Reproduction and larval development of the New Zealand scallop, *Pecten novaezelandiae*.

Neil E. de Jong

A thesis submitted to

Auckland University of Technology

in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

2013

School of Applied Science

Table of Contents

Table of Figures

Table of Tables

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:** 26/June/2013

Acknowledgments

Supervisors

I am very grateful to my supervisor Andrea C. Alfaro who made the thesis possible. For the time she has taken to ensure this project succeeded and her phenomenal support. Chris Pook who kept a watchful eye and supported me on technical matters.

Family

To my parents; my late father, Peter and Joan for their patience, humour and support. David, for you words of wisdom and Steven, for the warm meals.

Colleagues

I am very grateful for the wonderful support offered to me by the Aquaculture Biotechnology Group (ABG). A special thanks to Tim Young and Annapoorna Ganesan for their help. A special thanks to Henry Kaspar for you phenomenal insights, Aditya Kesarcodi-Watson and Achim Janke amongst the many people who aided me at Cawthron, Aquaculture Park.

Contributors

I am thankful to Liz and Graeme Clarke of Crail Bay Aquaculture Ltd. for initiating and funding the scallop experiments at Cawthron. The Ministry of Business, Innovation, and Employment, contract CAWX0802 (Adding Value to New Zealand's Cultured Shellfish Industry: Maximising Profit, Minimising Risk) contributed funds to the project.

A special thanks to the Marine Farmers Association for the "Andy Ritchie Scholarship".

I dedicate this thesis to my late father Peter.

His favourite quote of wisdom “They can never take away an education.”

Abstract

The New Zealand scallop *Pecten novaezelandiae* is an important fishery. Wild scallop spat are used extensively to enhance and maintain scallop beds. The current yields of scallops have decreased by over a third in the last decade. A potential solution to increasing the scallop yield and conserving stocks are hatchery grown spat. Most studies related to *P. novaezelandiae* have concentrated on their ecology and the process and effects of scallop enhancement. This study aimed to fill two gaps in our knowledge of *P. novaezelandiae* biology by exploring the effects of diet ration on the animal's gonad maturation and to characterise the larval development from zygote to pedivileger.

Wild scallops were collected and maintained in the laboratory for seven days and fed *ad libitum*. An initial sample of scallops was taken and reproductive condition was compared to that of scallops fed one of three algal diets for thirty days: The diet rations were 0.5g (low), 0.92g (medium) and 1.9g (high) of microalgae per day.

The results of the study showed there was a significant increase in the gonadalsomatic index (ANOVA; $F_3=49.3$; $p<0.05$; $n=91$), wet gonad weight (Steel, $p<0.05$), egg size (ANOVA, $F_3=2.8$; $p<0.05$; $n=3773$) and colour between the male and female portions of the gonad ($p<0.05$; $n=117$). However, colour, wet gonad weight and acini area were not significantly different after 30 days of conditioning.

Three lots of scallops were spawned to characterise the key phases of *P. novaezelandiae* from zygote to pedivileger. Two spawnings characterised *P. novaezelandiae* from gamete to D-larvae. The embryos were cultured in 900ml of fresh sea water, filtered to one micron for 3 days and sampled randomly. There was 100% mortality by day four. The third spawning saw fertilised eggs placed in a 170L conical tank for three days. The D-larvae were then transferred to six Cawthron Ultra Dense Larval rearing systems (CUDLs), from which larvae were selected every two days for imaging with Light Microscopy and Scanning Electron Microscopy.

A strong linear growth for P. novaezelandiae larvae diameter corresponded to 6.5895 × age in days + 74.337. A mean growth of 5.92 µm a day was experienced over 30 days for *P. novaezelandiae* larvae. A mean (±SE) clearance 65.4±2.59% of the Chlorophyll A was cleared per day over 30 days of feeding. P. novaezelandiae sperm morphology, 2.14 ±0.05 length and 61 ±0.07µm breadth, is similar to the *Pecten maximus.* Unknown micropores are visible on the nucleus of the sperm. *P. novaezelandiae* eggs are spawned with irregular shapes and experience meiosis in the first hour. A polar body emerges 15min after fertilisation. The embryogenesis of *P. novaezelandiae* is similar to other scallop bivalves comprising of a polar lobed, cleavage, cell division, blastula, gastrula and trochophore phases. D-larvae emerge after three days. However, an umbo veliger phase is not obvious and only emerges before around when an eyespot is visible around 23 days post fertilisation [PF]. Approximately 30 days PF a pedivileger emerges.

This is the first study in broodstock *P. novaezelandiae*, and which characterises key growth phases. It is also the first time semi quantitative colour measures have been conducted on the diet concentration of the gonad. Spawning was found to be a barrier to successfully spawning large quantities of scallops.

1 Chapter One: Introduction and literature review

1.1 Scallop Biology and Ecology

Scallops are distributed throughout the world, including in the northern to southern polar oceans, down to depths of 7,000m (Brand, 2006). Although there are over 400 known species in the Family Pectinidae, the most studied species are found inshore and are of commercial valued. In New Zealand, there are 18 scallop species recorded (Marsden & Bull, 2006). The New Zealand scallop, *Pecten novaezelandiae*, is found throughout the country and it is the primary scallop fishery (Marsden & Bull, 2006; Ministry of Fisheries Science Group, 2011).

The species, *Pecten novaezelandiae* belongs to the Phylum Mollusca, Class Bivalvia, Order Pectinoida, Family Pectinidea (Figure 1.1). This bivalve is asymmetrical in shape, with a convex right valve and a flat, or slightly concaved, left valve (Marsden & Bull, 2006). The shell has 17-22 ribs per valve and is coloured white, pink and brown (Marsden & Bull, 2006). The species inhabits subtidal areas, generally down to 60 m water depth throughout most of the country, but can reach down to 90 m in the Chatham Island (Bull, 1976). The New Zealand scallop inhabits substrates made up of sand, gravel and silt (Marsden & Bull, 2006; Morrison, 1999).

P. novaezelandiae are similar in morphology to the European king scallop, *Pecten.maximus* and the Australian, southern scallop, *Pecten fumatus* (Saavedra & Peña, 2004; Woodburn, 1998). Woodburn (1998) suggested that *P. fumatus* and *P. novaezelandiae* are separate species based on a few polymorphic enzymes. The life span of *P. novaezelandiae* has not been determined. The age of the king scallop, *P. maximus,* can be determined by the number of growth rings on it shell (Mason, 1958). *P. novaezelandiae* growth rings, however do not correspond to the scallops age (Bull, 1976).

It is estimated that the New Zealand scallop would live 6-7 years if left undisturbed in the wild (Marsden & Bull, 2006). Scallops are sexually mature at 65mm when measured ventral to dorsal (Williams & Babcock, 2005). Although scallops may mature within a year, they may not contribute to the cohort spawning of that year (Cryer, 2002; Mason, 1958). *P. novaezelandiae* is a hermaphrodite, containing male and female sexes within a single gonad. *P. novaezelandiae* is a broadcast

spawner, releasing its eggs and sperm into the water column for fertilisation. This species is highly fecund and can spawn several times a year (Williams & Babcock, 2004). Two main spawning events occur around October and mid-January in the Hauraki Gulf but may vary depending on environmental factors (Morrison, 1999; Williams & Babcock, 2004). About 15-50 million eggs are released per female and recruitment is highly variable with high mortalities (Cryer, 2002). Not all spawnings lead to successful spat settlement.

The lifecycle of *P. novaezelandiae* has not been fully described, but has been inferred through the development of other Pectinidae (Bull, 1976; Marsden & Bull, 2006; Nicholson, 1978). The eggs are fertilized in the water column. The embryos develop into trochophores and then veliger larvae after 48 h (Le Pennec, Paugam, & Le Pennec, 2003; Nicholson, 1978). After spending 3-4 weeks in the water column, the pedivileger or spat settle attach to filamentous material such as *Zostera* sp,. algae and hydroids, with their byssal threads (Bull, 1976; Mincher, 2008; Williams, 2005). They may also attach to artificial collectors such as synthetic rope and mesh (Food and Agriculture Organisation of the United Nations, 1987; Marsden & Bull, 2006). When the spat reach sizes around 5 mm, they release or break their byssal threads and the juvenile scallops settle on the sea floor. Juvenile scallops (2-9mm), may move long distances, by swimming or drifting (Marsden & Bull, 2006). Adult scallops are sedentary, and Morrison (1999) found that they seldom move more than 10m. Adult scallops can reach a diameter of 100 mm in 18 months in the Hauraki Gulf, North Island, whereas this size can take up to 2.5 years to achieve in the waters of New Zealand's South Island (Bull, 1976; Marsden & Bull, 2006; Morrison, 1999).

The New Zealand adult scallop can propel itself through the water by jettisoning water through their mantel to escape predators (Nicholson, 1978; Williams, 2005). Predators differ when the scallop is planktonic, free floating in the water, and when it is finally settled on the ocean floor. Most sea water filter feeders, such as mussels and oysters, consume planktonic scallop larvae. Once a scallop has settled on the seafloor, they are threatened by predators such as sea stars; such as *Astrostole scabra, Sclerasterias mollis, Astropecten polyacanthus* and *Costinasterias calamaria*; fish; such as snapper, *Chrysophrys auratus*, tarakihi, *Cheilodactylus macropterus,* bluecod, *Parapercis colias* Cephalopods; common octopus, *Octopus maorum*; and Elasmobranches; eagle rays ,*Myliobatus*

tenuicaudatus and skates to name a few (Bull, 1976; Talman, Norkko, Thrush, & Hewitt, 2004).

1.1.1 Diet

The scallop diet consists of particulate inorganic and organic matter from microalgae and detritus (Bull, 1976; Nesbit, 1999; Nicholson, 1978). Scallops may reposition their mantel them to make best use of the directional currents (Nicholson, 1978). They are filter feeders that capture particles in their mucus as water moves over the gills into densely ciliated troughs on the principle filaments (Beninger & Le Pennec, 2006). Particles are passed to the mouth via the ventral food groove (Beninger & Le Pennec, 2006; Nicholson, 1978). Some phytoplankton identified by Nicholson (1978) form the stomachs were *Peredinium* spp., *Ceratium* spp., *Pleurosigman* spp. *Cosinodiscus*, spp. P*hyzosolenia* spp and *Cocconeis* spp.

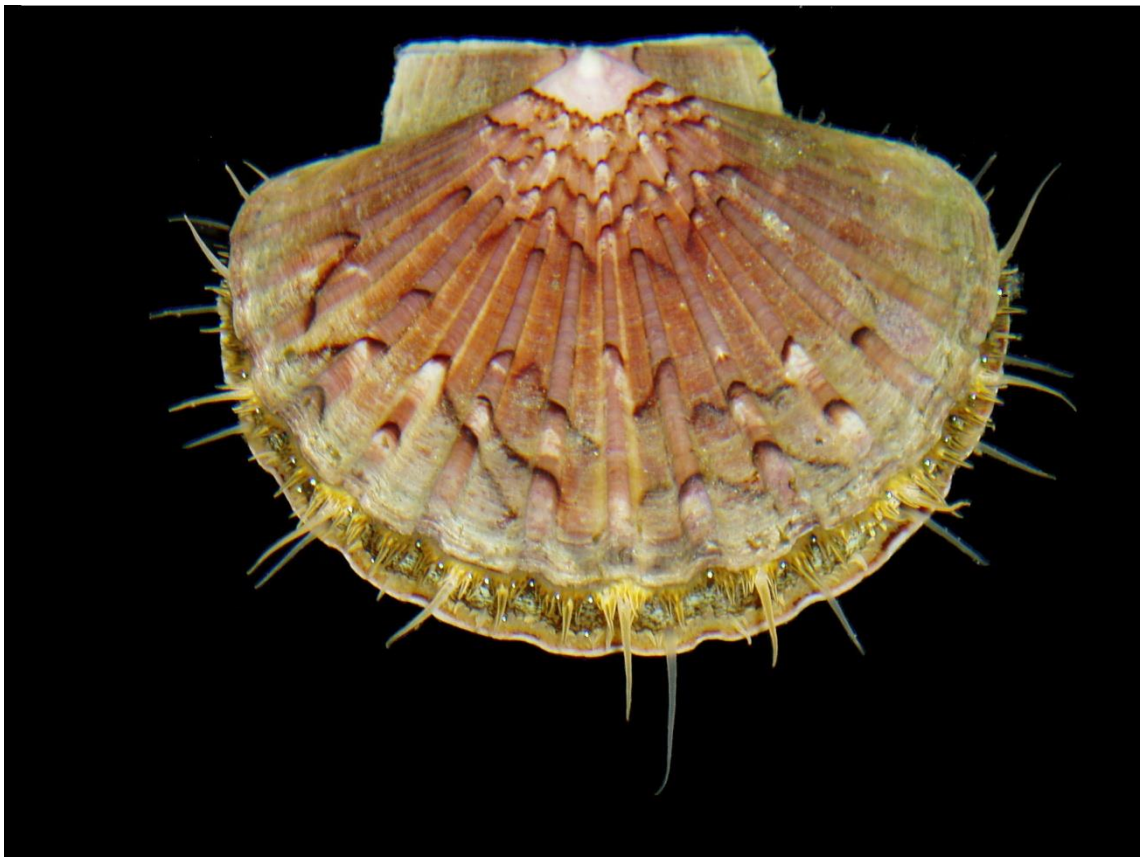


Figure 1.1. An adult *Pecten novaezelandiae* scallop, with its tentacles extend.

