is common in cultured bivalves (Bayne *et al*. 1978, Steele *et al*. 1999, Delgado *et al*. 2005). In the present study, gamete resorption was not observed in histological

sections, but there was an initial decrease in gonadal lipid content between 27th September and 13th October. This suggested that experimental scallops were stressed at the start of the experiment, which may have consequently affected their nutritional intake, as well as being affected by the different diet. This type of nutritional stress may have been responsible for the delayed gonadal maturation in the experimental animals.

In addition, Guerra *et al*. (2012) suggested that natural environments tend to support optimal growth for scallops, and noted that the scallop *A. ventricosus*, exhibited higher growth and reproductive investment in the wild, while experimental scallops died after the first spawning. Bayne *et al*. (1978) showed a simultaneous regression in gonad condition in the mussel *Mytilus edulis* under temperature stress or low food availability. In the present study, the temperature was maintained at a similar level and with similar fluctuations as those in the wild, so it is unlikely that temperature was responsible for the differences in maturation timing.

Nutrient utilization

The findings of this study indicate that experimental scallops had generally lower levels of carbohydrates, protein, lipids and total energy in their muscles compared to scallops from the wild. Adductor muscles are known to be the main storage organs in Pectinids (Mathieu & Lube 1993), and these stored nutrients can be made readily available for gamete production when needed. It is possible that the experimental animals in this study did not have enough nutrient ingestion to support

gametogenesis, and stored nutrients in the muscle were necessary to achieve maturity. Indeed, numerous studies have reported on the utilization of stored nutrient in adductor muscles to support gonad maturation (Barber & Blake 1981, Martinez *et al*. 2000a, Lodeiros *et al*. 2001), but under energy-limited conditions, energy investments can be prioritized for growth and maintenance (*P. magellanicus*) or for reproduction (*A. ventricosus*) depending on the species (MacDonald & Bayne 1993, Guerra *et al*. 2012). The general trend of muscular nutrient expense was observed in both wild and experimental scallops in the present study, although experimental animals had a more extreme depletion in carbohydrates, protein and overall energy levels in adductor muscles just prior to the gonads reaching peak maturity. This suggests that *P. novaezelandiae* utilizes adductor muscle nutrients for gonad maturation, and energy is prioritized for reproduction over somatic growth even when nutrient levels in the adductor muscle are low. The importance of carbohydrates and protein in adductor muscles in term of nutrient storage to support gametogenesis was also recognized in numerous scallop species, including *A. irradians concentricus* (Barber & Blake 1981), *A. purpuratus* (Martinez *et al*. 2000a) *A. irradian irrdians* (Epp *et al*. 1988), *Euvola zizac* (Brokordt *et al*. 2000), *Lyropecten nodosus* (Lodeiros *et al*. 2001) and *P. novaezelandiae* reported here in Chapter 3 . It is possible that these decreases in nutrient and energy reserves in the muscles of wild scallops could be attributed to mobility, such as those associated with predator escape activities. However, in this study, experimental animals were kept in protected and stable 4L tank environments, and thus it could be assumed that the nutrient and energy depletions in these animals would have been used almost entirely for reproduction.

Another observable pattern in the results of this study is that there was a lag time of <10 days from the depletion of nutrients and energy in the muscles to the increase in gametogenic condition for both wild and experimental scallops. This suggests that nutrient transfers from storage to reproductive organs are not instantaneous and nutrient accumulation in gonad tissues occurs gradually. Although the time frame for nutrient transfers from storage to reproductive organs has not been specifically reported before, several studies have illustrated that it takes about 1 week for nutrients to be transferred from the digestive gland to other organs, such as kidneys, gills and gonad (Allen 1970, Sastry & Blake 1971). Radiotracer experiments on *Mytilus edulis* indicated that nutrients require 1 week to be distributed from the digestive gland and accumulate in kidneys and gills (Allen 1970). Sastry and Blake (1971) demonstrated the transfer of nutrients from the digestive gland to the gonad in a ^{14}C-labelling study of *A. irradians*, and accumulation of nutrients was much higher than 1 week after radiotracer injection compare to 24 hours post injection. Nutrient transfer of *A. irradians concentricus* took place within a shorter period (4 days), and the authors suggested that this reflects a faster rate of nutrient assimilation and biochemical transformation compared to other species (Barber & Blake 1985). The lag time in nutrient transfer observed in this study has rarely been reported with respect to energy utilization studies of Pectinids. This is probably due to the fact that most experiments have used monthly sampling periods (Epp *et al*. 1988, Martinez *et al*. 2000a, Lodeiros *et al*. 2001), which may not be sufficient to elucidate these maturation lag times. With the relatively short sampling intervals of this study (6−16 days), our

results provide evidence that utilization of nutrients in gonad tissues that were previously stored in adductor muscles takes less than 10 days.

In contrast to the nutrient utilization in muscle tissues, digestive glands of wild scallops and experimental scallops did not have the same nutrient and energy changes. Nutrient contents in wild digestive glands (lipid levels in particular) were highly variable through the course of the study period, compared to the stable and consistent nutrition levels in the experimental animals. In bivalves, the digestive gland is responsible for storage and distribution of assimilated nutrients from food intake (Sastry 1968, Thompson *et al*. 1974). Therefore, changes in digestive gland nutrient contents are either due to variable food supplies or requirements to support reproduction. The minimum lipid levels in the digestive gland of wild scallops were recorded on 13th October, which coincided with the extreme low seston concentration for both size fractions. Furthermore, the fluctuations in digestive gland nutrient contents in experimental animal were low when food supplies were consistent and regular in the laboratory. Thus, it is likely that the digestive gland nutrient contents were mainly influenced by food intake for both wild and experimental animal, and that the fluctuations in nutrient content reflected remobilization to support gametogenesis. This result is in agreement with findings from 2012, that provide evidence that adductor mussel nutrient reserves, rather than nutrients from the digestive gland are used to produce a spring spawning in *P. novaezelandiae* (Chapter 3).

In the present study, the analysis of fatty acid composition within different tissues indicated distinct differences between wild and experimental animals for male

and female gonad and digestive gland tissues. In gonad tissues, C16, C20:5n3 (Eicosapentaenoic; EPA) and C22:6n3 (Docosahexaenoic acids; DHA) were the most dissimilar fatty acids between wild and experimental animals, with a difference of higher EPA in wild animals being the most pronounced. Palmitic acid (C16) is one of the main fatty acids in diatoms, and the high concentrations of this fatty acid in wild scallops is likely to indicate that diatoms were a significant food sources for these scallops. Conversely, experimental scallops were fed a diet containing a relatively small amount of one diatom species (20% of *Thalassiosira weissflogii*). EPA and DHA are considered to be essential fatty acids for survival and growth in molluscs (Ackman 1983). One of the nutritional factors that most affects the quality of spawning (size and number of spawned eggs) in bivalves is the content of EPA in female gonads (Hendriks *et al*. 2003), which is also the most abundant fatty acid in larvae during metamorphosis (Robinson 1992). While EPA is essential during embryonic development to provide energy, DHA is used for structural function throughout larval development (Helm *et al*. 1991, Marty *et al*. 1992). Although eggs were not collected from experimental scallops, as the animals were not induced to spawn in the present study, several studies have demonstrated that accumulated lipids and fatty acids (EPA and DHA) in gonad are transferred and used to release eggs during spawning (Trider & Castell 1980, Marty *et al*. 1992, Pazos *et al*. 1996, Wacker & Von Elert 2004). Thus, the much lower and progressively decreasing EPA levels in the gonads of the experimental animals in the present study indicates that spawning and egg quality, were negatively affected when *P. novaezelandiae* were held in the laboratory environment.

The main fatty acids responsible for the difference in digestive glands were C16, C16:1 and C18, which are dominant in algal fatty acids, originate from degradation of dietary chlorophyll, and are often found in digestive glands of bivalves (Joseph 1982). Comparably, C16, C16:1 and C18 were also the most differentiating fatty acids between the commercial microalgal diet and the small seston fraction. In previous studies on *P. novaezelandiae*, isotope analysis have indicated that small seston fractions (1.2−5 μm) provide the main diet for wild *P. novaezelandiae* (Chapter 3). Therefore, the different fatty acid compositions in wild and experimental digestive glands are likely to be due to the impact of dietary fatty acids (seston vs. artificial microalgal diet). The strong association of fatty acid composition between food sources and digestive gland tissues has been demonstrated by several laboratory experiments (Napolitano & Ackman 1992, Napolitano *et al*. 1993, Napolitano *et al*. 1997, Caers *et al*. 2003), as well as in field studies that suggest that changes in fatty acid composition within digestive glands is directly related to temporal variations in the composition and abundance of natural seston (Napolitano & Ackman 1992, Napolitano *et al*. 1997). For example, laboratory studies with *P. magellanicus* (Napolitano *et al*. 1993) and *Argopecten pupuratus* (Caers *et al*. 2003) showed that a 1-month feeding period significantly altered the fatty composition of digestive glands, and this variation was associated with the fatty acid composition of the diet provided. In field studies, fatty acids in *P. magellanicus* digestive glands during the pre-bloom period were characterised by C18:1n9, C18:4n3, and C22:6n3, which are common and often major constituents of dinoflagellate and prymnesiophyta lipids in the surrounding waters (Newfoundland, Canada), while those fatty acids exhibiting elevated concentrations during bloom and post-bloom periods

(i.e. C16:1n7, C16:1n4, and C20:5n3) are well established biochemical markers for diatoms (Napolitano *et al*. 1997). Similar to other bivalve species, the fatty acid composition of digestive glands in *P. novaezelandiae* were highly influenced by their food source.

In contrast to the notable difference in fatty acid composition in gonad and digestive gland tissues between wild and experimental animals, fatty acid profiles of adductor muscles were similar for scallops from both growing environments. Several studies have illustrated the fact that carbohydrates (glycogen) and/or protein, but not lipids, are the main energy reserves in adductor muscles. Consequently, changes in food availability and/or energy utilization within animals do not impact the lipid content of this organ (Barber & Blake 1981, Napolitano & Ackman 1992, Soudant *et al.* 1996). In the present study, similar results were obtained to those of *P. magellanicus* (Napolitano *et al*. 1993) and *A. pupuratus* (Caers *et al*. 2003), which showed that feeding on microalgal diets for ~1 month had a minimum effect on fatty acid composition of adductor muscles. As suggested by Napolitano *et al*. (1993), it is possible that muscular lipids are primarily for cellular structural function in Pectinidae. Therefore, adductor muscles may have less lipid metabolism compared to other high lipid content tissues, such as gonads and digestive glands.

Summary

The findings in the present study show that nutrient composition varies between wild and experimental *P. novaezelandiae*. Wild scallops spawned within the

study period, while the experimental animals reached gonad maturation (comparable reproductive condition to wild scallops prior to spawning) at the end of the experiment. The delay in gonad maturation in experimental animals is likely to be due to nutritional stress associated with the change in environment and subsequent feeding with the artificial diet provided in the laboratory. Somatic nutrient storage (adductor muscle carbohydrate and protein) was remarkably lower in experimental animals compared to field counterparts. Nevertheless, muscular nutrient expenses used for reproductive activity were similar for scallops from both growing environments. Hence, *P. novaezelandiae* may prioritize energy for reproduction at the cost of muscle energy storage, regardless of the level of nutrient reserve in the animals. Polyunsaturated fatty acid (PUFA) concentrations were much lower and progressively decreased in gonads from experimental animals. This suggests that scallop spawning may have been negatively affected in the experimental culturing conditions. The results of the present study suggest that *P. novaezelandiae* under laboratory conditions (using the commercial microalgal diet) was able to achieve gonad maturation, but largely supported by previously stored nutrients from other tissues, such as muscles and digestive glands. Thus, while the present study shows that *P. novaezelandiae* can be successfully cultivated in the lab with a commercial diet, the optimal condition for culturing this species has not been identified and requires further investigation.

Table 4-1 ANOVA analyses of condition index for gonad, muscle and digestive gland tissues in wild and experimental scallops. Bold p-values indicate significant differences, at $p < 0.05$.