1.2 Fisheries and Aquaculture

Scallops are an important fishery in New Zealand. Only two scallop species have been commercially harvested in New Zealand, *Pecten novaezelandiae* and the Queen Scallop, *Chlamys delicatula*. Scallop yields are recorded in greenweight, that is the weight of marine organism before processing. *C. delicatula* was experimentally harvested with the aim of establishing a market. Consistently small yields were harvested with the maximum haul of 233 tons greenweight in 2001/2. Ultimately *C. delicaltula* has not developed into an established fishery (Marsden & Bull, 2006; Ministry of Fisheries Science Group, 2011).

Scallops were introduced to the Quota Management System [QMS] in 1996 under the *Fisheries Act* of 1996, and fully implemented in 2001 (Mincher, 2008). Provisions under the act allow for commercial, recreational and customary fishers. There are three main commercial fisheries (Figure 1.2) in New Zealand: 1- Northland, between Reef Point (Ahipara) and Cape Rodney; 2- Coromandel, between Tauranga and Cape Rodney and 3- Southland, Golden Bay, Tasman Bay, and the Marlborough Sounds, with some fishing on the Chatham Island and Steward Island (Bull, 1976; Cryer, 2002; Morrison, 1999). Scallops are commercially harvested by dredging. Dredging for scallops has two severe consequences: incidental mortalities of juvenile scallops and environmental degradation (Morrison, 1999; Talman et al., 2004).

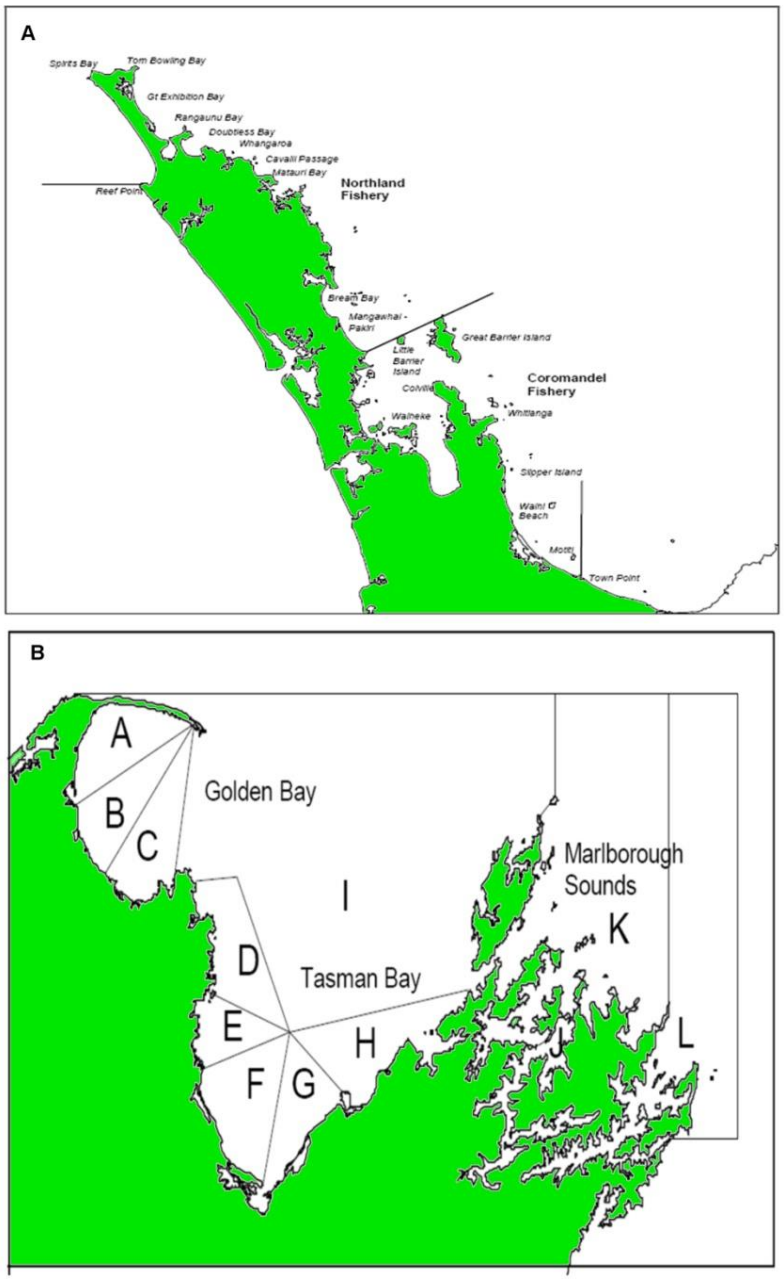


Figure 1.2. Map of North Island and South Island Scallop Fisheries;SCA 1, SCA2 (A) and SCA7(B) in New Zealand.

Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants (Food and Agriculture Organisation of the United Nations, 2013). Healthy scallop spat, from which healthy adults can develop, are the key to successful commercial scallop production. There are two methods of acquiring spat; collection of wild spat settled on to spat bags placed in the marine environment or spat spawned and grown in hatcheries (Food and Agriculture

Organisation of the United Nations, 1987; Hardy, 2006). These methods- extensive and intensive production, respectively- reflect the two contrasting approaches to marine farming. Once consistent culturing protocols are established, hatcheries breed the best traits of scallops from egg to spat, using broodstock conditioning to maximize the survival of larvae (Delaunay, Marty, Moal, & Samain, 1993; Helm, Bourne, & Lovatelli, 2004; Utting & Millican, 1997). When scallop larvae develop into spat they are transferred to farms where they can be grown out on long lines or in cages (Figure 1.3).

A preliminary economic assessment for scallop farming in New Zealand was conducted by the Ministry of Agriculture and Fisheries in 1983 (Cameron, 1983). The preliminary findings concluded that a hanging culture would not be economically viable. The intensive capital start-up costs and the frequent anti-fouling of nets from mussel spat fouling and other marine organisms would increase costs. Furthermore, purchasing hatchery reared spat was not a viable option in New Zealand. Scallop enhancement was trialled in the early 1980's in the southern fishery and yielded good results.

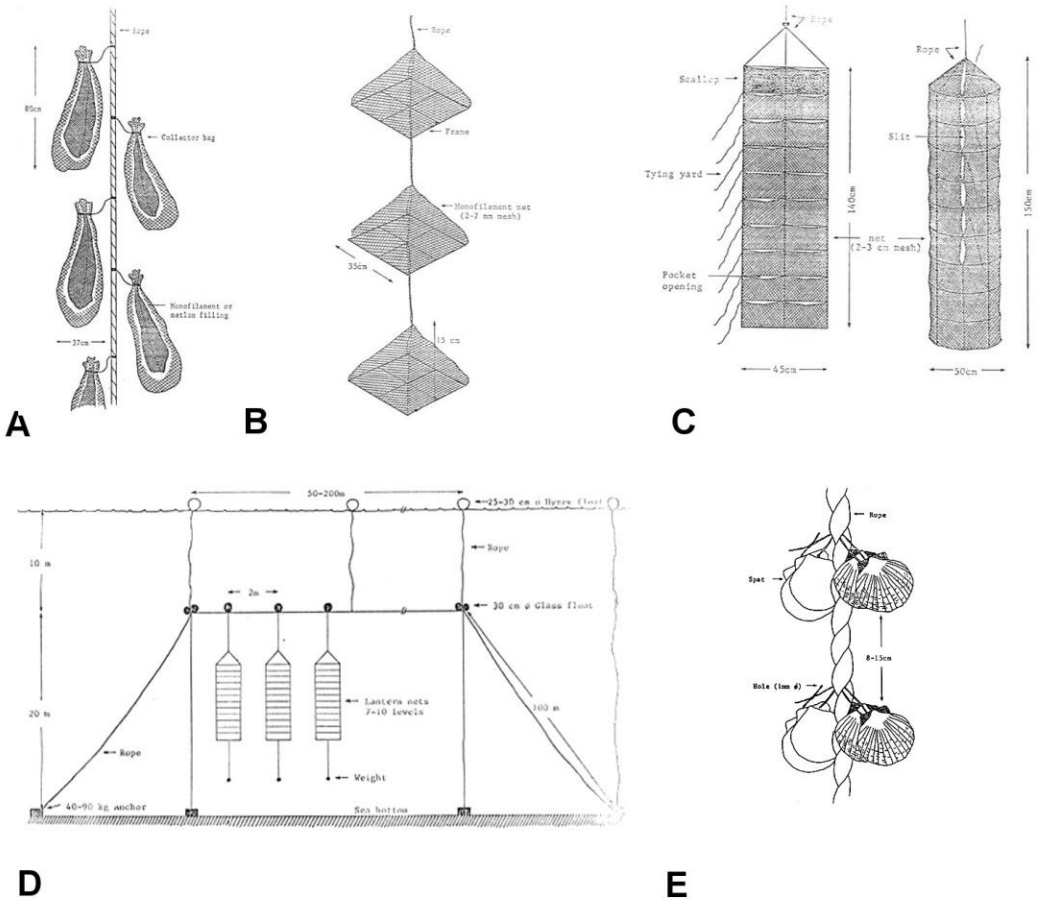


Figure 1.3. Images of scallop rearing equipment (Food and Agriculture Organisation of the United Nations, 1987)

A. Spat bags

B. Pearl lanterns nets

C. Pocket and lantern nets

D. Long line hanging culture

E. Ear hanging

1.2.1 Scallop Enhancement

Scallop enhancement or sea ranching is practiced in New Zealand by the collection of wild scallop spat that settle on to bags of plastic mesh placed in the sea water column. These are then transferred to existing scallop beds where they release from the bags to settle and grow naturally on the sea bed (Drummond,

2008; Mincher, 2008). Spat bags, with an inner and outer bag (Figure 1.3 A), are attached to a long line rope and buoy and moored in high areas of spat incidence. The outer bag has a smaller mesh size than the inner bag. Scallop spat attach themselves with a byssal thread on the inner and outer bags. When the scallop spat from the inner mesh release or break their byssal threads, they are trapped within the outer bag. The outer spat release and fall to the ocean floor. There are two spat capturing phases in the reseeding process: initial capture of spat by bag and secondary recruitment whereby collecting escaped spat that have settled on the ocean floor beneath the spat bags (Mincher, 2008). These spat are dredged and then relocated to the scallop beds.

Reseeding or scallop enhancement trials began in 1982 with the New Zealand Ministry of Agriculture in conjunction with the Overseas Fisheries Cooperation Foundation of Japan. Using 44,000 catch bags, 35 million spat were caught and released in the first year (Marsden & Bull, 2006). It was identified that over 20% of the spat would survive from the primary capture and that secondary spat attached to the outside of the spat bags would also fall to the seafloor. Secondary spat could be captured and further reseeding would take place on existing scallop beds (Marsden & Bull, 2006; Mincher, 2008). The enhancement program in the Southern fishery proved to be cost effective and yielded good biological results (Drummond, 2008; Marsden & Bull, 2006). Scallop Quota Holders Association was formed in 1993 (Drummond, 2008). The Challenger organisation consisted of quota members who contributed a levy to the running and interests of the scallop fishery. The scallop yields in the southern fishery initially increased from the crash of the early 1980's (Figure 1.4). In 1984, 250t were harvested with the catch steadily increasing until it reached its plinth in 1995, harvesting 850t. The success of scallop enhancement in the southern fishery trials led to a similar project being initiated in the Northland fishery (Morrison & Cryer, 2003), Coromandel (Bartrom, 1990) and the Chatham Islands (Marsden & Bull, 2006). The same results have not been achieved and the projects were deserted due to extremely high spat mortalities. Possible reasons for the poor results were attributed to bad weather, poor site selection and heavy predation by fish (Bartrom, 1990) .