Condition Index

Source	*df*	Gonad Index		Adductor muscle Index		Digestive gland Index	
		F	p	*F*	p	*F*	p
Environment	1	0.01	0.09	12.22	**<0.01**	0.28	0.60
Time	2	3.02	0.05	24.09	**<0.01**	1.98	0.14
Environment x Time	2	3.01	0.05	5.78	**<0.01**	3.16	0.05
Error	88						

Table 4-2 ANOVA analyses of biochemical composition for wild and experimental scallop tissues**.** Bold p-values indicate significant differences, at $p < 0.05$.

Carbohydrates

Source	*df*	Female gonad		Male gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p	*F*	p
Environment	1	0.95	0.33	0.22	0.64	56.98	**<0.01**	10.01	**<0.01**
Time	2	3.45	**0.04**	2.20	0.12	3.93	**0.02**	0.34	0.71
Environment x Time	2	2.14	0.13	4.86	**0.01**	5.25	**0.01**	15.80	**<0.01**
Error	88								

Protein

Source	*df*	Female gonad		Male gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	P	*F*	P
Environment	1	0.14	0.71	9.84	**<0.01**	9.84	**<0.01**	0.27	0.60
Time	2	4.50	**0.02**	15.53	**<0.01**	15.53	**<0.01**	10.90	**<0.01**
Environment x Time	2	10.11	**<0.01**	24.37	**<0.01**	24.37	**<0.01**	6.27	**<0.01**
Error	88								

Lipids

Source	*df*	Female gonad		Male gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	P	*F*	P
Environment	1	0.14	0.71	0.28	0.60	13.91	**<0.01**	19.07	**<0.01**
Time	2	4.97	**0.01**	4.18	**0.02**	29.69	**<0.01**	28.28	**<0.01**
Environment x Time	2	4.19	**0.02**	1.00	0.36	18.97	**<0.01**	15.57	**<0.01**
Error	88								

Table 4-3 ANOVA analyses of energy content for wild and experimental scallop tissues, in kJ per tissue. Bold numbers indicate significant differences, at $p < 0.05$.

Energy							
Source	*df*	Gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p
Environment	1	0.85	0.36	36.55	**<0.01**	13.01	**<0.01**
Time	2	0.91	0.41	7.84	**<0.01**	14.99	**<0.01**
Environment x Time	2	2.79	0.07	11.01	**<0.01**	14.20	**<0.01**
Error	88						

Table 4-4 Average dissimilarity of fatty acid proportions of wild seston (1.2−5µm, >5µm) and microalgal diet, seston collected from Prospect Bay between 27th September — 29th October 2013. The cut-off is shown at 80%.

Seston (1.2−5µm) & Seston (>5µm)	
Average dissimilarity: 52.69	
Fatty Acids	Cum.%
C16:0	27.18
C18:0	43.01
C16:1	58.65
C14:1	72.01
c20:5n3	79.62
C22:1n9	84.29

Seston (1.2−5µm) & Microalgal diet		Seston (>5µm) & Microalgal diet	
Average dissimilarity: 75.14		Average dissimilarity: 49.35	
Fatty Acids	Cum.%	Fatty Acids	Cum.%
C18:0	25.04	C18:0	21.65
C16:0	45.61	C14:1	35.91
C16:1	57.89	C14:0	48.81
C14:0	65.69	C18:3n3	56.21
c20:5n3	71.96	C18:2n6c	62.63
C18:2n6c	77.58	C18:1n9c	68.48
C18:3n3	82.43	C22:1n9	73.94
		C22:6n3	79.07
		C22:0	83.89

Table 4-5 Average dissimilarity of fatty acid proportions of wild and experimentalscallop tissues (female gonad, male gonad, adductor muscle and digestive gland), between 27th September — 29th October 2013. The cut-off is shown at 80%.

Female gonad		Male gonad	
Average disimilarity: 18.33		Average dissimilarity: 12.95	
Fatty Acids	Cum.%	Fatty Acids	Cum.%
c20:5n3	30.2	c20:5n3	24.8
C16:0	53.8	C16:0	44.2
C22:6n3	65.7	C22:6n3	58.6
C16:1	74.5	C18:0	67.7
C18:0	81.1	C16:1	75.7
		C14:0	82.2

Adductor muscle		Digestive gland	
Average dissimilarity: 8.86		Average dissimilarity: 40.43	
Fatty Acids	Cum.%	Fatty Acids	Cum.%
C22:6n3	22.4	C16:0	42.2
C16:0	42.0	C16:1	55.6
c20:5n3	58.4	C18:0	66.5
C18:0	66.7	C14:0	77.3
C16:1	72.0	C18:1n9c	82.4
C14:0	77.2		
C18:3n6	82.0		

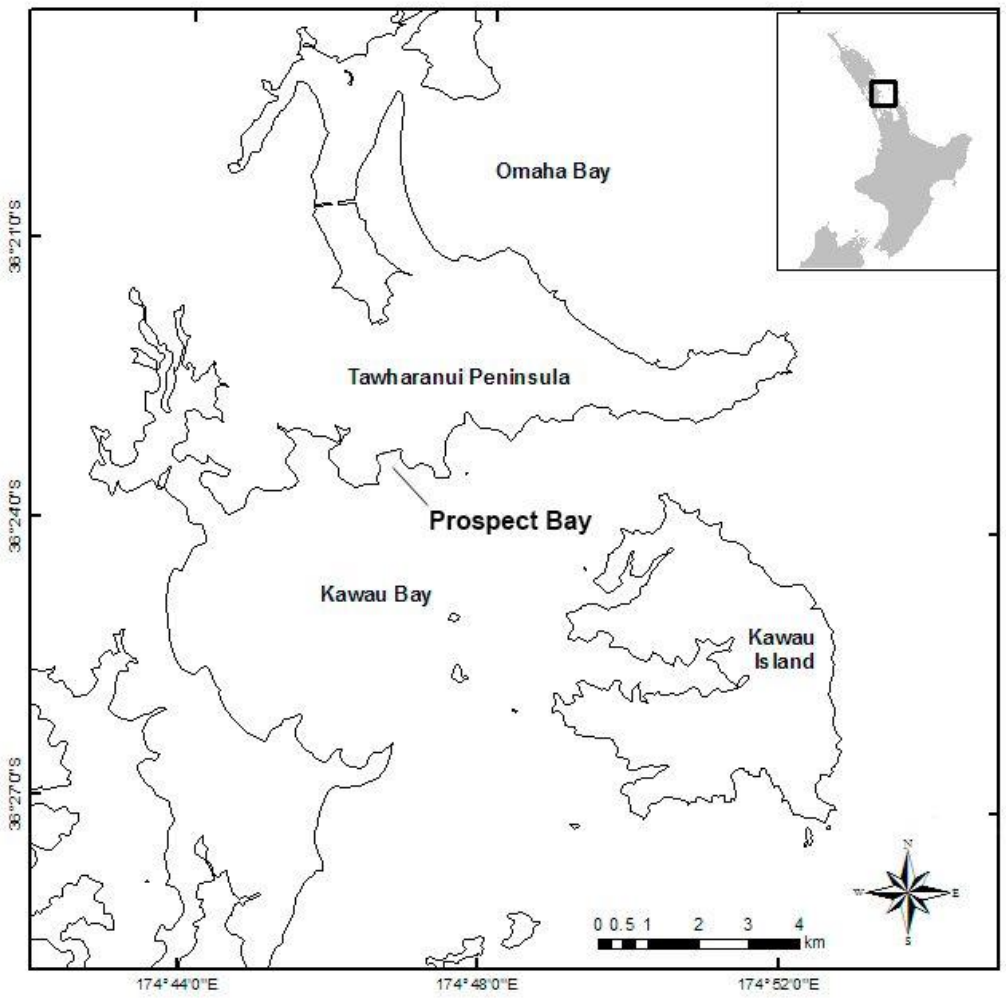

Figure 4-1 Study site, Prospect Bay, southern Tawharanui Peninsula, Hauraki Gulf, New Zealand.

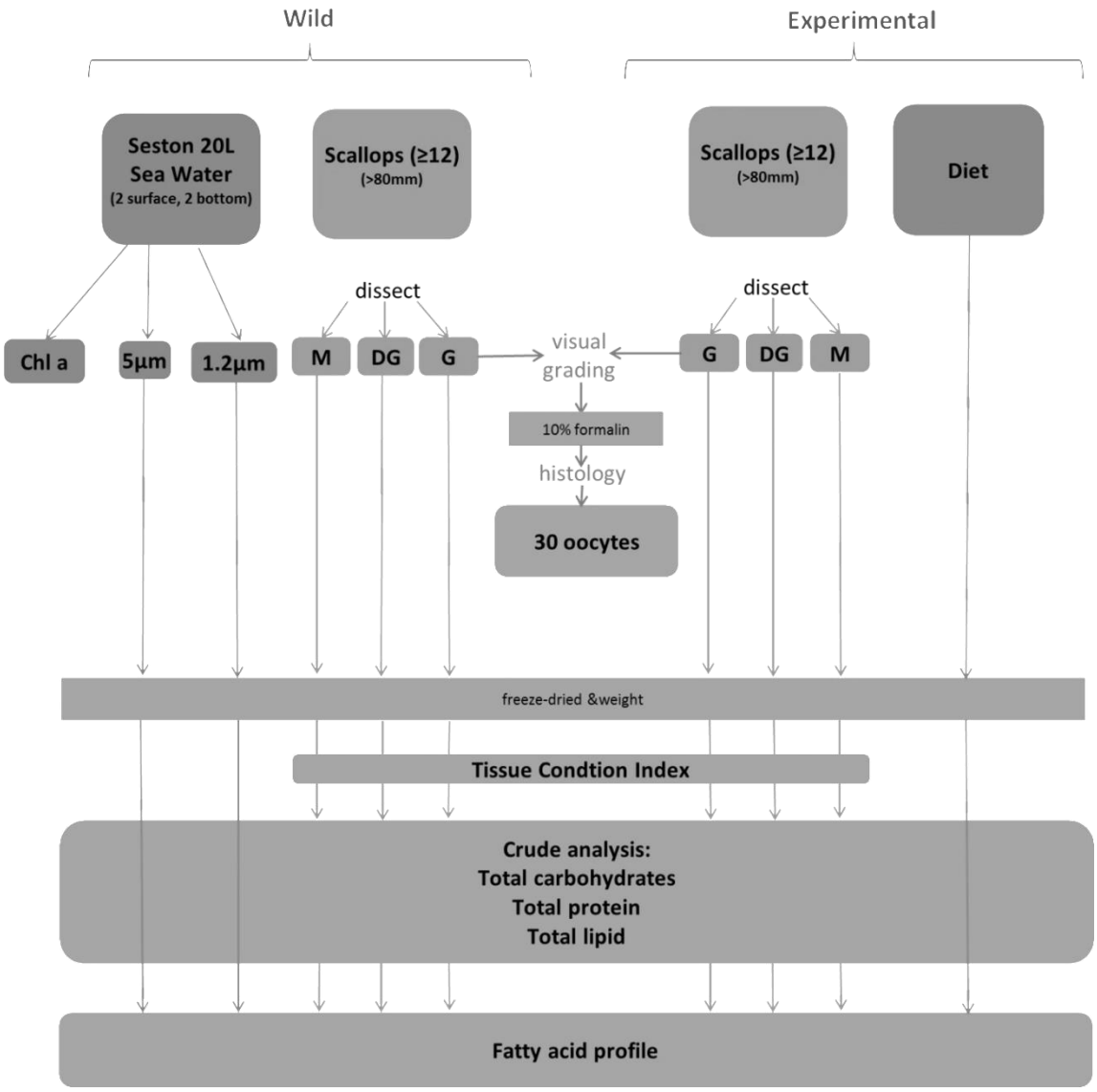

Figure 4-2 Flow chart depicting sample collections and analyses to compare reproductive condition and nutritional content of wild and experimental scallops. G: gonad M: adductor muscle, DG: digestive gland.