From 1995 the harvests have steadily decreased in the southern fishery to 104t by 2008/9 (Ministry of Fisheries Science Group, 2011). Such drastic decreases

may be signalling the demise of the scallop fishery. A potential solution to improving scallop yields would be hatchery supplied scallop spat.

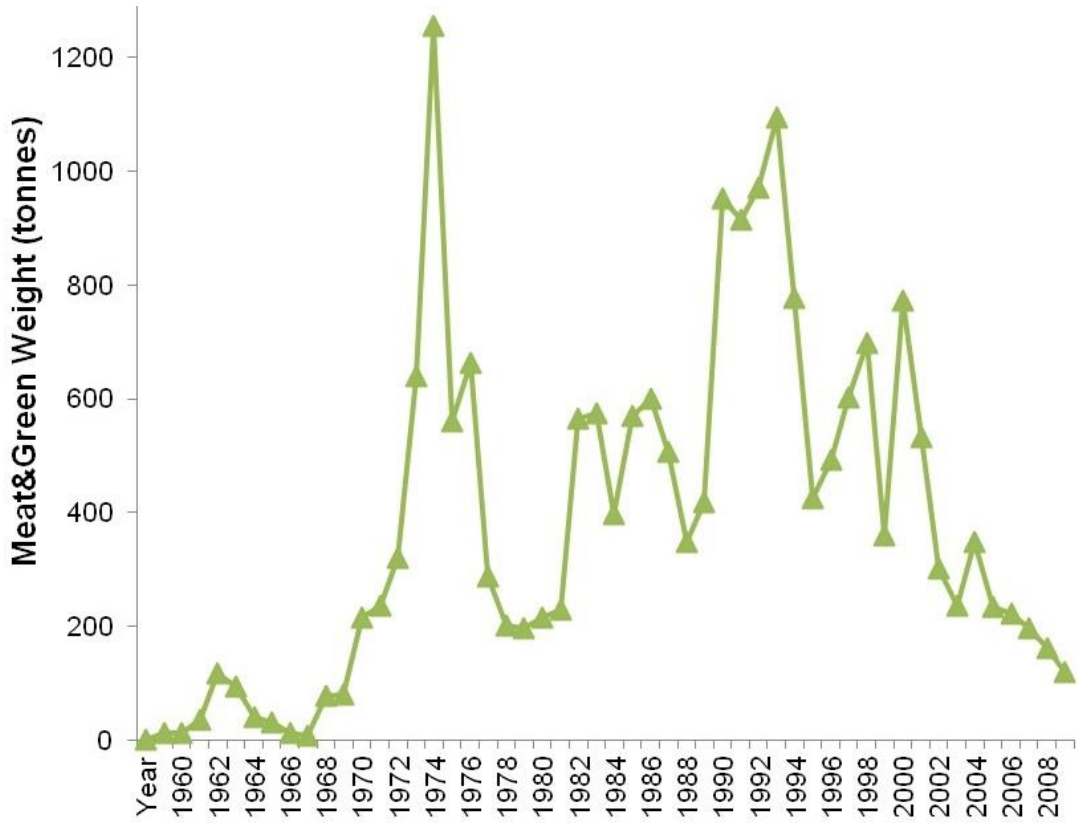


Figure 1.4. Meat & Greenweight landings of *P. novaezelandiae* from 1960 – 2010 (Ministry of Fisheries Science Group, 2011).

1.2.2 Hatcheries

Hatcheries could provide a consistent spat supply, in and out of traditional spat catching seasons. Environmental variables, such as temperature, food supply, and water quality control, assist in producing a consistent spat supply when a suitable protocol has been established. Scallop culturing has been practiced in Europe; England, France and Norway; Asia, Japan, China; Americas; Canada, Chile, Brazil; Caribbean and Australia (Andersen, Christophersen, & Magnesen, 2011; Kleinman, Hatcher, Scheibling, Taylor, & Hennigar, 1996; von Brand, Merino, Abarca, & Stotz, 2006; Zhongguo & Huang Hai shui chan yan jiu suo, 1991). Research and development has been in progressing since the 1970s for the king scallop, *Pecten maximus* (Andersen et al., 2011). Traditionally, scallops

were collected from the wild and conditioned for spawning (Andersen et al., 2011; Heasman, O'Connor, & Frazer, 1994b; Utting & Millican, 1998). Once spawned, the eggs are fertilized and placed in tanks. When the larvae grow to D-larvae, they are transferred to larger tanks and stored at 2 larvae/ml. Water is exchanged every two days and when the larvae are ready to settle the spat are placed in settlement tanks (Helm et al., 2004). Antibiotics are routinely used in the process of rearing the larvae in Europe. However, some antibiotics, such as chloramphenical, have been banned in Europe (Torkildsen, Coyne, Samuelsen, Magnesen, & Bergh, 2002). Commercial hatcheries are not obligated to share their protocols (Andersen et al., 2011). Each hatchery will have unique environmental challenges and solutions which in turn will impact upon larvae rearing protocols.

Scallop hatcheries do not exist in New Zealand. The Cawthron Institute has successfully cultured the green lipped mussel *Perna canaliculus* and Pacific oyster, *Crassostrea gigas* (Ragg, King, Watts, & Morrish, 2010). The New Zealand government has proposed a billion dollar industry by 2025. Declining scallop takes and a potential scallop collapse of the Southern fishery may be counter balanced with hatchery supplied spat and scallop farming (Ministry of Fisheries Science Group, 2011; Moore, 2012; New Zealand Aquaculture, n.d.).

1.3 Aims of this thesis

- The aim of this study is to investigate the effects of diet quantity on gonad condition of the broodstock of the New Zealand scallop, *Pecten novaezelandiae*.

- To characterise larval development (morphological, physiological characteristics) of the New Zealand scallop, *Pecten novaezelandiae* from D-larvae to the pediveliger stage.

2 Chapter Two: Broodstock conditioning of scallops.

2.1 Introduction

The scallop, *Pecten novaezelandiae,* is an important recreational and commercial fishery in New Zealand. However, there has been a consistent decrease in wild stock recruitment over the last decade (Food and Agriculture Organisation of the United Nations, 2011). Globally, scallop recruitment is erratic, and the New Zealand scallop follows the same trend. The re-seeding of scallop beds with wild spat has not halted the declines over the last decade (Ministry of Fisheries Science Group, 2011). Seeding scallop beds with hatchery reared scallop juveniles may potentially provide the solution to the natural unpredictability of spat supply and recruitment in the wild. However, hatchery production of scallop spat relies on well-conditioned adults that can be readily spawned to produce large quantities of healthy gametes in a controlled environment. This study focuses on developing the gonad condition through a rationed concentration of mixed algae and evaluating the outcomes.

2.1.1 Biology

Pecten novaezelandiae is a functioning hermaphrodite that can be found below the sub tidal zone to 96m in depth (Bull, 1976). *P. novaezelandiae* is a broadcast spawner that can spawn up to seven times a year (Williams, 2005). This species normally has a main spawning event between November and January, and may have a second event in March. Some populations also may have additional spawning events throughout the year (Williams, 2005). Spawning appears to be associated with cold water upwelling (Bull, 1976; Morrison, 1999). Scallop larvae remain in the water column for 3-4 weeks before attaching with byssal threads to filamentous hydroids and algae, and sea grasses, such as *Zostera (Marsden & Bull, 2006).* Once attaining the size of 5mm, they release from their attachment point and can be found freely settled above the sea bed, which may consist of sand, shell gravel and grit (Morrison, 1999). It can take 18 months to 3 years for scallops to reach a harvestable size of 90-100mm, depending on environmental conditions (Marsden & Bull, 2006).

2.2 Broodstock conditioning

Scallop broodstock are animals that have been collected from the wild and maintained in the laboratory for the purpose of producing larvae for aquaculture stocking. Conditioning is the manipulation of an organism's environment and diet in order to promote gametogenesis. The conditioning of broodstock displays positive effects on the scallop eggs and the growth and survival of the resulting larvae (Heasman et al., 1994b; Uriarte, Farías, Hernandez, Schäfer, & Sorgeloos, 2004; Utting & Millican, 1998).

2.2.1 Measures of gonad condition

Measures for the determination of successful gonad conditioning can be divided into three categories: destructive, non-destructive and post spawning. Destructive measures require that the scallop be destroyed. Wet and dry weights, gonad weight, histology and gonadalsomatic indices have been used in studies to determine changes in the gonad condition (Andersen & Ringvold, 2000; Barber & Blake, 2006; Williams & Babcock, 2004). Histology is an accurate measure to assess the status of the gonad by quantitative measures, acini and egg size or areas measured, from the stains (Auffret et al., 2003; Duinker & Nylund, 2002; Williams & Babcock, 2004).

Visual or macroscopic observations are non-destructive and are qualitative. The number of visual grades of gonads can vary from four to nine categories (Barber & Blake, 2006). The categories of the gonad status are: Ia) virgin, un spawned gametes; Ib) developing gametes; II) differentiated; assessment of the gonad sex; III) recovering; IV) filling; V) half full; VI) full and VII) spent. Various authors have described the macroscopic and microscopic level changes the gonad experiences during maturation (Barber & Blake, 2006; Cochard & Devauchelle, 1993; Heasman, O'Connor, & Frazer, 1996; Naidu, 1970; Sühnel et al., 2010; Williams & Babcock, 2005).These include changes in colour, size and shape of the whole organ as well as histological changes in tissue structure, gamete size and abundance. However, each of these studies was conducted in specific scallop species and so the categorical systems reported in one species may not

apply to another. Heasman (1996) derived eight different categories of gonad condition for *P. fumatus* for the purposes of optimising scallop reproduction in aquaculture. *P. fumatus* is morphologically similar to *P. novaezelandiae*, enabling Williams & Babcock (2004) to adapt Heasman's index for a study of the reproduction in wild populations of this latter species. Williams and Babcock showed that the histology and gonad mass of the two species correlated significantly.

2.2.2 Colour

Some gonad visual grading indices incorporate assessments of the gonad's colour. The hermaphroditic scallop *P. novaezelandiae* has male (creamy white) and female (orange) portions that are clearly distinguishable. Sühnel (2010) showed a strong relationship between reproductive stages and carotenoid astaxanthin in the female portions of the lion's paw scallop, *Nodipecten nodosus*. Tang (1941) used colour changes, confirmed by histology, to catagorise the gonad into five stages; a) recovering b) filling; c) full stage; d) spawning and e) spent, for *P. maximus.* Roe (1971) used ovarian colour to determine the degree of maturation through visual observation by seasonal variation for the calico scallop (*Argopecten gibbus*. Further works by Miller et. al. (1981) used colour categories based on visual observations to determine gonad condition for the calico scallop. All these methods are qualitative, however, and so the results should be considered subjective.

2.2.3 Spawning and post spawning

Scallops are alleged to be synchronous spawners. In the wild spawning events are triggered by environmental cues. In the laboratory one conventional technique for obtaining gametes from bivalve molluscs, such as oysters and mussels, is "strip spawning" (Helm et al., 2004; Slaughter, McCartney, & Yund, 2008). This involves collection of loose eggs and sperm by pipette through incisions in the gonad wall. Strip spawning has not proven to be an effective

method of extracting gametes from scallops (Heasman, 2007). Injections of the neurotransmitter serotonin have been used successfully to stimulate the release of sperm, but not eggs (Heasman et al., 1994b; Nesbit, 1999; Williams, 2005). Thermal shocking, the temporary exposure of organisms to elevated water temperatures, is another common method of inducing spawning in bivalves. This technique has been reported to successfully induce the release of both eggs and sperm in scallops (Heasman et al., 1994b; Helm et al., 2004; Williams, 2005). Following spawning the success of the broodstock conditioning process is indicated by the number of eggs, fertilisation success, larval development and survival of the larvae (Andersen & Ringvold, 2000; Barber & Blake, 2006).

Assuming all environmental variables are conducive to sustaining scallops within an aquaculture facility sea water system; three significant environmental factors would be required for successful broodstock conditioning: photoperiod, temperature and diet.

2.2.4 Photoperiod

Little is known about the effects of photoperiod on gametogenesis and gonad conditioning. Utting & Millican (1997) suggested photoperiod of 15h light: 9h dark would bring a greater number of scallops *P. maximus,* to spawning. Experiments by Duinker (2000) found a constant photoperiod of 12h, increasing to up to 17h, a day over a period of 45 days, resulted in larger gonads in the *P. maximus*. Mallet & Carver (2009) found a 16h light regime to be optimum for the *Argopecten irradians irradians* and the minimum photoperiod required for gametogenesis was 8h.