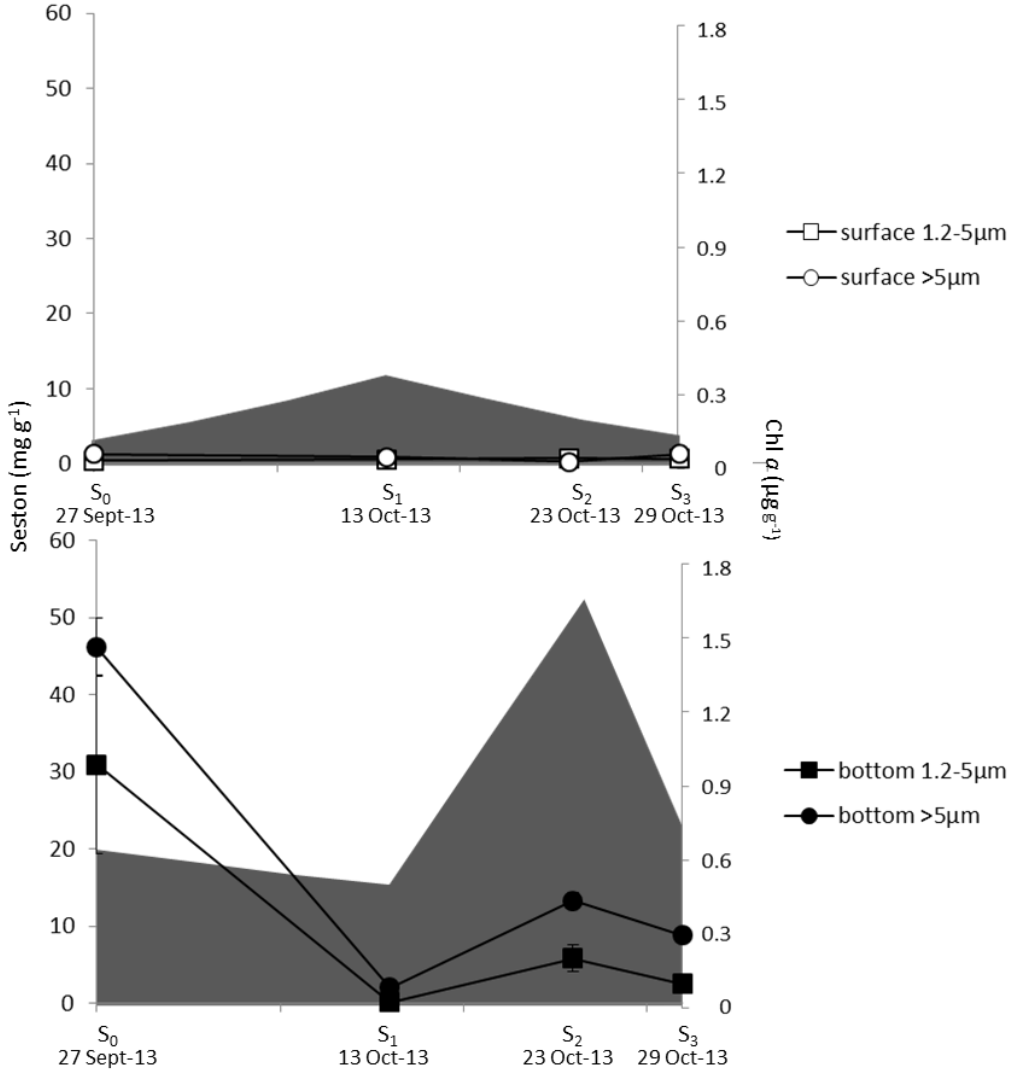

Figure 4-3 Temporal changes in surface and bottom seston of two size classes (1.2−5µm and >5µm), and chlorophyll *a* concentrations (grey area) between 27th September– 29th October 2013. Mean values ± standard error.

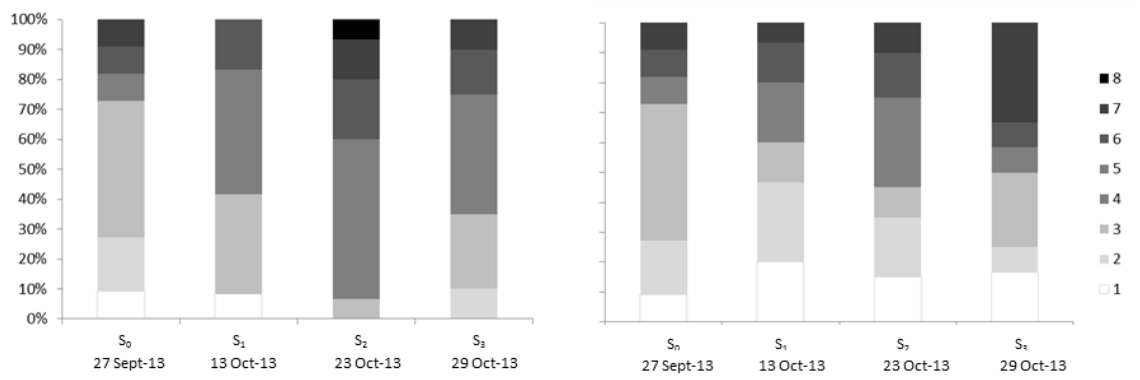

Figure 4-4 Frequency distribution of eight visual gonad index (VGI) classes for scallops. Wild (left) and experimental (right) scallops between 27th September and 29th October, 2013. Mean values ± standard error.

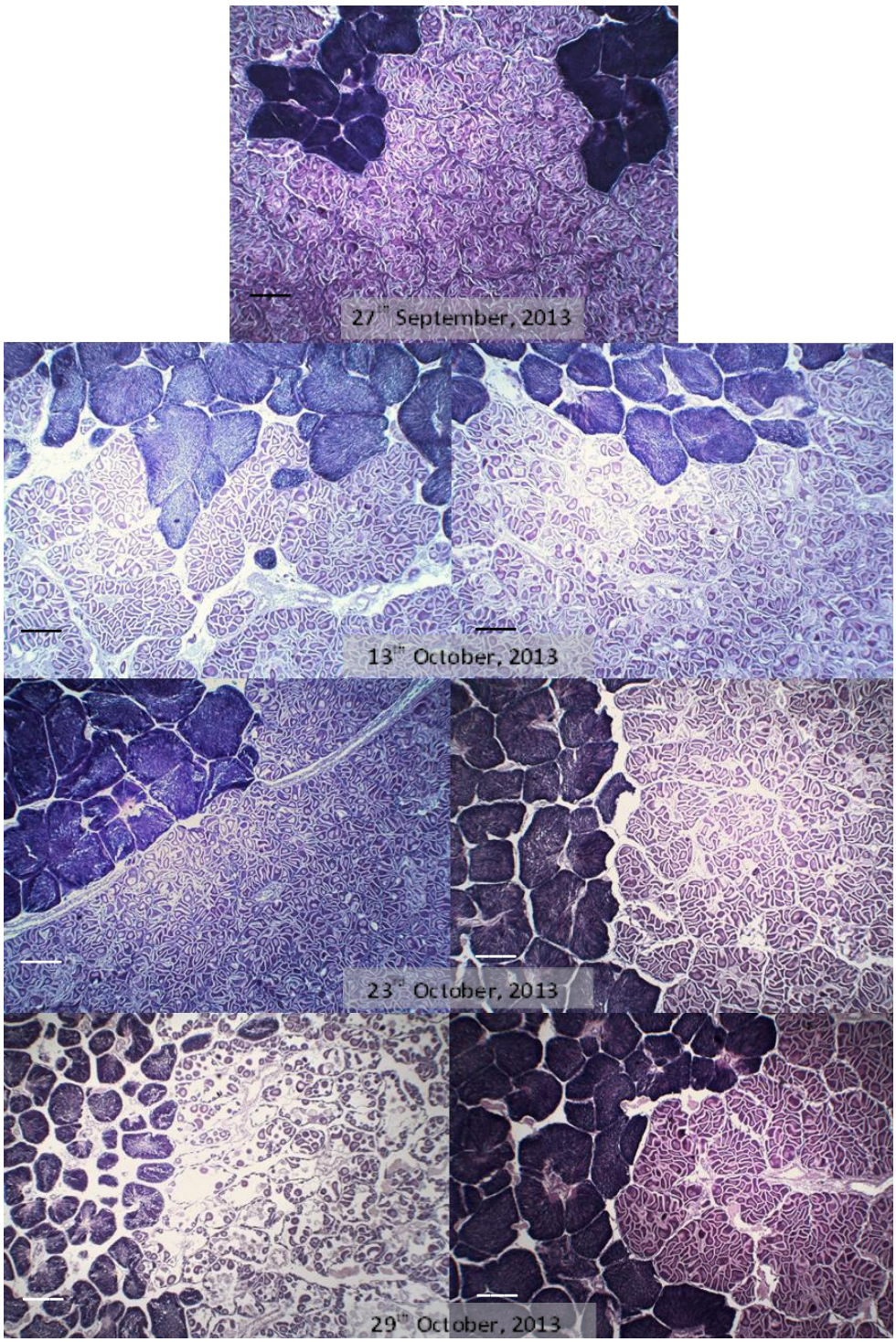

Figure 4-5 Photomicrographs of gonadal sections from wild (left) and experimental (right) scallops; scale bar: 100µm. Sperm are stained in dark purple and eggs in pink. Gonad appearance, visual grade index (VGI), gonad index (GI), gonad wet weight and scallop shell length are detailed in Appendix VI.

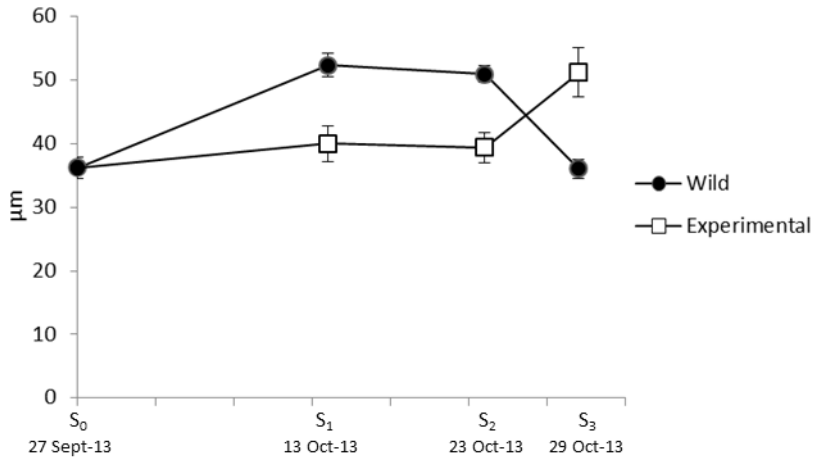

Figure 4-6 Oocyte diameters of wild and experimental scallops between 27th September and 29th October, 2013. Mean values ± standard error.

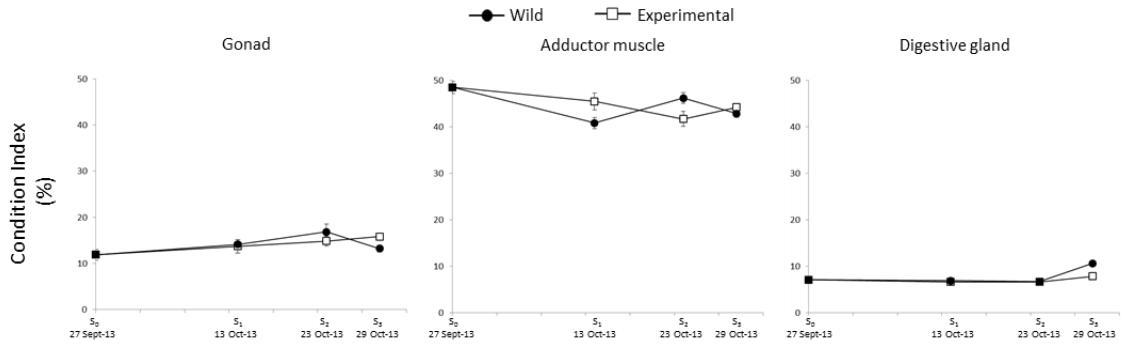

Figure 4-7 Variations in condition index of wild and experimental scallop tissues (gonad, adductor muscle and digestive gland) between 27th September — 29th October 2013. Mean values ± standard error.

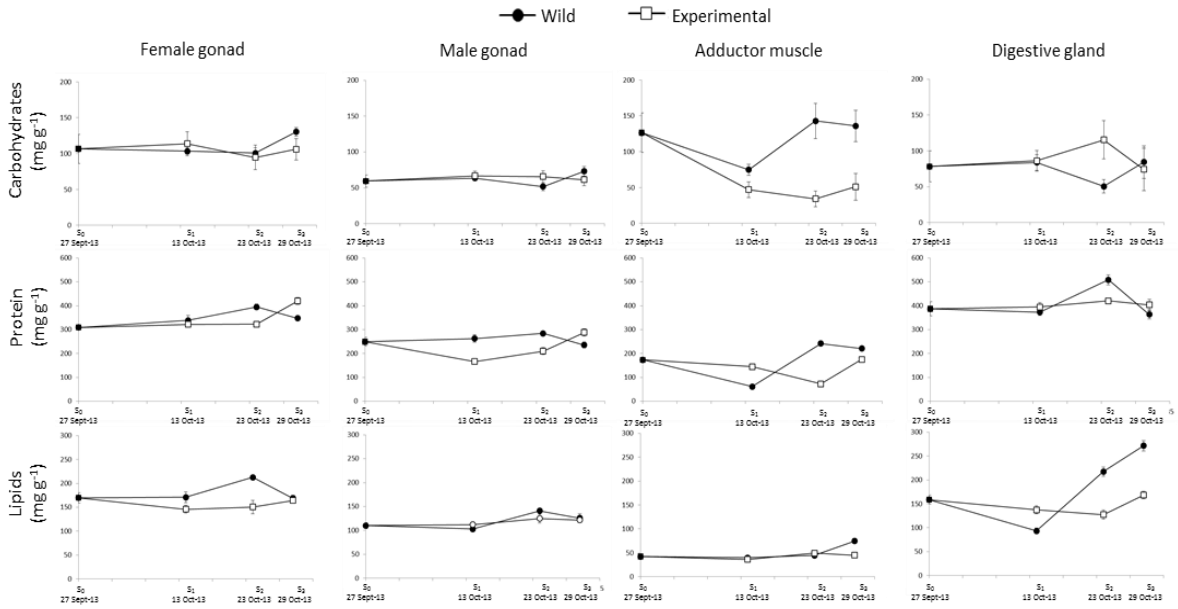

Figure 4-8 Variation in biochemical composition (carbs: carbohydrates, lipids and proteins) of tissues (female gonad, male gonad, adductor muscle and digestive gland) in wild and experimental scallops between 27th September and 29th October 2013. Mean values ± standard error.

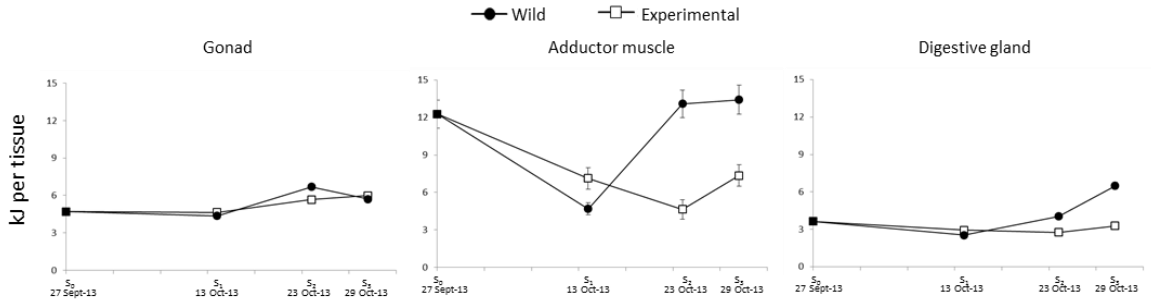

Figure 4-9 Variation in energy content of wild and experimental scallop tissues (gonad, adductor muscle and digestive gland) between 27th September — 29th October 2013, expressed in kJ per tissue. Mean values ± standard error.

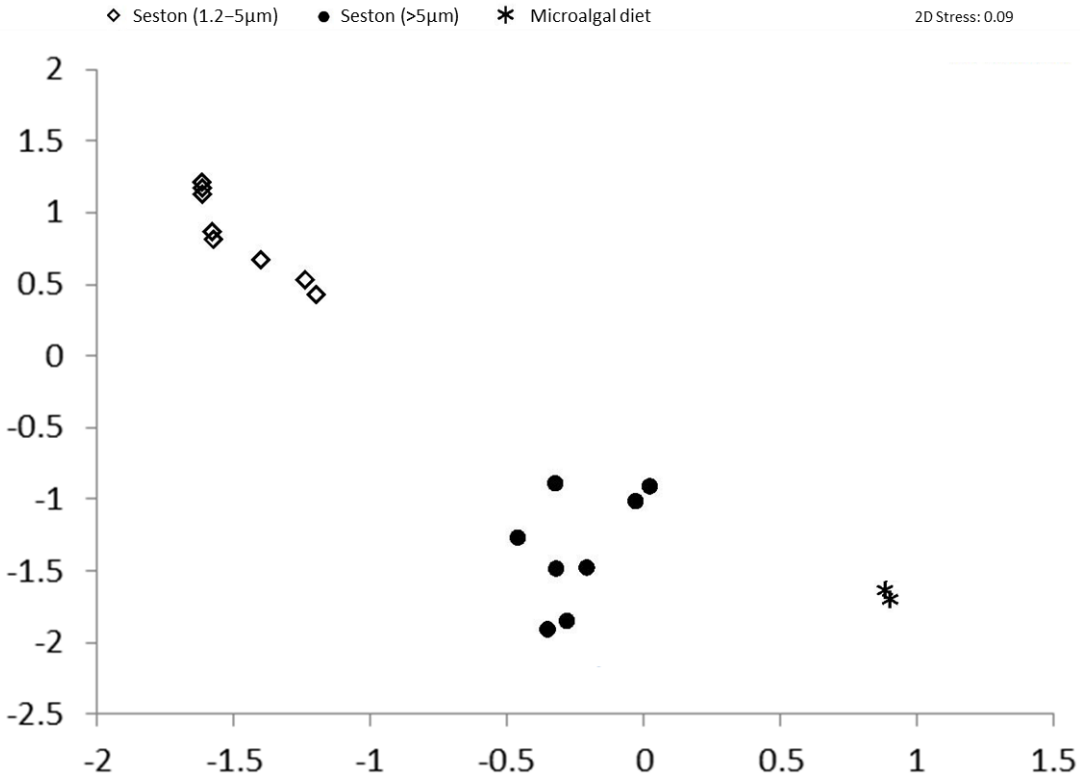

Figure 4-10 MDS plot of fatty acid composition of wild seston (1.2−5µm, >5µm) and microalgal diet, seston collected from Prospect Bay between 27th September — 29th October 2013.

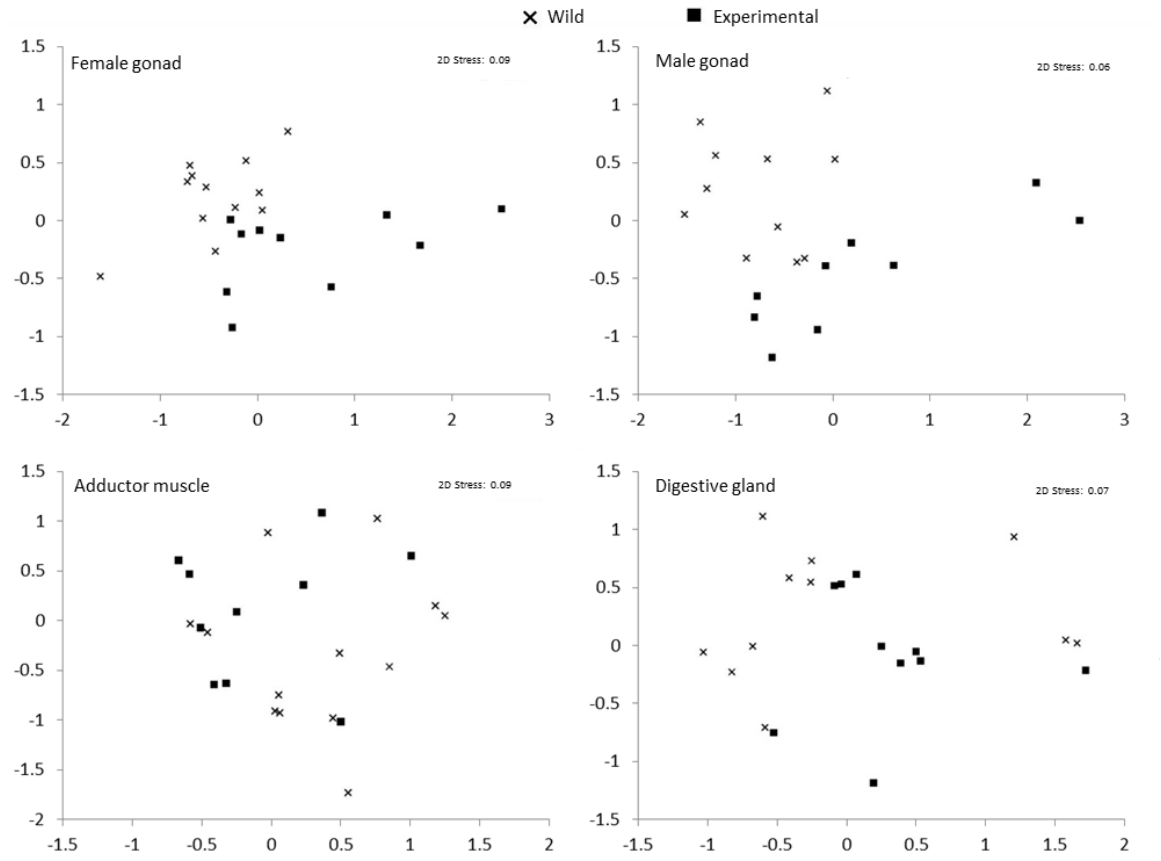

Figure 4-11 MDS plot of fatty acid composition of wild and experimental scallop tissues (female gonad, male gonad, adductor muscle and digestive gland), between 27th September — 29th October 2013.

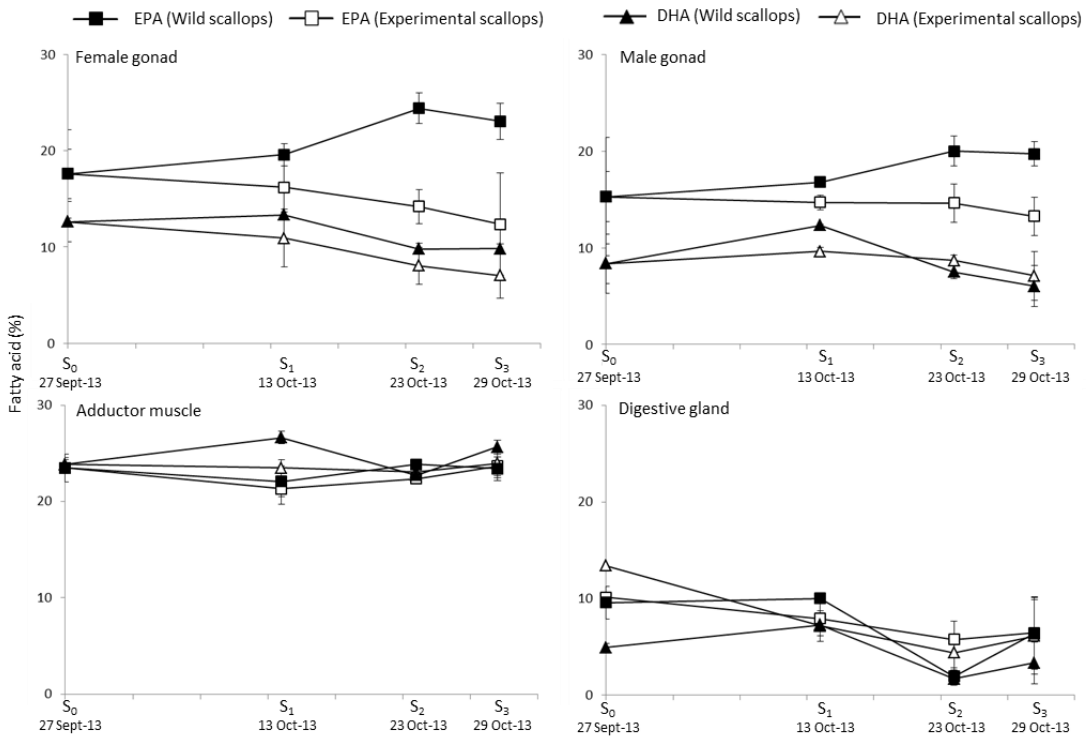

Figure 4-12 Variation in eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) within wild and experimental scallop tissues (gonad, adductor muscle and digestive gland), between 27th September – 29th October 2013, expressed in % of total fatty acids. Mean values ± standard error.