2.2.5 Temperature

Sastry (1963, 1966) observed the effects of seawater temperature on *Aequpecten irradians* Lamark and concluded that temperature influenced the initiation of gametogenesis through latitudinal gradients and laboratory experiments. The scallop *Aequipecten irradians* Lamark develops oogonia when conditioned at 15°C, but it is unable to produce oocytes when nutrients are withheld (Sastry & Blake, 1971). Blake's (1972) (as sighted in (Barber & Blake, 2006)) found that starving scallops and the outer parameters of extreme temperature, ±±5 and 15°C, can result in the re-absorption of the germinal epithelim. In a review by Utting & Millican (1998) found that starving scallops and temperature can result in the re-absorption of the germinal epithelim. Heasman (1996) found the optimal temperature for broodstock with *P. fumatus* to be 15°C, although within the range of 12°C to 18°C broodstock conditioning still successfully conditioned scallops. The results of these studies indicate that temperature is a significant factor in conditioning scallop gonads.

2.2.6 Diet

To ensure that the scallops receive the optimum diet a multi microalgal diet strategy is best (Heasman, 2007; Utting & Millican, 1997). Studies conducted on the *P. fumatus* for broodstock found a diet of $6x10^9$ cells per scallop per day of three differing microalgae (*Chaetoceros calcitrans, Pavlova lutheris, Tahitian Isochrysis* and *Chroomonas sauna)* yielded rapid gonad development (Heasman et al., 1994b). Anderson & Ringvold (2000) using a combination of micro-algae diets, Tahitian *Isochrysis galbana, Pavlova lutheri, Skeletonema costatum* and *Chaetoceros gracilus,* observed the outcomes between feed diets and seasonal variation. There was no significant difference between the three day old veligers that were spawned in the winter and those in the summer. Anderson (2000) also noted that eggs and larval survival rates were not a good measure of diet quality, as few veligers may result from spawning. Further studies showed that mono specific algal diets fed over four weeks to *Mimachlamys asperrima,* doughboy scallop*,* did not reflect fecundity (O'Connor, Heasman, & O'Connor, 2000).

Little information is available on the broodstock conditioning of *P. novaezelandiae* within a hatchery environment. Most studies for this species have focused on the environmental factors that affect wild stocks and fisheries *(Bull, 1976; Morrison, 1999; Nicholson, 1978; Williams, 2005)*.

2.3 Aim

The aim of this study was to investigate the effects of diet quantity on gonad condition of the broodstock of the New Zealand scallop, *Pecten novaezelandiae.*

2.4 Methods and Materials

2.4.1 Animal collections

A total of 110 scallops were collected from Jones Bay, Tawharanui Peninsula, Auckland (36°22’05”S 174°49’12”E) at water depths of 10 to 17 meters using SCUBA. All individuals collected were between 70mm and 100mm in shell width. The scallops were transported in a cooler bin with a thin layer of seawater to the Auckland University of Technology Aquaculture laboratory. The scallops were then scrubbed with a wire brush to remove any fauna or flora attached to the shells. Any polychaetes that remained attached to the shells were destroyed by removing their tubes. The scallops were individually tagged, and placed in a re-circulating seawater system to acclimatize for a month before the start of the broodstock conditioning experiment.

2.4.2 Feeding experiment

Nine round tanks with 40L of seawater each were used for the conditioning experiments. Three ten-litre header tanks were constantly supplied by a 200L reservoir of seawater that was re-circulated through biological filters and a protein skimmer. The water was chilled to 17°C. The seawater was filtered through a one-micron filter before accessing the header tank (Figure 2.1). Three different microalgal concentrations (about 1.5%, 3% and 6% of mean scallop dry weight) over 30 days were introduced into each of the three header tanks with a peristaltic pump. Each header tank represented a different feeding treatment and supplied three replicate experimental tanks. After acclimatization to the re-circulating system, 81 scallops were randomly selected and placed in one of nine 40L experimental tanks. A seawater flow rate of 1.5L per minute was used to fill each experimental tank. Aeration was applied to the three header tanks and all replicate tanks with air stones in each container. Ten percent of the seawater was exchanged weekly or when a noticeable change in pH, salinity or water turbidity occurred. A 16h light and 8h dark regime was maintained throughout the experiment.

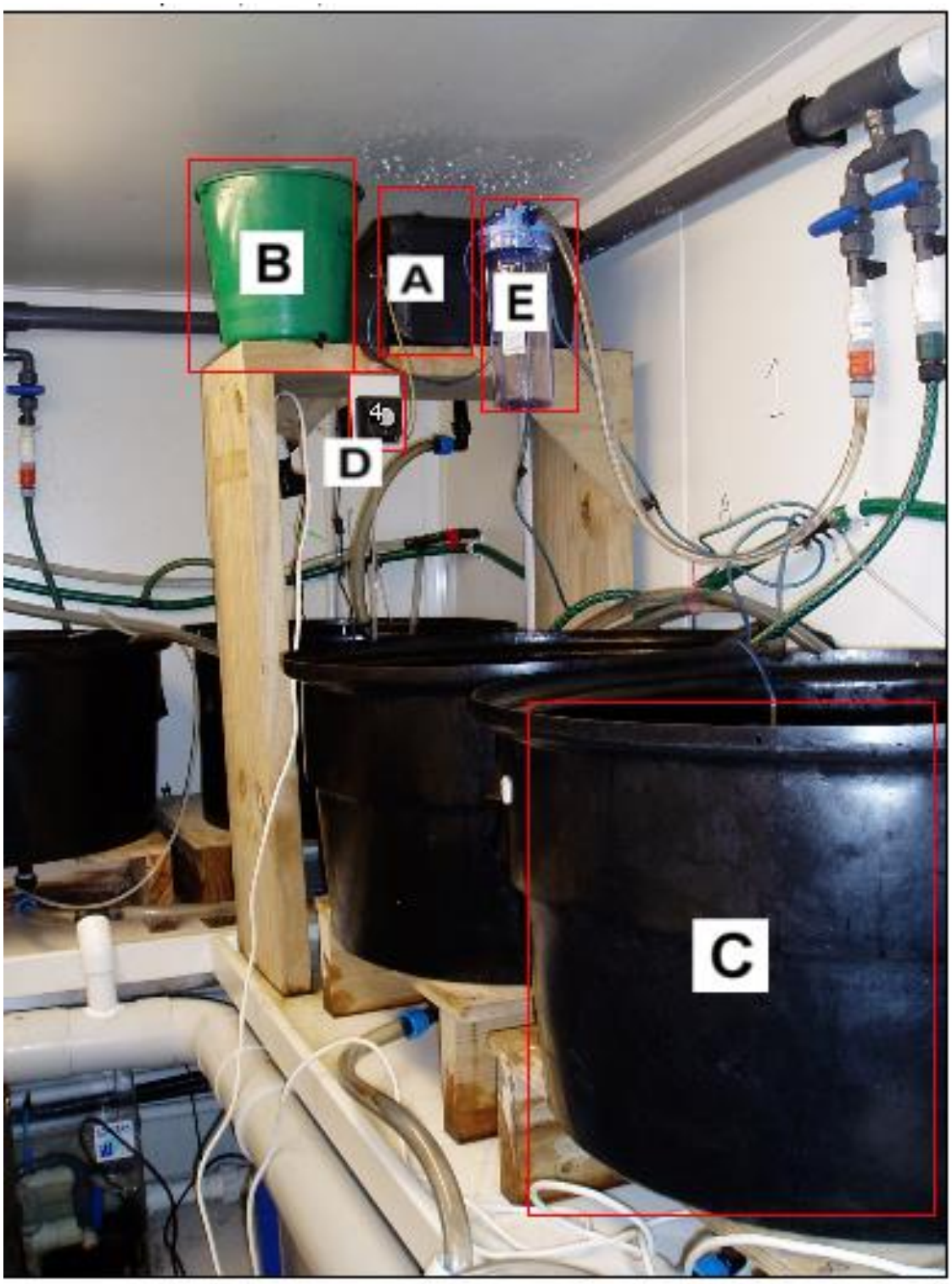


Figure 2.1. A.U.T. re-circulating water system.

A- Header tank.

B- Concentrated microalgal supply.

C- Experimental tanks.

D- Peristaltic pump.

E- Inlet filter).

2.4.3 Feeding rations

The microalgal feed used in these experiments was the commercial liquid diet 'Reed Mariculture 'Shellfish Diet 1800' (RMSD). This diet consisted of four microalgae (*Isochrysis* sp. 40%, *Pavlova* sp. 15%, *Tetraselmis* sp. 25%, and *Thalassiosira weissflogii 20%*). The three treatment concentrations were calculated at 1.5%, 3% and 6% of the estimated net dry weight of the scallop based on a previously described formula.

$$\text{Ration (g per day per adult)} = \text{percentage of net dry weight (g) x mean dry adult meat weight (g)}/100$$

The mean RMSD dry weight was obtained by dispensing 15 replicate 250µL liquid RMSD samples onto individually pre-weighed watch glasses. The RMSD samples were dried in an oven for 48 hours at 80°C and re-weighed. The average dry weight per adult scallop was determined by drying the tissues of 20 scallops. This value was used to estimate the amount of food needed per tank for each of the three feeding treatments. Feeding was maintained continuously with the dosing pumps throughout the experimental period of 30 days.

2.4.4 Weight measurements

Measurements of wet and dry tissue weight were recorded for 20 representative animals at the start of the experiment. The same measurements were recorded on all the experimental animals at the end of the experiment. The specific measurements taken were: wet weight of all the soft tissues, wet weight of gonad only, and wet and dry weight of the soft tissues without the gonad. The shells of these animals were removed and the wet weight [ww] of the total tissue of each animal was recorded after blotting the tissue with paper towels to remove excess water and weighing to the nearest 0.01g. The gonadalsomatic index [GSI] was calculated using this formula:

$$\mathrm{GSI} = (\text{gonad weight}/(\text{soft body weight} - \text{Gonad weight})) * 100$$

The gonad was carefully removed, weighed and stored in Davidson's solution (glycerin, formaldehyde, ethanol, filtered seawater) for further histological analysis (Howard & Smiith, 1983). The remaining tissue was weighed and then placed in an oven for 48 hours at 80°C and then re-weighed to obtain the dry weight [dw].

2.4.5 Gonad colour analysis

One of the benefits of using Davidson's solution is that the original colour of the gonads does not leach out. This enabled the analysis of colour as another quantitative assessment of gonad condition. Use of a colourimeter allows subtle differences between samples to be detected. A Colourimeter Hunberlab ColorFlex was used to record the colour of the portion of gonad tested, based on three measurements within the visual light spectrum: light to dark (L*; 0-100), green to red (a*, + red - green) and yellow to blue (b*; + yellow - blue) (Young & Whittle, 1985). The ColorFlex aperture was modified by covering the aperture with black Perspex with a 15mm diameter hole drilled in the centre (Figure 2.2). Colour sample plates were used to confirm that ColorFlex operations had not been compromised. The male and female portion of each scallop was analysed separately. The differences between gonad male and female portions were calculated by only using the developing gonad categories (VGI 4-8, as described in the methods). The mean of the initial gonads (I) were taken to calculate the ΔE (the distance between the standard and the sample) as described in Young & Whittle (1985).

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$$

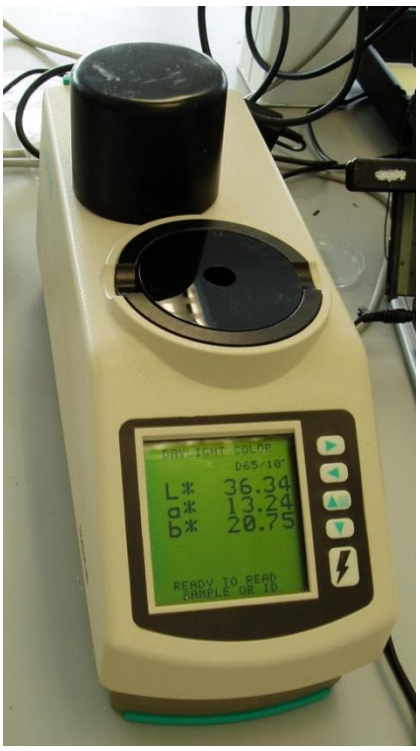


Figure 2.2. Hunter Lab ColorFlex with the modified aperture.