Chapter 5 : General discussion

This thesis investigates the nutrient utilization of *Pecten novaezelandiae* in relation to scallop reproductive condition in the Hauraki Gulf. The results complement the existing knowledge on scallop nutrient cycles among species and population. In addition to understanding the general biology (Bull 1976), feeding behaviour (Nicholson 1978), population distribution (Morrison 1999), spat biology (Nesbit 1999) and reproduction (Williams 2005) of *P. novaezelandiae*, knowledge now exists on how nutrients are utilized within this species, with the ultimate goal of maintain and maximize the potential of the scallop fisheries in New Zealand.

Reproductive cycle of *P. novaezelandiae*

The reproductive cycle of *P. novaezelandiae* from Prospect Bay was characterized by two major spawning periods, in spring (November) and summer (March). Timing for the spring spawning tended to be consistent for this population (end of October/November) in 2012 (Chapter 3) and 2013 (Chapter 4). Nevertheless, the spawning of *P. novaezealandiae* is not necessarily predictable in the Hauraki Gulf. Chapter 2 reported on the variation in timing of spawning/gonad maturation (reflected by VGI and gonad lipid contents) between populations: populations in Prospect Bay were significantly different to populations in Tiritiri Matangi and Otata Island. Indeed, environmental conditions, such as water depth, temperature, food availability, are

known to play an important role in reproduction of bivalves (Barber & Blake 2006). Williams (2005) recorded a trend that *P. novaezelandiae* from shallow waters (Tiritiri Matangi Island, 5 m depth) tend to have higher fecundity than scallops from deeper waters (Jones Bay, 18 m). Given the high variability of nutritional quality of seston (due to changes in length of day, season of the year, or geographic locality), it is difficult to define a 'typical natural diet' for wild scallops. However, laboratory experiments in this study have clearly demonstrated that diet quantity and quality have a strong influence on scallop reproduction. Experiments using *Pecten fumatus* (Heasman *et al*. 1996) and *P. novaezelandiae* (Nebsit 1999) indicated that gonad condition is strongly affected by temperature, whereas gonad size varies more with diet quantity. Scallop beds with similar water depths were chosen in Chapter 2, and sea water temperature were similar between sites, thus, variations in gonad maturation/spawning time between populations recorded in here are unlikely to be influenced by water depth and/or seawater temperature. Food availability or other environmental factors (e.g. seabed habitat, water current and predation) are more likely to be responsible for variations in spawning timing and number of spawning events in the Hauraki Gulf.

Nutrient expense on reproduction

Interestingly in Chapter 2, nutrient content (gonad lipids, adductor muscle carbohydrate and digestive gland lipid) had high contribution in explaining the variance of scallop VGI. This is likely to indicate that VGI might be a good indicator for scallop nutrition condition. The classification of scallop reproductive stages based on

macroscopic and external feature, similar to VGI, was used as early as 1950s (Gibson 1956, Mason 1958). The practicability of VGI on P. novaezelandiae was confirmed by significant correlation between VGI and quantitative measurement of histology and gonad mass (William & Babcock 2004). Since scallop nutrient content is highly associated with gonad maturity, VGI also represented the nutritional state of the animal to a certain expense. Unfortunately, accessing the feasibility of VGI was not the main focus of the present study, but the potential of this non-destructive technique on estimate nutrition content for P. novaezelandiae should be further investigated.

Based on the one year monitoring of *P. novaezelandiae* nutrient content in relation to reproductive condition (Chapter 3), it was concluded that gametogenesis started at the expense of accumulated carbohydrates in adductor muscle in winter, when temperature and food availability is low. In spring and summer when temperature increase and food is abundant, energy assimilated from food to digestive gland is high, and the spring spawning is supported by the expense of digestive gland protein. Thereafter, gonad recovery is relying on the availability of digestive gland lipid contents. During autumn, when food availability decreases, assimilated food within the digestive gland is used for maintenance expenses, and excess energy is stored in adductor muscle.

The importance of stored carbohydrates to support gametogenesis is documented in various Pectinids and bivalves species (Barber & Blake 1981, Martinez *et al.* 2000a, Lodeiros *et al*. 2001). The utilization of carbohydrates for gametogenesis normally involves the conversion to lipid, in a manner similar to the glucose-fatty acid

cycle in vertebrates (Gabbott 1975, 1976). Gabbott (1976) suggested that it is due to the considerable advantage of storing lipids in eggs and larvae (condensed energy source, buoyancy), which outweigh the cost of converting glycogen to lipid. The existence of a functional Kreb's cycle in bivalves is generally accepted, as evidenced by the presence of the carboxylic acid intermediates and enzymes involved in the cycle (Gabbott 1976). An increase in glucose-6-phospate dehydrogenase activity associated with sexual development (Mori 1967) suggests operation of the pentose phosphate pathways which would provide NADPH for fatty acid synthesis. In addition, Bayne *et al*. (1982) have documented a decline in the volume of adipogranular (glycogen storing) cells in the mantle of mussel *M. edulis* during gametogenesis.

The use of protein in scallop reproduction involves breaking down of tissue cell to provide energy for metabolism, rather than gamete production (Barber & Blake 2006), thus supporting gametogenesis indirectly. Protein is laid down as new somatic tissue during growth periods, and is utilized over the gametogenic period, most commonly after other reserves have been depleted (Bayne 1976, Adachi 1979, Perez-Camacho *et al*. 2003).

Utilization of lipids, on the other hand, is achieved by direct transfer of stored and/or recently ingested fatty acids to developing ova in the gonad. Although the exact pathways of lipid transport among tissues is still controversial and awaits further research. Radiotracer experiments with *Crassostrea gigas* (Allen & Conley 1982, Caers *et al*. 2000) and the freshwater mollusc *Diplodon delodontus* (Huca *et al*. 1984, Pollero *et al*. 1985, Pollero & Heras 1989) illustrated the existence of intra-organ

hemolymphatic lipid transport via hemocytes and plasma. Furthermore, the transfer of lipids from the digestive gland to the maturing female gonad was also demonstrated in the scallops *Argopecten irradians concentricus* (Barber & Blake 1985) and *Chlamys hericia* (Vassallo 1973), using ^{14}C-labelling. Caers *et al*. (2003) also revealed the contribution of dietary fatty acid in accumulated lipids of maturing female gonad (via digestive gland) in Chilean scallop *Argopecten purpuratus*.

The nutrient expense of stored adductor muscle carbohydrates was shown in wild scallop (Chapter 3 & 4), as well as "experimental" animals (Chapter 4), and reinforced the importance of muscular carbohydrate reserves in reproduction of *P. novaezelandiae*. Reproduction is dependent to various degrees on energy reserves and available food levels in different temperate marine bivalves. In *Chlamys opercularis* (Taylor and Venn 1979), *Pecten maximus* (Comely 1974) and *Placopecten magellanicus* (Robinson *et al*. 1981) for example, energy reserves were utilized for both the initiation of gametogenesis and gonadal growth. Reserves were also apparently required for both these activities in the intertidal mussel, *Mytilus edulis* (Gabbott 1975). In other bivalves, however, intake of food is necessary for vitellogenesis and gonadal growth and often for the initiation of gametogenesis. Gonadal growth in *A. irradians* cannot occur without feeding, since reserve material from the digestive gland and other body tissues are not adequate to sustain maturation (Sastry 1966, 1968, 1970). In the Chapter 3 and 4, the initiation of gametogenesis was shown to occur at the expenses of adductor muscle carbohydrates in wild *P. novaezelandiae*, and this reserved nutrient was also utilized for gonadal maturation in both wild and laboratory animals.

Although various spawning times of *P. novaezelandiae* were recorded by several studies, most studies (if not all) have corresponded well with the pre-Christmas spawning of this species (Bull 1976, Nicholson 1978, Morrison 1999, Williams 2005). It is possible that because this main spawning is supported by the utilization of accumulated nutrient (mostly adductor muscle carbohydrates) as suggested by this thesis, environmental condition plays a less important role in gametogenesis. Therefore the pre-Christmas (spring) spawning was generally consistent, for scallop populations in the Hauraki Gulf (Nicholson 1978, Morrison 1999, Williams 2005), and even in those that were 500 kilometres away (Marlborough Sounds) (Bull 1978). Nevertheless, gonad recovery relied on digestive gland nutrient, which is highly influenced by quantity and quality of food source, thus further spawning is unpredictable and can vary among populations.

Future of New Zealand scallop fisheries

"Where are they from"

The success of Southern scallop enhancement programme has proven the benefits in re-seeding wild scallop. If re-seeding is to be carried out in the North Island, consistent supply of scallop spat is necessary. Currently, much of the world's bivalve culturing, including New Zealand, relies on seed collection from wild sources (Chavez-Villalba *et al*. 2002), and the success of wild seeding collection is at the mercy of environmental, biological and anthropogenic factor that are beyond the aquaculturist's control (Lubet *et al*. 1991). Although Morrison (1999) has demonstrated that spat

collection in the Hauraki Gulf is achievable in commercial quantities (>300 spat/bag), the number of spat collected fluctuated largely among years. In the 3 years of spat collections, the author collected up to 3000 animals per bag in 1992, while the densities depleted to 10−100 spat in 1993. This suggests that spat collection from the wild may not be a reliable source. Environmental factors such as phytoplankton abundance (Chí & Chí 2001), phytoplankton type (Bricelj & MacQuarrie 2007), short-term weather severity (Strasser *et al*. 2001) and long-term climate changes (Philippart *et al*. 2003) all impact larval growth and survival and ultimately limit seed procurement from the wild. In order to overcome the many problems associated with wild seed collection, hatchery-reared spat is the next option.

The practice of bringing adult scallops into spawning condition in a hatchery environment (i.e. broodstock conditioning) is not a new technology, it has been practised in North America at least since 1930 (Loosanoff & Davis 1952). Physical and nutritional factors are manipulated to initiate gonad development and gametogenesis in order to control the maturation process and maximise the fecundity, making it possible for the production of viable offspring for commercial purposes at virtually any time of the year (Utting 1993). In broodstock conditioning, the composition of diet has been discussed as the main factor determining the reproductive capacity and on the quality of their gametes (Robinson 1992, Uriarte *et al*. 2004). The present study (Chapter 4) on *P. novaezelandiae* has indicated that a mixed microalgal diet was not sufficient to promote gonad development, reflected on the depleted gonad PUFA levels in scallop kept in laboratory environment for 1 month. Therefore, a clear next

step in scallop fisheries is to enhance the knowledge on the requirement for conditioning this species.