2.4.6 Visual grading index

The initial 20 scallops were used as a control group at the beginning of the experiment for comparisons with the animals subjected to the feeding experiment. The condition of all initial and experimental individuals was determined with an established visual grading index [VGI] (Table 1.1) according to Williams & Babcock (2005). Each individual was visually assigned to one of eight categories according to the gonadal state.

Table 2.1 Visual Grading Index System (Williams, 2005).

Visual Grade Index	**Gonad appearance**	**Gonad Condition**
1	Gametes are absent or largely so. Gonad is small, thin, flaccid, and translucent. Ovarian and testicular tissues are difficult to differentiate. Intestinal loop is clearly visible.	Spent
2	Gonad is much reduced in size compared to visual grade 3 and has lost turgor. Ovary appears mottled or lattice-like, presumable due to the majority of acini that have been voided. Intestinal loop is usually visible.	Partially spawned
3	Ovarian tissue of gonad is uniform in colour (similar to visual grade 7 or 8) but is interspersed with isolated specs of translucent (voided) acini. Gonad may still be large, but turgor is reduced.	Active
4	Gonad small. Separate acini are clearly apparent and gamete material lines the acini walls. Male (white) and female (orange) portions of the gonad are distinguishable. Intestinal loop is easily visible.	Active
5	Gonad is larger than visual grade 4 and is increasing in turgor. Gonad is less granular in appearance as acini begin to fill. Intestinal loop is partially obscured	Active
6	Ovarian tissue appears uniform in colour and texture as acini fill. Very little of intestinal loop visible (usually only a small portion of the ascending limb at the distal extremity of the gonad)	Active
7	Gonad is large and thick. Ovarian tissue is bright, uniform in colour and turgid. Separate acini are not apparent with little if any intestinal loop visible. Gonoducts are prominent.	Ripe
8	Gonad is very large and thick, as if ready to burst. Ovarian tissue is bright, uniform in colour, glossy and highly turgid. Gonoducts are usually large and conspicuous. Acini are not apparent and intestinal loop is not visible.	Ripe

2.4.7 Histological analyses

Gonad samples retrieved from the scallops at the start and end of experiment were fixed in Davidson's solution without acetic acid (Appendix

A). The omission of acetic acid to Davidson's solution has been found acceptable for the storage of bivalve tissue (Howard & Smiith, 1983). The gonad samples were fixed following standard histological protocols, including dissection, fixation, embedding, sectioning and staining. Gonad samples were held in Davidson's solution for no more than a month before being fixed. Gonad samples larger than the embedding cassettes were sectioned to 5mm thickness and placed in an individual cassette. The thickness of the tissue was used to determine the length of the embedding schedule. A 5mm long tissue specimen was considered optimum. A Shandon Citadel 2000 tissue processor with twelve baths was used to embed the specimens using the embedding schedule described in detail in Appendix B. Briefly, this process included sequential washes in baths containing gradual increases in ethanol to dehydrate the specimen followed by immersion in Xylol and finally setting in a paraffin wax block. After dehydrating and saturating the sample with paraffin wax, the tissue was removed from the cassette and was embedded into a paraffin wax block using a Thermo Scientific HistoStar* Embedding Workstation. The embedded tissue was microtomed to 5µm thickness and placed in a water bath at 40°C. The floating paraffin slices were mounted onto histology slides. The samples were then placed in an oven at 100°C for 15 minutes to melt the excess wax. The tissues were stained using haematoxylin and eosin and finally preserved with DPX mounting adhesive and a coverslip for microscopic and image analysis (Howard & Smiith, 1983). Based on these histological sections, all female and male portions of the gonad samples were assigned a histological gonad index [HGI] value, which followed the same categories in Table 1.

2.4.8 Acini and egg analysis

In order to specifically assess the state of egg development, measurements of acini and egg areas (sizes) were obtained from histological sections. Pictographs were obtained with a Leica DM2000 microscope and Leica DFC290 microscope camera. The size of objects was verified using an Improved Neubauer haemocytometer as a template. The pixel to line ratio was calculated and standardized using ImageJ Software (Abramoff, Magelhaes, & Ram, 2004). The number of pixels to object establishes a comparative size. These parameters allow for the calculation of the area and feret of the acini and eggs with the software. The software package was used to trace the outlines of the acini (the berry like structure from which the primordial gametogenesis eventuates (Beninger & Le Pennec, 2006; Williams, 2005), eggs, feret diameter (the longest? distance between two parallel tangents), and area and number of eggs (Figure 2.3) (Abramoff et al., 2004). Three random acini per gonad were selected and their eggs were counted. All the eggs within each acini also were traced individually to determine their size, documented in the Region of Interest [ROI] manager and the results appended to a separate file (Figure 2.4). Based on these acini and egg data, scallops were assigned into three categories: spawned (all acini were void of eggs), partially spawned (some acini were void of eggs), and not spawned (no indication of egg release from acini).

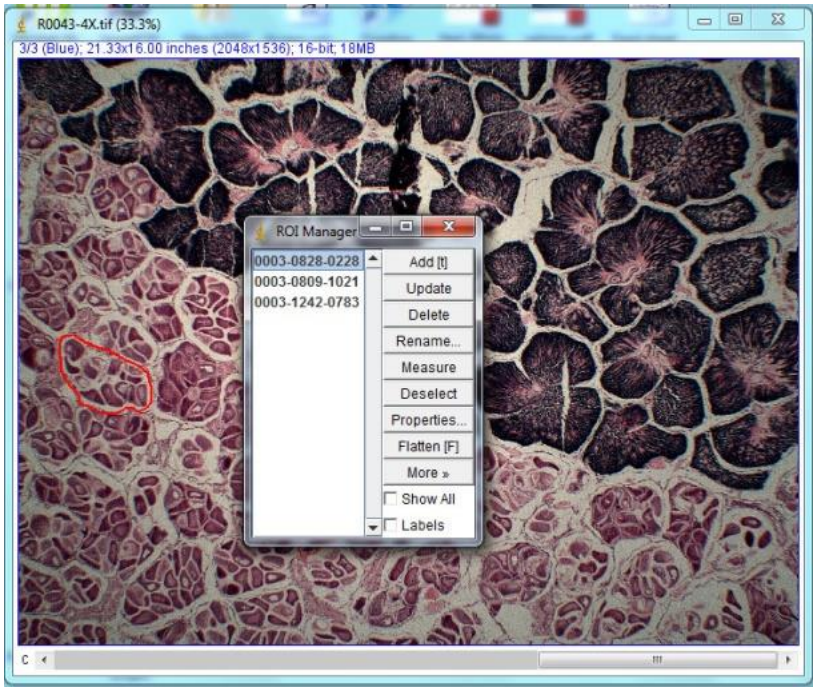


Figure 2.3. Outlined acini of the eggs and the Region of Interest (ROI) manager dialog using ImageJ software.

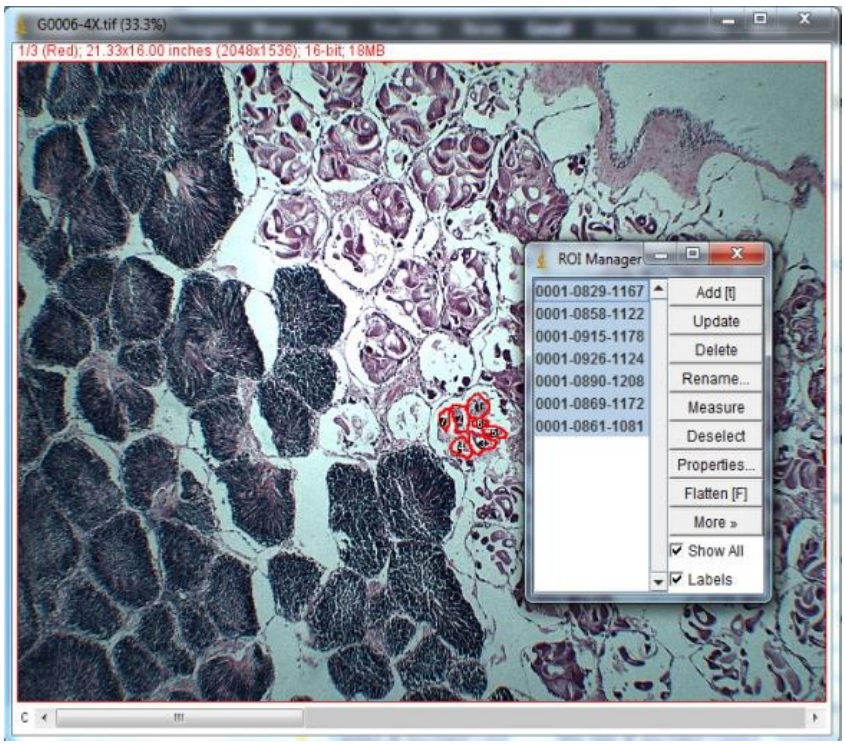


Figure 2.4. Outline of eggs and the reference position captured in the ROI using ImageJ software.

2.4.9 Statistics

The data were analysed using R Statistics. All confidence levels were calculated at 95%. If data showed homogeneity of variance before or after an appropriate transform they were analysed by one-way analysis of variance. If the data were found to be significant, a post hoc Tukey Honest Significance Difference test was conducted. Data that exhibited heterogeneity of variance were analysed using a non-parametric testing with a Kruskal-Wallis and Steele or Fischer-Behrens post hoc analysis.

2.5 Results

2.5.1 Feeding rations

The feeding rations for each of the low, medium, and high microalgal concentration treatments were 0.5, 0.92, and 1.9 g of dry microalgal feed per tank per day, respectively. These rations were maintained constant over the 30-days experimental period.

2.5.2 Mortality

There was one scallop mortality during the course of the experiment. The dead scallop was replaced by a live one of a similar size to maintain the same density within the tank, but the replacement scallop was not included in the data analysis.

2.5.3 Total tissue wet weight

There was a negative trend from the onset of the experiment to the low, medium and high rations, respectively. The twenty scallops that were sacrificed at the onset of the experiment had a mean (±SE) tissue wet weight (shell excluded), of 40.99±0.155g (Figure 2.5). At the end of the 30-day feeding experiment, the mean (±SE) tissue wet weight of animals within the low, medium and high food concentration treatments were 37.31±2.71g, 29.67±2.49g, and 27.53±1.96g, respectively. There was a significant difference between pair analyses across the rations (Kruskal-Wallis; F_3=22.099; $p<0.05$) but not between Initial and Low. A test for homogeneity failed with a Levene test (F=0.035). Transformation of the data did not generate a homogenous set of values. A Kruskal-Wallis, rank sum non-parametric test, was conducted with a Steel post hoc analysis.

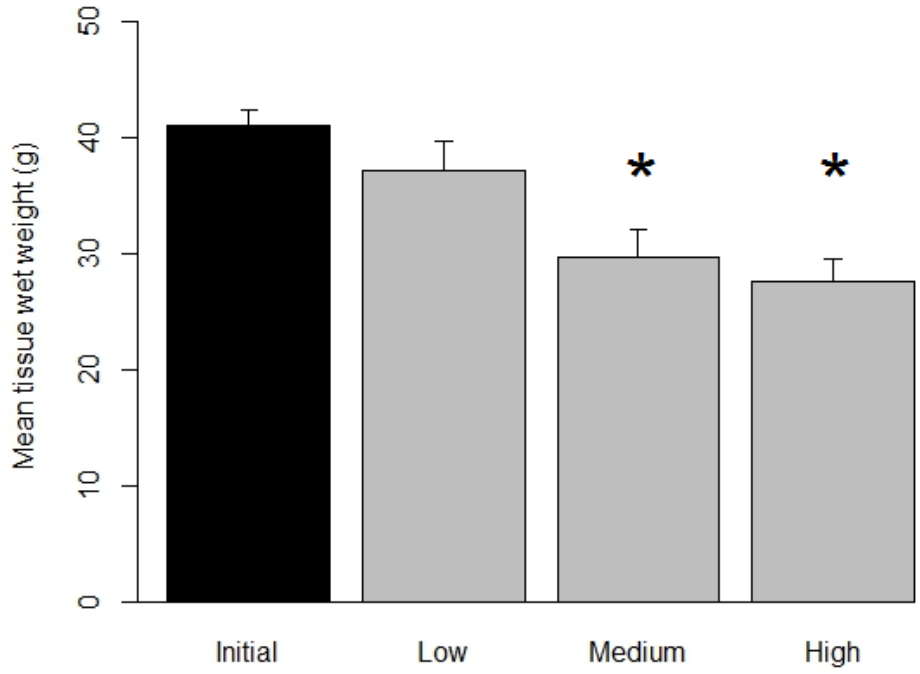


Figure 2.5. Mean (±SE) scallop tissue wet weight (g) with the initial (I) group and the treatments after 30 days of feeding (L = low, M = medium, H = high food concentration rations). A single star over a bar represents a significant difference between the treatments.

2.5.4 Net tissue dry weight

There was a positive trend in dry tissue weight between the low, medium and high rations. An analysis of variance between the food rations resulted in no significant differences between the rations (ANOVA; F3=0.51; p>0.05) (Figure 2.6). The starting mean net dry weight excluding the gonad was (I) 2.5 ±0.25g and ended with 2.57 ±0.21g (low), 2.8 ±0.30 (medium) and 3.08 ±0.35g (high). A Levene test resulted in homogenous net tissue dry weight data. An analysis of variance resulted in non-significant differences among the diet rations (ANOVA; F3=0.534; p>0.05).

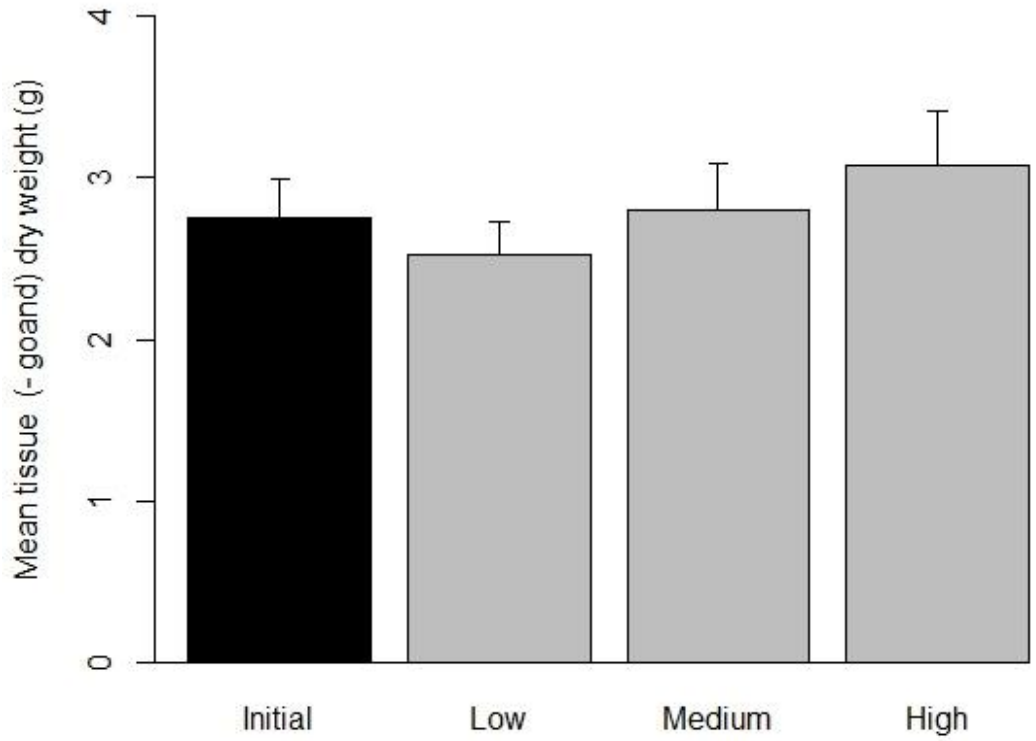


Figure 2.6. Mean (±SE) net tissue dry weight of the scallops (excluding the gonad) with the initial (I) group and the treatments ($p > 0.05$) (L = low, M = medium, H = high food concentration rations). There were no significant differences between treatments.

2.5.5 Gonad wet weight

There was an increase in the wet gonad weight from the initial starting weight of 3.15±0.16g (initial) to 5.488±0.73g (low). The wet gonad weight then plateaus to the medium diet (29.67±29.67g) and then decreased to 4.983±0.55g for the high diet. A Kruskal-Wallis test for non-parametric data (Kruskal-Wallis; F_3=15.99; p<0.01) with a *post hoc* Steel analysis was calculated (Logan, 2010). There was a significant difference between the starting wet gonad weight (I) and the medium and high wet gonad weight at the end of the experiment (p<0.05) (Figure 2.7).

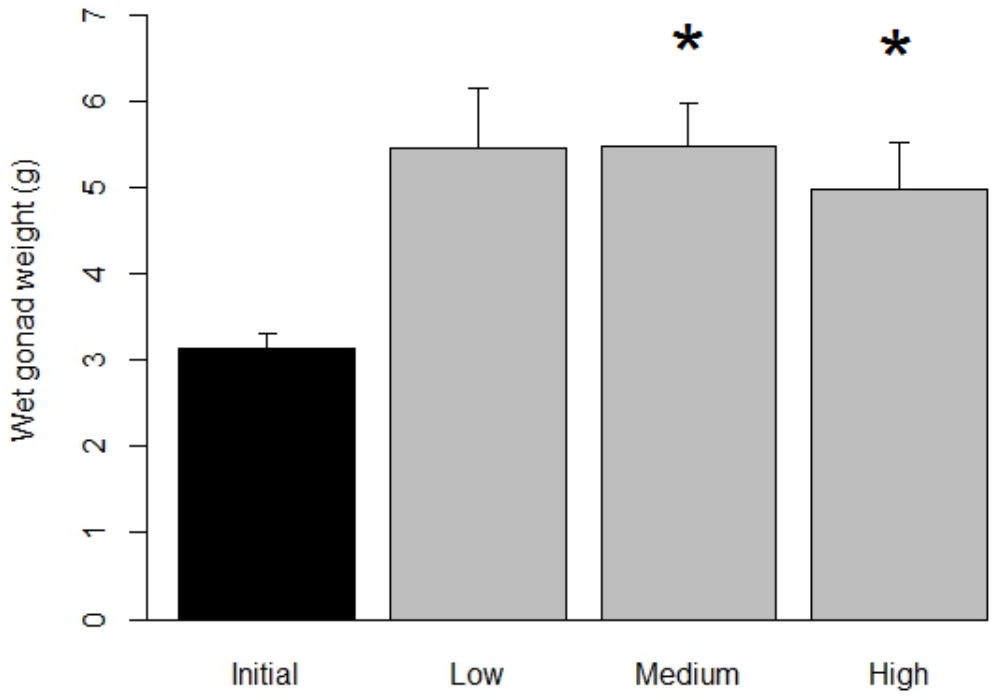


Figure 2.7. Mean (±SE) gonad wet weight with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations). A single star over a bar represents a significant difference between the treatments.

2.5.6 Gonadalsomatic index

The gonadalsomatic index (GSI) increased from 8.28±0.29% (I) to 16.75±1.93% (low), to 22.95±1.62% (medium) and finally decreased slightly to 23.27±1.81% (high). There was no significant difference between the medium and high diet treatments, but significant differences were observed among the initial, low and medium rations ($p<0.05$) (Figure 2.8). A Levene test of homogeneity was conducted on the results. The data were found to be heterogeneous and were therefore transformed. An analysis of variance [ANOVA] was then conducted on the data and the GSI was found to be significant (ANOVA; $F_3=49.3$; $p<0.05$; $n=91$). A TukeyHSD test was executed for pair diet ration comparisons.

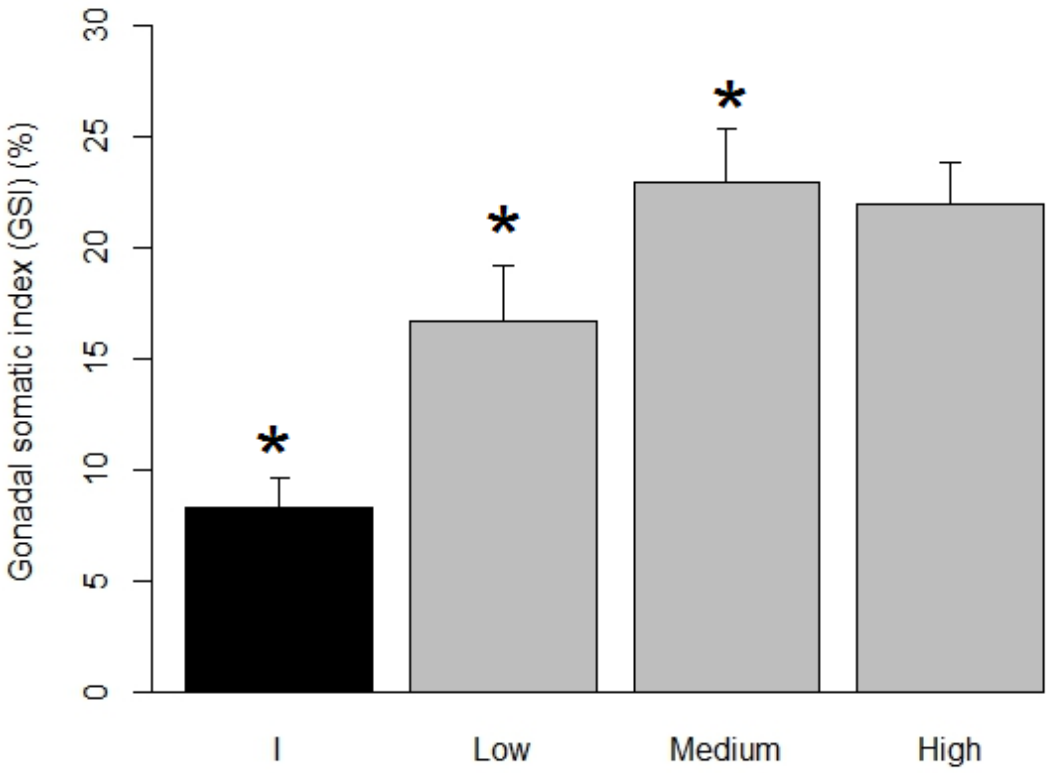


Figure 2.8. Mean (±SE) gonadalsomatic index (%) with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations). A single star over a bar represents a significant difference between the treatments.

2.5.7 Colour

There was a significant difference between mean±SE ΔE between the male (11.19±.65) and female portion (15.40±0.74 ΔE) of the gonad ($p<0.05$) (Figure 2.9). A Levene test found the data be homogenous ($F=0.88$; $p<0.05$; $n=117$).

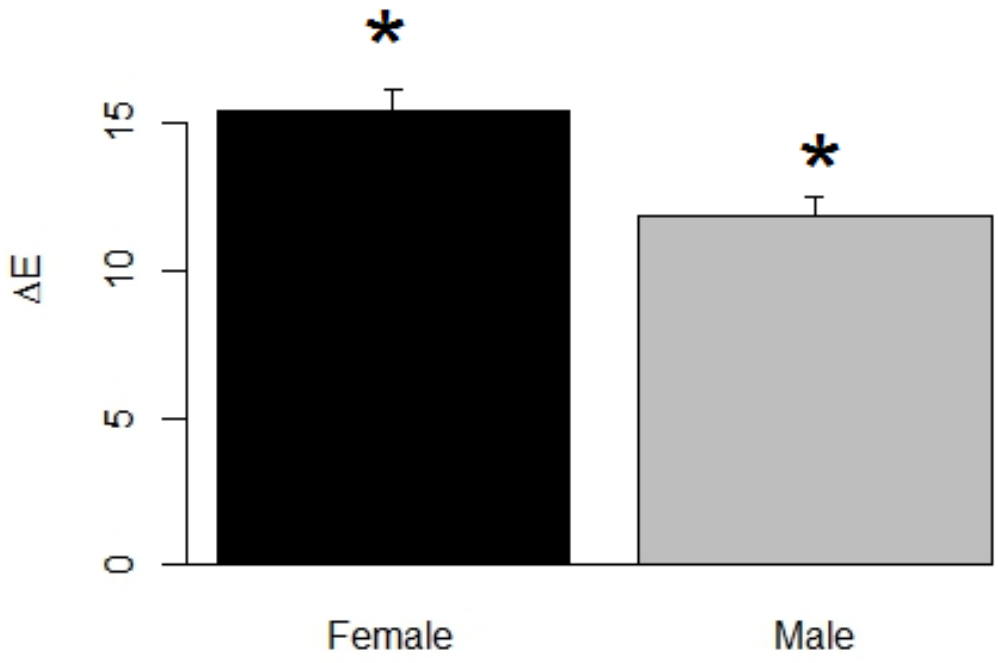


Figure 2.9. Mean (±SE) difference between the ΔE male portion and female portion of the gonad.). A single star over a bar represents a significant difference between the treatments.