"What would they eat"

Providing appropriate diet is the key to successfully bringing bivalves into spawning condition in a hatchery (broodstock conditioning). While formulated microalgal diet shown to be insufficient for *P. novaezelandiae*, culturing wild seston from the scallops' natural habitat could be an alternative from relying on artificial diet. Success in identifying the 'magic' ingredient in the wild seston will be a key to optimizing the culturing condition for broodstock conditioning. The result from this study suggested that further investigation on nutrition quality of artificial diet compare to wild seston might be carried to identify appropriate diet for *P. novaezelandiae*.

In addition, according to Helm *et al.* (1991), the survival of larvae of *Ostrea edulis* was related to the lipid content of the larvae at the time of release. Other authors have also reported lipids playing a significant role in the development of gonad, larvae and juveniles (Gallager & Mann 1986, Robinson 1992, Coutteau *et al*. 1996). Recent research has emphasized the importance of lipids or fatty acids supplement in the diet for broodstock scallops. Martinez *et al.* (2000a) demonstrated that a 30% substitution of emulsions enriched with n-3 PUFA into the microalgal diet during reproductive conditioning of *A. purpuratus* broodstock produced higher fecundity and better responses to spawning induction. In addition, the highest percentage of ripe *A. purpuratus* and highest larval survival rate occurred in individuals

fed with a diet of microalgae supplemented with lipids independent to water temperature (Navarro *et al*. 2000). Since PUFA (EPA in particular) levels were much lower in experimental *P. novaezelandiae* in the present study (Chapter 4), it is suggested that lipid supplements to a microalgal diet will be beneficial in culturing this species, and warrant further investigation.

Although dietary lipids play an important role in gonad maturation, gametogenesis of *P. novaezelandiae* occurred at the expense of stored carbohydrates in the adductor muscle, and this expense is even more dramatic in experimental animals. This may suggest the importance to improve nutrient reserve when conditioning this species. Gabbott (1975) reported that the gametogenic cycle of marine bivalves is coupled to synthesis of lipid during vitellogenesis at the expense of stored glycogen. Martinez *et al*. (2000a) recorded less depletion of muscular carbohydrates during gonad maturation when starch supplements were provided in broodstock diet for *A. purpuratus*. Results from this thesis have consistently shown evidence of the utilization of muscular carbohydrate reserve to support the reproduction of *P. novaezelandiae*. Therefore, in addition to lipids supplement, it is suggested that starch supplement may also be useful in culturing this species, and recommend further investigation.

“Where would they live”

Chapter 2 recorded variation in timing of spawning/gonad maturation between populations. This reinforced the notion that environmental conditions play an

important role in *P. novaezelandiae*'s reproduction. Williams (2005) recorded greater fecundities and more spawnings at Pembles Island and Tiritiri Matangi Island (up to 6 events per year) than Challenger Island and Jones Bays (2−3 events per year), suggesting that gonad redevelopment (expect pre-Christmas spawning) depends on the suitability of local environmental condition. Thus, it is necessary to take environmental conditions into consideration when selecting reseeding location for *P. novaezelandiae*. Unfortunately this thesis did not monitor multiple scallop populations to access the optimal location for potential scallop reseeding in the Hauraki Gulf. The importance in choosing areas that promote fecundities (e.g. Pemble Island and Tiritiri Mantangi Island) is stressed, and it is suggested that further investigation is conducted to identify the main environmental conditions that encourage gonad development for this species.

Predictive models have been recently recognised as an important tool to understand and assess the potential interactions of marine ecosystems (Austin 1998, 2002, Guisan & Zimmermann 2000). A wide variety of statistical and machine-learning methods have been introduced, often in conjunction with geographic information systems (GIS) and remote-sensing (Pereira & Itami 1991, Carroll *et al*. 1999, Mortensen *et al*. 2009). The vision of this thesis is that when future investigations are to be carried out to identify the key environmental parameter(s) that affect the reproduction of *P. novaezelandiae*, GIS modelling (cooperating the interaction of environmental parameters and reproductive development of *P. novaezelandiae*) will be a great tool to predict optimum reseeding site for this species.

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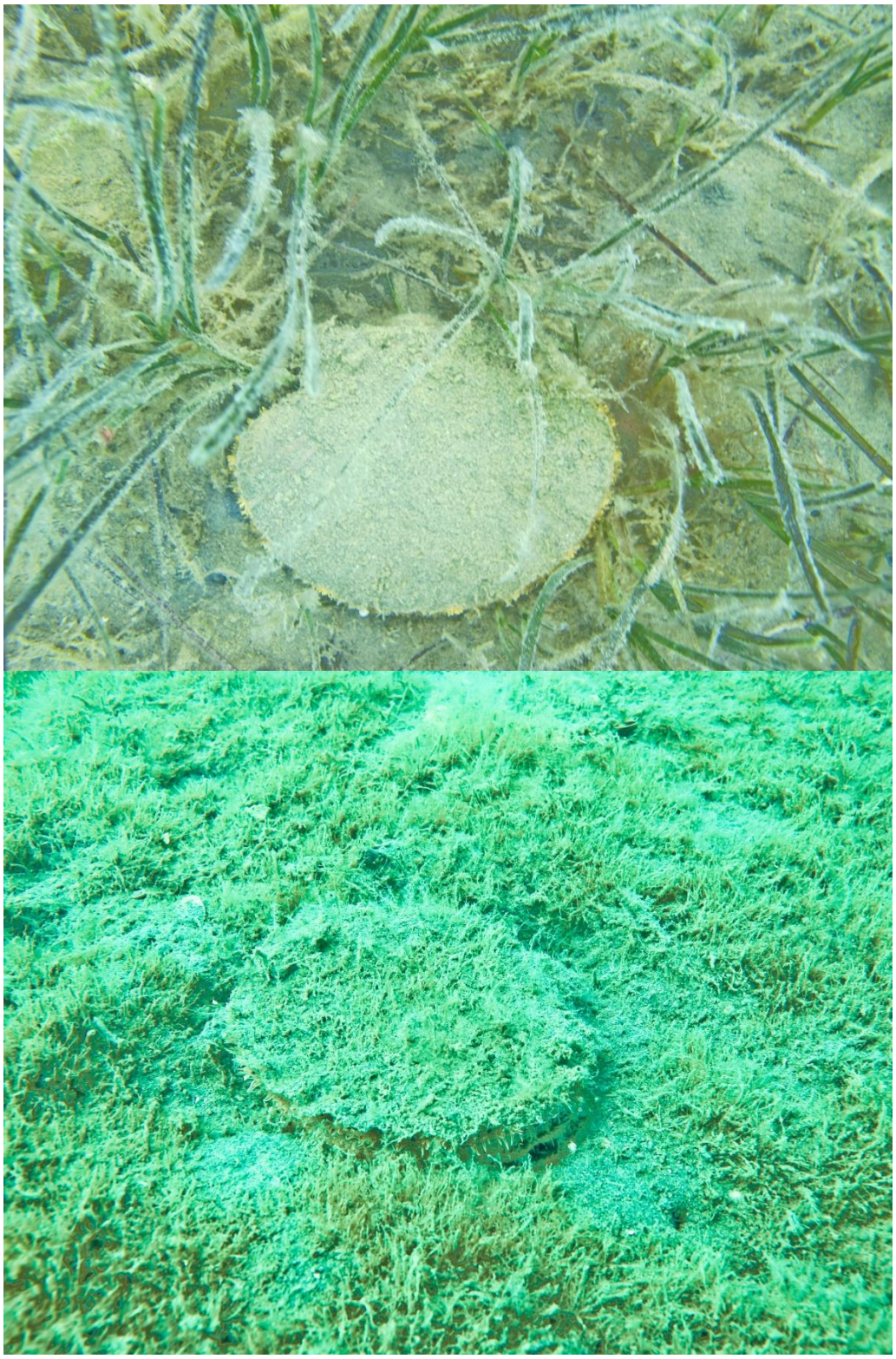
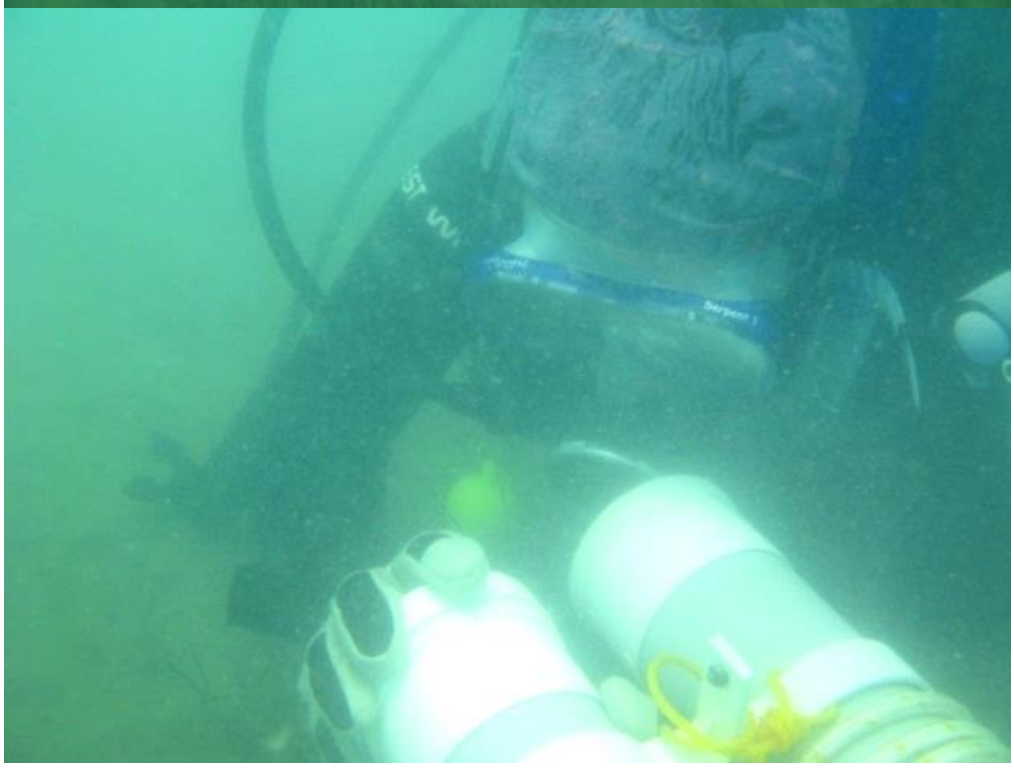
Appendices

Appendix I Site 1: seagrass habitat (Top); Site 2: muddy-sand habitat (Bottom).



Appendix II Bottom water sample collection.



Appendix III Gonad appearance, visual grade index (VGI), gonad index (GI), gonad wet weight, scallop shell length, collection site and date of each gonadal section photomicrograph presented in Figure 3-6.

Stage I

VGI: 1
Gonad Index: 4.49
Gonad wet weight: 0.89g
Shell length: 99mm
Collected at: Site 1,
May 2012

Stage II

VGI: 2
Gonad Index: 5.53
Gonad wet weight: 0.92g
Shell length: 99mm
Collected at: Site 1,
July 2012

Stage III

VGI: 5
Gonad Index: 6.51
Gonad wet weight: 1.97g
Shell length: 98mm
Collected at: Site 1,
August 2012

Stage IV

VGI: 8
Gonad Index: 18.54
Gonad wet weight: 7.43g
Shell length: 108mm
Collected at: Site 1,
October 2012

Stage V:

VGI: 6
Gonad Index: 14.27
Gonad wet weight: 3.34g
Shell length: 89mm
Collected at: Site 1,
November 2012

Appendix IV Seasonal variations of fatty acids (w/w% of fatty acids) of *P. novaezealandiae*: female gonad, male gonad, adductor muscle, digestive gland

	Site 1																					
Seston (> 5µm)	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13		
C14:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	9.48	±	1.18	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C16:0	38.30	±	1.28	37.56	±	1.68	33.26	±	2.37	34.32	±	0.01	29.93	±	6.59	43.05	±	0.96	39.20	±	3.94
C17:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C18:0	8.51	±	1.42	8.14	±	1.61	7.33	±	0.27	6.97	±	0.90	6.81	±	0.54	6.91	±	1.07	8.96	±	0.90
C24:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
∑Saturated	46.81	±	2.70	45.70	±	3.29	40.59	±	2.64	50.77	±	2.09	36.74	±	7.13	49.96	±	2.03	48.16	±	4.83
C16:1	26.47	±	3.20	29.47	±	3.56	28.34	±	0.64	19.98	±	0.61	29.49	±	0.56	21.52	±	0.95	22.27	±	4.96
C18:1n9c	3.20	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	5.18	±	1.18	4.50	±	0.50	6.65	±	0.49
C20:1n9	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
∑Monoenoics	29.67	±	3.20	29.47	±	3.56	28.34	±	0.64	19.98	±	0.61	34.67	±	1.74	26.02	±	1.46	28.93	±	5.45
C18:2n6c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00
C18:3n6	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	1.10	±	1.10
C20:2	7.44	±	0.78	11.31	±	1.08	10.99	±	0.67	7.69	±	0.69	12.61	±	0.45	8.27	±	0.71	5.75	±	2.85
C20:4n6	8.46	±	0.22	8.76	±	1.42	11.16	±	0.31	6.94	±	0.50	9.94	±	2.40	8.16	±	0.18	7.63	±	1.39
C20:5n3	6.06	±	0.06	3.06	±	3.06	6.88	±	0.47	12.51	±	1.19	3.25	±	3.25	6.50	±	0.52	6.97	±	0.87
C22:6n3	1.56	±	0.14	1.71	±	0.14	2.05	±	0.55	2.18	±	0.30	2.82	±	0.20	1.14	±	0.30	1.45	±	0.15
∑Polyenoics	23.52	±	1.20	24.84	±	5.70	31.08	±	2.00	29.33	±	2.67	28.61	±	6.30	24.06	±	1.71	22.91	±	6.36

	Site 2																							
Seston (> 5µm)	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13					
C14:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	5.10	±	0.34	4.82	±	0.82	4.68	±	1.63	0.00	±	0.00			
C16:0	37.52	±	2.58	39.81	±	5.00	39.60	±	4.63	33.45	±	3.50	35.29	±	0.69	44.00	±	0.81	34.96	±	2.53			
C17:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	1.11	±	1.11	0.91	±	0.91			
C18:0	4.83	±	1.26	2.88	±	2.88	8.94	±	0.52	6.68	±	0.99	5.82	±	0.70	7.31	±	2.54	7.80	±	1.28			
C24:0	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00			
∑Saturated	42.35	±	3.84	42.69	±	7.89	48.54	±	5.15	45.23	±	4.83	45.93	±	2.21	57.10	±	6.08	43.67	±	4.72			
C16:1	26.59	±	2.54	27.87	±	4.37	31.54	±	3.24	29.60	±	3.24	23.66	±	2.11	23.80	±	3.78	26.23	±	3.27			
C18:1n9c	2.67	±	0.00	2.42	±	2.42	0.00	±	0.00	2.64	±	2.64	2.19	±	2.19	0.00	±	0.00	2.96	±	2.96			
C20:1n9	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	1.12	±	0.00	0.00	±	0.00			
∑Monoenoics	29.26	±	2.54	30.30	±	6.80	31.54	±	3.24	32.24	±	5.88	25.85	±	4.31	24.92	±	3.78	29.20	±	6.23			
C18:2n6c	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	1.17	±	1.17	0.00	±	0.00			
C18:3n6	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	1.27	±	1.27	1.35	±	1.35			
C20:2	12.68	±	0.98	14.54	±	0.16	11.47	±	0.07	10.26	±	7.53	15.58	±	0.54	5.21	±	1.98	10.22	±	0.37			
C20:4n6	7.49	±	1.52	8.13	±	0.58	7.71	±	0.79	7.12	±	1.44	7.14	±	1.71	3.14	±	1.31	5.53	±	3.58			
C20:5n3	6.33	±	0.40	2.94	±	2.94	0.00	±	0.00	2.74	±	2.74	3.54	±	1.47	6.09	±	0.02	8.38	±	0.58			
C22:6n3	1.89	±	0.19	1.40	±	0.40	0.75	±	0.15	2.40	±	0.20	1.95	±	0.35	1.10	±	0.20	1.65	±	0.15			
∑Polyenoics	28.39	±	3.09	27.01	±	4.07	19.92	±	1.01	22.52	±	####	28.22	±	4.07	17.99	±	5.95	27.13	±	6.04			

	Site 1								Site 2							
Female gonad	Oct-12			Nov-12			Jan-13			Oct-12			Nov-12			Jan-13	
C14:0	1.32	±	0.13	3.09	±	0.25	4.02	±	0.08	4.99	±	0.14	3.68	±	0.22	3.76	±	0.08
C16:0	34.28	±	0.49	25.40	±	0.46	23.87	±	0.31	22.67	±	0.18	24.41	±	0.43	23.43	±	0.37
C17:0	2.83	±	0.09	1.77	±	0.06	1.61	±	0.03	1.41	±	0.03	1.56	±	0.04	1.63	±	0.04
C18:0	1.87	±	0.03	8.18	±	0.16	8.31	±	0.14	6.73	±	0.30	7.51	±	0.23	8.64	±	0.38
C24:0	0.85	±	0.02	0.99	±	0.04	0.94	±	0.02	0.75	±	0.05	0.90	±	0.05	1.08	±	0.07
∑Saturated	41.14	±	0.76	39.44	±	0.97	38.75	±	0.58	36.55	±	0.70	38.06	±	0.97	38.55	±	0.93
C16:1	8.91	±	0.13	6.27	±	0.45	8.93	±	0.22	13.23	±	0.55	7.89	±	0.71	7.11	±	0.90
C18:1n9c	2.81	±	0.25	3.03	±	0.18	2.51	±	0.10	2.22	±	0.05	2.96	±	0.10	2.59	±	0.12
C20:1n9	2.19	±	0.15	1.97	±	0.23	1.35	±	0.04	1.11	±	0.04	1.41	±	0.04	1.29	±	0.16
∑Monoenoics	13.90	±	0.53	11.27	±	0.87	12.79	±	0.36	16.56	±	0.65	12.26	±	0.85	10.99	±	1.18
C18:2n6c	1.79	±	0.11	1.47	±	0.09	1.45	±	0.03	1.40	±	0.03	1.57	±	0.05	1.28	±	0.09
C18:3n6	1.29	±	0.08	2.54	±	0.15	1.84	±	0.12	1.67	±	0.06	2.60	±	0.14	1.90	±	0.15
C20:2	1.99	±	0.08	0.75	±	0.09	0.51	±	0.02	0.41	±	0.01	0.54	±	0.03	0.53	±	0.02
C20:4n6	6.43	±	0.25	2.21	±	0.07	2.53	±	0.07	2.65	±	0.07	2.23	±	0.07	2.61	±	0.13
C20:5n3	26.84	±	0.50	22.52	±	0.47	22.98	±	0.25	24.63	±	0.31	23.97	±	0.34	22.60	±	0.38
C22:6n3	6.61	±	0.15	19.80	±	0.77	19.16	±	0.25	16.13	±	0.57	18.77	±	0.77	21.54	±	0.67
∑Polyenoics	44.95	±	1.16	49.29	±	1.65	48.46	±	0.74	46.89	±	1.04	49.68	±	1.39	50.46	±	1.45

	Site 1						Site 2					
Male gonad	Oct-12	Nov-12		Jan-13			Oct-12	Nov-12		Jan-13		
C14:0	5.07 ± 0.32	1.504 ± 0.21		1.867 ± 0.07			1.99 ± 0.07	1.234 ± 0.07		1.832 ± 0.08		
C16:0	26.03 ± 0.55	22.64 ± 0.34		21.57 ± 0.18			21.11 ± 0.31	21.53 ± 0.24		20.7 ± 0.32		
C17:0	1.41 ± 0.05	1.594 ± 0.04		1.439 ± 0.03			1.56 ± 0.04	1.387 ± 0.04		1.527 ± 0.05		
C18:0	7.54 ± 0.34	9.719 ± 0.37		10.48 ± 0.17			10.78 ± 0.31	9.168 ± 0.21		10.6 ± 0.29		
C24:0	0.46 ± 0.07	1.206 ± 0.07		1.533 ± 0.14			1.34 ± 0.11	1.169 ± 0.05		1.66 ± 0.09		
∑Saturated	40.50 ± 1.33	36.66 ± 1.04		36.89 ± 0.58			36.78 ± 0.84	34.49 ± 0.62		36.33 ± 0.83		
C16:1	12.71 ± 0.74	3.393 ± 0.39		4.893 ± 0.15			5.87 ± 0.21	3.057 ± 0.14		4.223 ± 0.58		
C18:1n9c	2.09 ± 0.03	1.927 ± 0.15		2.051 ± 0.05			2.08 ± 0.11	1.851 ± 0.06		2.162 ± 0.08		
C20:1n9	1.34 ± 0.21	3.426 ± 0.25		2.59 ± 0.12			2.57 ± 0.1	3.648 ± 0.09		2.704 ± 0.13		
∑Monoenoics	16.14 ± 0.99	8.75 ± 0.79		9.53 ± 0.32			10.53 ± 0.41	8.56 ± 0.29		9.09 ± 0.79		
C18:2n6c	1.49 ± 0.07	0.927 ± 0.1		1.125 ± 0.04			1.06 ± 0.03	0.911 ± 0.03		1.134 ± 0.04		
C18:3n6	1.50 ± 0.07	1.507 ± 0.18		1.086 ± 0.05			1.14 ± 0.07	1.416 ± 0.04		1.16 ± 0.13		
C20:2	0.45 ± 0.06	1.167 ± 0.07		0.753 ± 0.05			0.66 ± 0.02	1.193 ± 0.05		0.732 ± 0.05		
C20:4n6	1.22 ± 0.27	2.919 ± 0.13		3.549 ± 0.09			4.14 ± 0.24	3.057 ± 0.08		3.679 ± 0.13		
C20:5n3	22.98 ± 0.63	24.03 ± 0.42		23.55 ± 0.32			24.65 ± 0.68	25.18 ± 0.36		22.21 ± 0.46		
C22:6n3	15.73 ± 0.41	24.04 ± 0.64		23.51 ± 0.26			21.05 ± 0.56	25.2 ± 0.54		25.67 ± 0.39		
∑Polyenoics	43.36 ± 1.51	54.59 ± 1.54		53.58 ± 0.81			52.69 ± 1.61	56.96 ± 1.11		54.58 ± 1.20		