2.5.8 Male gonad colour

There was no significant difference between the ΔE spermatozoa coloured portion of the gonad and the different diet treatments (ANOVA; $F_5 = 0.94$; $p > 0.05$) (Figure 2.10). Further comparisons between diet treatments and colour were conducted using a Levenes test for homogeneity and an AOV followed by a post hoc TukeyHSD. There was no significant difference between the treatments.

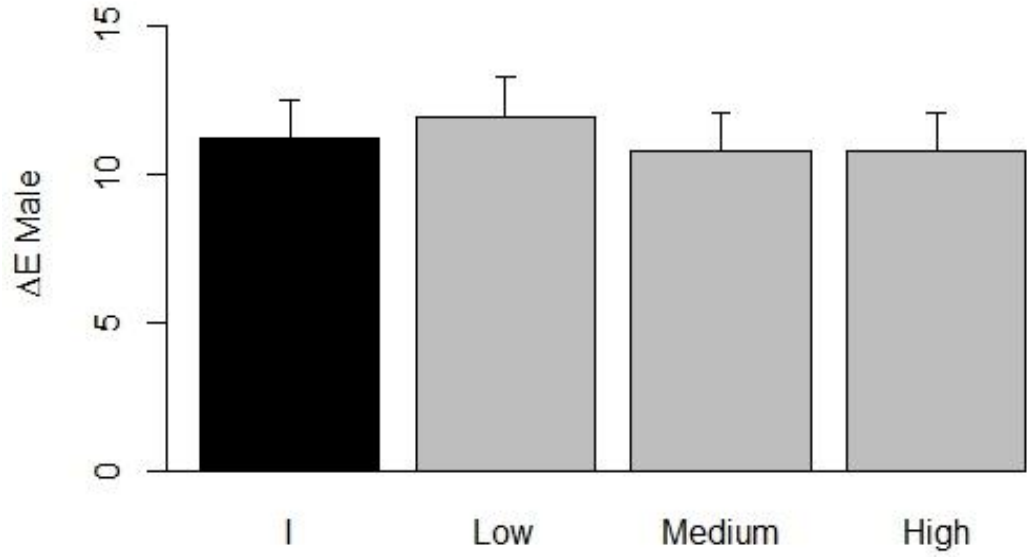


Figure 2.10. Mean (±SE) male gonad colour represented by ΔE with initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations. There were no significant differences between treatments.

2.5.9 Female gonad colour

A convex pattern of female gonad colour was observed through the initial, low, medium and high rations (Figure 2.11). A TukeyHSD indicated that there was a significant difference between the initial colour of the female portion of the gonad and the low and medium rations However, there were no significant difference between the different diet rations based on colour (ANOVA; F_5=0.83; p>0.05).

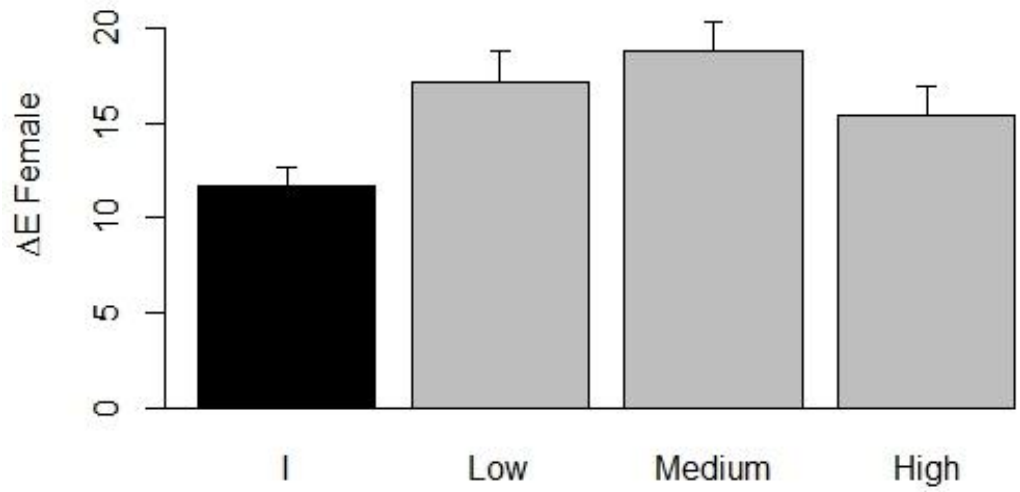


Figure 2.11. Mean (±SE) female gonad colour represented by ΔE with initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations. There were no significant differences between treatments.

2.5.10 Visual gonad index

A visual assessment of the male portion of the gonads for all samples resulted in the initial group of scallops mostly in a developing phase with 42.10% VGI 5 and 57.89% VGI 6 (Figure 2.12). At the end of the experiment, the majority of male portioned scallops were matured across the low 82.35% (VGI 7), medium 76.19% (VGI 7) and high 85% (VGI 7) diet treatments.

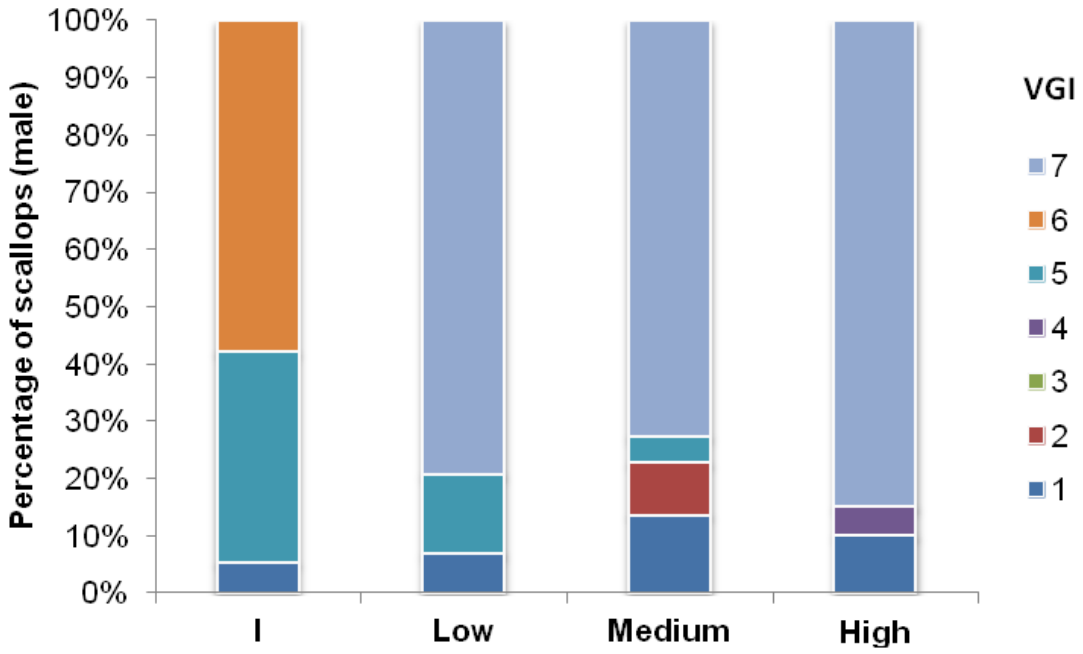


Figure 2.12. Percentage of scallops (male portion) VGI with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations).

A visual assessment of the female portion of the gonads for all samples resulted in the initial group of scallops mostly in a developing to mature phase with VGI 6 (Figure 2.13). At the end of the experiment, the majority of female portioned scallops were developoing across the low 47.83% (VGI 6) and 4.35% (VGI 7); medium 40% (VGI 6) and 12% (VGI 7); and high 41% (VGI 6) and 4.17 (VGI 7) diet treatments. However, there were more spawned (VGI 1) and partially spawned (VGI 2) gonads across the low 17.4%, medium 34% and high 20.83% than at the start of the study.

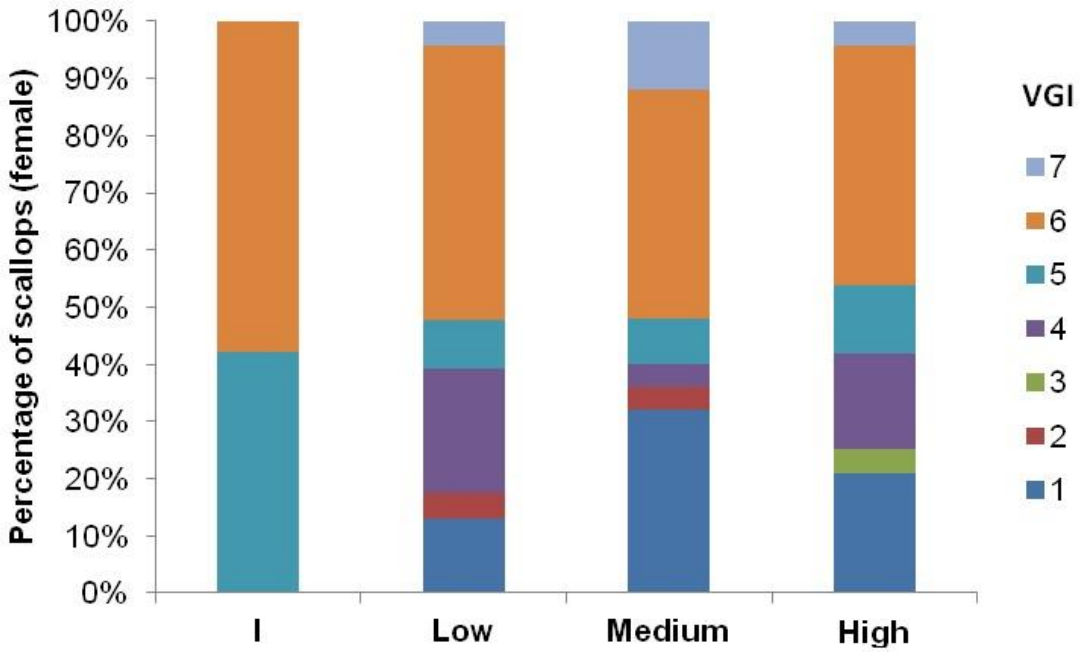


Figure 2.13. Percentage of scallops (female portion) VGI with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations).

2.5.11 Histological gonad index

Assessment of the male portions of the gonads for all scallop samples, including initial, low, medium, and high food concentration treatments resulted in 80% mature (HGI 7), 10% (HGI 5) developing and 10% spawned (HGI 1) (Figure 2.14). A wider spectrum of gonadal state was identified for the female portion of the gonads. For the initial group of scallops, the gonad status was 23% fully spawned (HGI 1); no scallops had partially spawned (HGI 2); 15% were developing (HGI 5); 38% were developing and close to maturity (HGI 6); and 23% were mature (HGI 7) (Figure 2.15Figure 2.13). On the low diet treatment, 13% of the scallops were fully spawned (HGI 1) and 30% were partially spawned (HGI 2); 4% were developing gonads (HGI 4); 4% developing immature gonads (HGI 5); 26% developing mature gonads (HGI 6); 17% mature gonads (HGI 7) and 4% were unidentifiable after the 30 days of the feeding experiment. On the medium diet treatment, 8% of the scallops were partially spawned (HGI 2);17% were fully spawned (HGI 1); 12% were in developing immature gonads (HGI 4); 4% developing gonads (HGI 5); 38% developing mature gonads (HGI 6); 20% mature

gonads (HGI 7) by the completion of the feeding experiment. On the high diet treatment, 27% of the scallops were partially spawned (HGI 2); only 5% were fully spawned (HGI 1); 4% were mortalities; 4% were developing immature gonads (HGI 4); 55% mature developing gonads (HGI 5); 5% mature gonads (HGI 7) at the completion of the experiment.