	Site 1																	
Adductor Muscle	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13		
C14:0	1.58	±	0.09	0.80	±	0.02	0.92	±	0.06	9.25	±	1.23	5.43	±	1.17	1.17	±	0.10	1.28	±	0.09
C16:0	19.51	±	0.26	18.07	±	0.23	17.32	±	1.41	18.57	±	0.40	18.13	±	0.32	20.4	±	0.18	18.43	±	0.20
C17:0	1.78	±	0.05	1.47	±	0.04	1.62	±	0.05	1.36	±	0.06	1.43	±	0.05	1.64	±	0.05	1.08	±	0.04
C18:0	10.33	±	0.15	10.27	±	0.19	10.59	±	0.21	9.05	±	0.11	9.59	±	0.18	10.7	±	0.12	10.31	±	0.14
C24:0	1.45	±	0.02	1.52	±	0.04	1.13	±	0.06	1.21	±	0.04	1.28	±	0.03	1.29	±	0.05	1.35	±	0.03
∑Saturated	34.65	±	0.56	32.13	±	0.52	31.59	±	1.79	39.45	±	1.84	35.86	±	1.75	35.20	±	0.50	32.45	±	0.43
C16:1	1.02	±	0.02	0.82	±	0.05	1.15	±	0.11	0.96	±	0.05	1.07	±	0.05	1.34	±	0.08	1.34	±	0.08
C18:1n9c	1.88	±	0.10	1.96	±	0.06	2.04	±	0.05	1.79	±	0.05	1.76	±	0.06	1.79	±	0.06	1.85	±	0.05
C20:1n9	1.32	±	0.05	1.45	±	0.09	1.36	±	0.07	1.14	±	0.07	1.14	±	0.08	1.41	±	0.11	1.86	±	0.04
∑Monoenoics	4.22	±	0.17	4.23	±	0.20	4.55	±	0.23	3.89	±	0.18	3.97	±	0.19	4.54	±	0.25	5.05	±	0.17
C18:2n6c	0.87	±	0.01	0.97	±	0.03	1.01	±	0.03	0.80	±	0.03	0.88	±	0.03	0.81	±	0.03	0.83	±	0.04
C18:3n6	0.82	±	0.01	1.08	±	0.07	1.09	±	0.05	0.82	±	0.05	0.82	±	0.06	0.64	±	0.02	0.86	±	0.08
C20:2	0.46	±	0.02	0.51	±	0.03	0.49	±	0.02	0.43	±	0.01	0.47	±	0.01	0.53	±	0.01	0.49	±	0.02
C20:4n6	3.38	±	0.07	3.52	±	0.15	3.65	±	0.12	3.07	±	0.10	3.37	±	0.11	3.79	±	0.09	3.58	±	0.06
C20:5n3	17.64	±	0.15	18.43	±	0.15	19.60	±	0.46	17.64	±	0.27	18.79	±	0.22	17	±	0.22	17.84	±	0.18
C22:6n3	37.95	±	0.38	39.12	±	0.23	38.02	±	0.85	33.92	±	0.58	35.83	±	0.53	37.4	±	0.30	38.95	±	0.40
∑Polyenoics	61.13	±	0.64	63.64	±	0.66	63.87	±	1.54	56.66	±	1.03	60.17	±	0.96	60.26	±	0.68	62.55	±	0.78

	Site 2																	
Adductor Muscle	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13		
C14:0	1.50	±	0.03	1.41	±	0.02	0.83	±	0.04	0.7	±	0.06	0.71	±	0.05	1.06	±	0.09	1.34	±	0.02
C16:0	19.12	±	0.21	18.1	±	0.28	18.9	±	0.19	19	±	0.36	18.3	±	0.13	19.8	±	0.27	19.32	±	0.22
C17:0	1.62	±	0.02	1.32	±	0.03	1.49	±	0.05	1.49	±	0.08	1.45	±	0.04	1.42	±	0.05	1.49	±	0.04
C18:0	10.74	±	0.20	10	±	0.16	9.51	±	0.12	9.06	±	0.21	10	±	0.14	10.7	±	0.22	10.22	±	0.15
C24:0	1.39	±	0.06	1.69	±	0.06	1.15	±	0.06	1.38	±	0.08	1.02	±	0.07	1.24	±	0.06	1.58	±	0.07
∑Saturated	34.37	±	0.53	32.61	±	0.55	31.90	±	0.46	31.67	±	0.79	31.47	±	0.43	34.26	±	0.69	33.95	±	0.50
C16:1	0.96	±	0.01	0.93	±	0.05	0.9	±	0.04	0.99	±	0.05	0.95	±	0.06	1.42	±	0.08	1.24	±	0.03
C18:1n9c	1.74	±	0.06	2.07	±	0.07	1.91	±	0.04	1.78	±	0.04	1.88	±	0.08	1.78	±	0.06	1.76	±	0.08
C20:1n9	1.43	±	0.04	1.57	±	0.12	1.47	±	0.10	1.21	±	0.11	1.33	±	0.08	1.69	±	0.14	1.58	±	0.09
∑Monoenoics	4.12	±	0.12	4.57	±	0.24	4.28	±	0.18	3.98	±	0.20	4.16	±	0.22	4.89	±	0.28	4.58	±	0.20
C18:2n6c	0.82	±	0.03	0.96	±	0.03	0.95	±	0.02	0.88	±	0.02	0.84	±	0.04	0.74	±	0.04	0.85	±	0.04
C18:3n6	0.98	±	0.04	1.15	±	0.05	1.09	±	0.04	0.97	±	0.04	0.78	±	0.05	0.84	±	0.06	0.80	±	0.03
C20:2	0.39	±	0.01	0.46	±	0.02	0.53	±	0.02	0.47	±	0.03	0.53	±	0.01	0.56	±	0.04	0.58	±	0.02
C20:4n6	2.76	±	0.08	3.23	±	0.13	3.4	±	0.12	3.17	±	0.16	3.66	±	0.08	3.51	±	0.15	3.43	±	0.10
C20:5n3	16.77	±	0.20	18.1	±	0.16	18.6	±	0.15	18.7	±	0.19	19.4	±	0.16	16.7	±	0.26	17.23	±	0.02
C22:6n3	39.80	±	0.30	38.9	±	0.31	39.3	±	0.22	40.2	±	0.30	39.1	±	0.27	38.5	±	0.46	38.57	±	0.29
∑Polyenoics	61.51	±	0.65	62.82	±	0.70	63.82	±	0.56	64.35	±	0.74	64.37	±	0.61	60.85	±	1.02	61.46	±	0.50

	Site 1																	
Digestive gland	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13		
C14:0	3.98	±	0.25	4.31	±	0.20	5.18	±	0.35	7.57	±	0.34	6.01	±	0.39	7.15	±	0.32	3.00	±	0.30
C16:0	31.01	±	1.33	29.42	±	1.06	30.26	±	1.59	32.37	±	1.38	25.42	±	0.78	25.19	±	0.44	21.90	±	0.78
C17:0	2.45	±	0.14	2.22	±	0.05	1.99	±	0.08	1.94	±	0.04	1.65	±	0.06	1.88	±	0.04	2.38	±	0.08
C18:0	18.83	±	0.90	15.46	±	0.32	11.67	±	0.49	14.67	±	0.31	11.97	±	0.55	12.80	±	0.36	14.33	±	0.48
C24:0	1.27	±	0.09	1.93	±	0.11	1.18	±	0.09	1.14	±	0.08	1.03	±	0.06	1.26	±	0.06	2.05	±	0.16
∑Saturated	57.54	±	2.70	53.34	±	1.74	50.29	±	2.62	57.69	±	2.15	46.08	±	1.84	48.29	±	1.22	43.66	±	1.79
C16:1	2.74	±	0.23	5.80	±	0.23	5.63	±	0.35	10.16	±	0.35	11.41	±	0.80	12.16	±	0.71	3.41	±	0.43
C18:1n9c	4.50	±	1.10	3.42	±	0.21	4.10	±	0.19	3.17	±	0.14	3.23	±	0.19	2.72	±	0.13	3.09	±	0.13
C20:1n9	2.08	±	0.16	1.92	±	0.05	1.80	±	0.06	1.70	±	0.05	1.59	±	0.09	1.87	±	0.11	2.60	±	0.07
∑Monoenoics	9.32	±	1.50	11.13	±	0.49	11.53	±	0.60	15.03	±	0.54	16.23	±	1.09	16.75	±	0.95	9.09	±	0.62
C18:2n6c	1.48	±	0.08	1.44	±	0.10	2.00	±	0.13	1.11	±	0.05	1.63	±	0.11	1.40	±	0.07	1.31	±	0.10
C18:3n6	2.66	±	0.18	2.40	±	0.23	3.65	±	0.27	1.37	±	0.10	1.88	±	0.21	1.68	±	0.12	1.92	±	0.15
C20:2	1.22	±	0.08	0.86	±	0.03	0.73	±	0.04	0.72	±	0.04	0.63	±	0.04	0.50	±	0.04	0.61	±	0.03
C20:4n6	2.33	±	0.23	2.34	±	0.20	1.97	±	0.20	1.68	±	0.15	2.11	±	0.16	2.95	±	0.15	4.98	±	0.40
C20:5n3	10.09	±	0.97	13.37	±	0.65	13.59	±	0.92	12.96	±	0.82	17.66	±	0.69	15.68	±	0.46	14.16	±	0.24
C22:6n3	15.36	±	1.72	15.12	±	0.99	16.24	±	1.42	9.45	±	0.68	13.77	±	1.84	12.75	±	0.85	24.28	±	0.87
∑Polyenoics	33.14	±	3.26	35.53	±	2.20	38.18	±	2.98	27.29	±	1.84	37.69	±	3.04	34.96	±	1.70	47.25	±	1.80

	Site 2																							
Digestive gland	May-12			Jul-12			Aug-12			Oct-12			Nov-12			Jan-13			Mar-13					
C14:0	4.02	±	0.25	5.01	±	0.33	5.72	±	0.34	7.68	±	0.40	5.46	±	0.35	5.61	±	0.25	2.86	±	0.15			
C16:0	34.46	±	1.46	35.50	±	2.07	33.17	±	1.55	30.54	±	1.19	23.03	±	0.95	22.21	±	0.39	21.06	±	0.48			
C17:0	2.67	±	0.10	2.33	±	0.08	2.04	±	0.07	1.72	±	0.02	1.54	±	0.05	1.61	±	0.05	2.25	±	0.06			
C18:0	18.60	±	0.43	14.35	±	0.42	12.65	±	0.46	13.70	±	0.34	11.90	±	0.45	12.04	±	0.50	13.76	±	0.30			
C24:0	2.60	±	0.15	1.25	±	0.12	1.14	±	0.11	1.17	±	0.08	1.16	±	0.08	1.25	±	0.10	2.24	±	0.12			
∑Saturated	62.35	±	2.39	58.45	±	3.02	54.73	±	2.53	54.80	±	2.04	43.09	±	1.88	42.73	±	1.29	42.17	±	1.11			
C16:1	3.59	±	0.16	6.00	±	0.30	5.73	±	0.27	10.88	±	0.53	9.36	±	0.69	10.20	±	0.70	2.67	±	0.14			
C18:1n9c	4.66	±	0.18	4.12	±	0.23	4.33	±	0.17	3.25	±	0.16	2.99	±	0.26	2.48	±	0.20	2.84	±	0.10			
C20:1n9	2.27	±	0.10	1.67	±	0.07	1.78	±	0.05	1.47	±	0.06	1.64	±	0.08	1.57	±	0.12	2.39	±	0.07			
∑Monoenoics	10.52	±	0.45	11.79	±	0.60	11.83	±	0.48	15.60	±	0.75	13.99	±	1.04	14.25	±	1.02	7.91	±	0.30			
C18:2n6c	1.56	±	0.06	1.50	±	0.12	1.83	±	0.12	1.25	±	0.06	1.55	±	0.09	1.28	±	0.12	1.15	±	0.07			
C18:3n6	2.02	±	0.12	2.48	±	0.25	3.29	±	0.27	1.57	±	0.15	1.77	±	0.17	1.52	±	0.14	1.80	±	0.11			
C20:2	0.80	±	0.05	0.62	±	0.05	0.74	±	0.04	0.78	±	0.03	0.67	±	0.05	0.50	±	0.04	0.51	±	0.04			
C20:4n6	1.85	±	0.16	1.93	±	0.26	1.71	±	0.19	1.77	±	0.17	2.75	±	0.24	3.41	±	0.21	5.11	±	0.23			
C20:5n3	8.43	±	0.65	10.75	±	0.95	11.81	±	0.70	14.02	±	0.77	19.35	±	1.03	19.39	±	0.42	14.90	±	0.13			
C22:6n3	12.47	±	1.23	12.48	±	1.34	14.05	±	1.06	10.21	±	0.81	16.83	±	1.17	16.92	±	0.89	26.45	±	0.53			
∑Polyenoics	27.13	±	2.28	29.76	±	2.96	33.43	±	2.39	29.60	±	1.98	42.92	±	2.73	43.03	±	1.82	49.92	±	1.11			

Appendix V Experimental setup in the aquaculture facility. Top: overview of the aquaculture facility. Bottom left: recirculation set up for scallop rearing. Bottom right: Individual *P. novaezelandiae* in 4L black tank.

Appendix VI Gonad appearance, visual grade index (VGI), gonad index (GI), gonad wet weight and scallop shell length of each gonadal section photomicrograph presented in Figure 4-5.