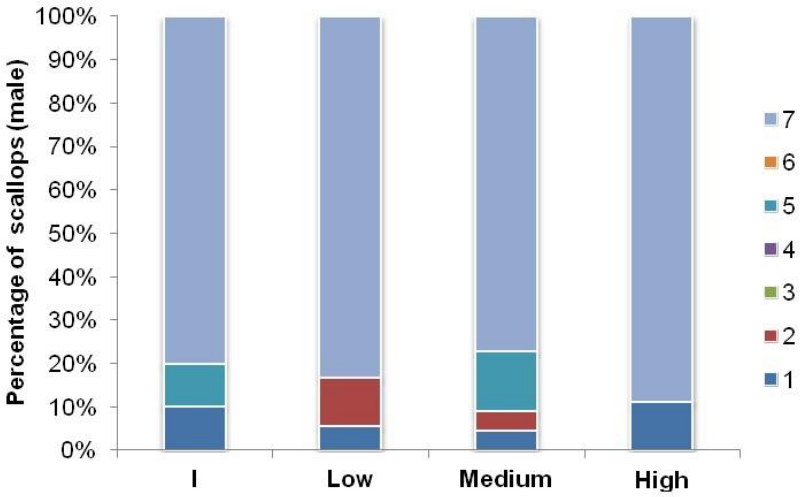


Figure 2.14. Percentage of scallops (male portion) HGI with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations)

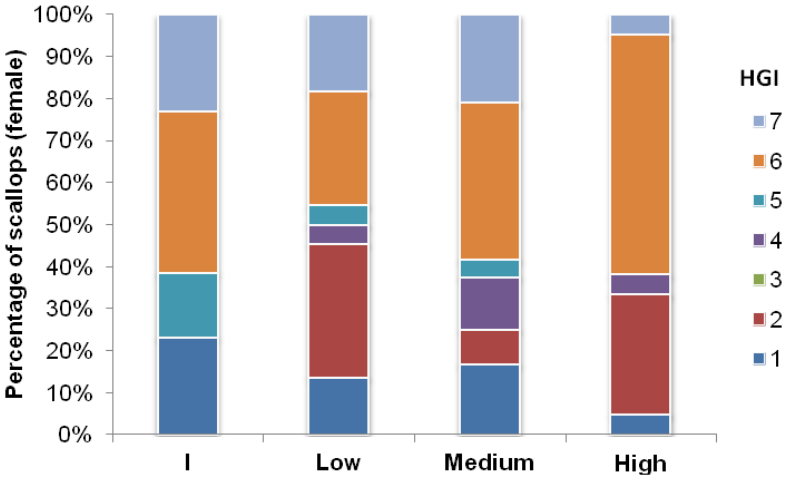


Figure 2.15. Percentage of scallops (female portion) HGI with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations)

2.5.12 Acini area

There were differences in the area of acini within scallops in the different diet treatments. However, there was no significant difference ($p>0.05$) between the mean acini area at the start of the experiment (55.4mm2 ± SE5.68mm2) and the low (45.14mm2 ± SE3.43mm2), medium (49.73mm2 ± SE4.60 mm2) and high (58.58mm2 ± SE3.99mm2) rations (Figure 10). A Levene test for homogeneity resulted was found significant ($p<0.05$).A Kurskal-Wallis test for non-parametric data was performed and a *post hoc* analysis for non-parametric data, Behrens-Fischer, were used to detect significance among rations.

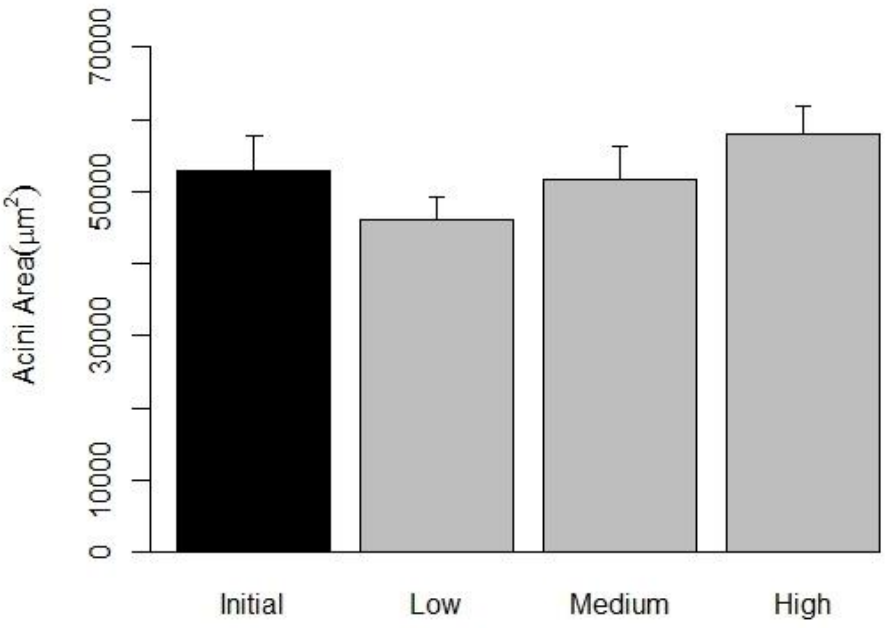


Figure 2.16. Mean (±SE) acini area (mm2) with the initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations). There were no significant differences between treatments.

2.5.13 Egg area

There were no clear trends in the egg size data. However, the eggs sizes were found to be significantly different in scallops among the diet treatments. The initial (I) eggs area size was 1,057.56±470.76µm² and increased over the next thirty days. The low diet culminated in 1.669.60±186.31µm² (L); 1,424.22±107.89 µm² (M) for the medium diet and 1,524.87±112.00µm² for the high diet (H) (Figure 2.17). A Levene test indicated that the data were homogenous. An AOV inferred the data to significant (ANOVA, F_3=2.8; p<0.05; n=3773). A Tukey Honest Significant Difference shows a significant difference between the Initial eggs size and the low diet.

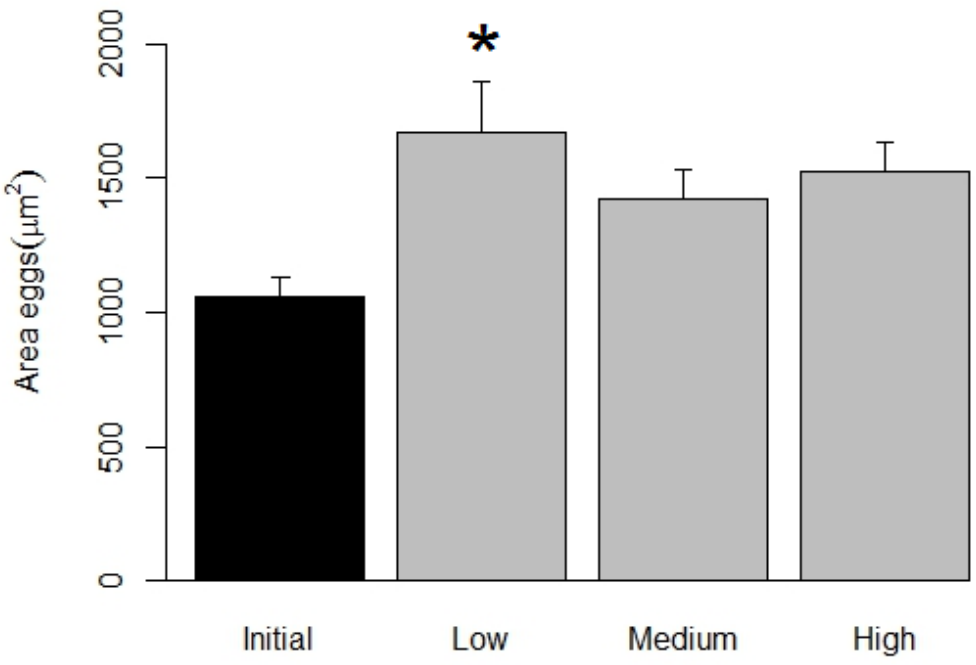


Figure 2.17. Mean (±SE) egg size area (mm2) with initial (I) group and the treatments (L = low, M = medium, H = high food concentration rations). A single star over a bar represents a significant difference between the treatments.

2.5.14 Spawning

More scallops had spawned and partially spawned when fed a low concentration diet, followed by the high concentration diet. Scallops within the initial samples and those exposed to a medium concentration diet had the lowest spawning activity.

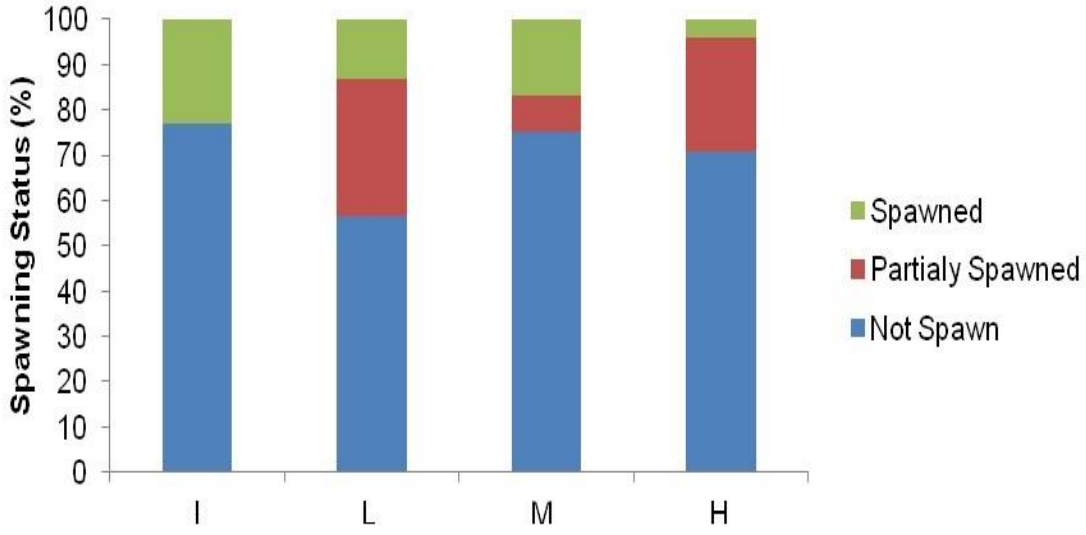


Figure 2.18. Percentage of spawned, partially spawned, and not spawned scallops within initial samples (I), and low (L), medium (M), and high (H) concentration rations.

Table 2.2. Summary of significant outcomes

Description	Significant	Increase / Decrease or static	Significant
GSI	Y	↑	Y
Wet Weight	Y	↓	N
Wet Gonad	Y	↔	N
Dry Weight	N	↑	N
Acini	N	↑	N
Eggs	Y	↑	Y
M/F Colour	Y		Y
Colour Male	N	↔	N
Colour Female	N	↔	N

Legends: ↑ signifies an increase; ↓ a decrease and ↔; no significant difference.

2.6 Discussion

This is the first study to report on the effects of diet on conditioning of *Pecten novaezelandiae*. The results of this study will aid in the assessment and enhancement of reproductive condition of *P. novaezelandiae* for aquaculture production. Specific trends in conditioning state were observed among rations with different microalgal concentrations.

2.6.1 Weight analyses

The mean total tissue wet weight in scallops within the initial food ration group was higher than the low, medium and high food ration treatments, respectively. This trend is commonly observed in animals undergoing gamete development (Laurén, 1982). During this process energy resources are re-allocated from somatic tissue (adductor muscle and digestive gland) to reproductive tissue. Once matured and gametes are released, an overall weight decrease is observed. Indeed, in this study, gonad wet weight and somatic index increased in the scallops exposed to the food rations compared to the initial group. A similar trend was apparent with the gonad dry weight data, although these were not statistically significant. However, the combined gonad weight data do not reflect an increase in weight with increasing food ration, as was expected. This is probably a result of the relatively short duration of the experiment, which did not allow for elucidation of these patterns. In addition, scallops within each tank may have had significantly different food consumption rates, which could not be measured or standardized in this experiment.

2.6.2 Gonad development

The new quantitative method of gonad state assessment with the Hunter Flex colourimeter was used successfully to identify the male and female portion of the gonad. However, this method was not able to identify differences in development among scallops within the initial and food ration treatments for the male portion

of the gonad. This may be due to the fact that sperm development was fast and the majority of male gonad samples were mature. Conversely, the gonad colour analysis did pick up significant differences between the female gonad portion of the initial group compared with those in the low and medium food ration treatments. These results are in agreement with the slower gonad development and broader range of developmental stages observed in the female gonads. Based on these results, this method appears to have potential for use in aquaculture as a semi-quantitative non-destructive method to assess broostock conditioning. However, since this is the first time that this method has been applied for gonad tissues, further studies will be needed to refine this methodology.

Results from the non-destructive visual gonad index data indicate that this is a generally good method for quick assessment of gonadal state, especially with regard to distinguishing differences between male and female developmental states. This visual assessment method also was used successfully by Williams and Babcock (2004) for this species. In this study, the male portion of the gonads were mostly matured (ripe) within the feeding treatments, and slightly less developed (ripe plus active) in the initial group. Also, these individuals had a low proportion of sperm within the early developmental stages (VGI 3-5), which suggests a rapid developmental processes for sperm production. This pattern has been observed for most bivalves, including clams (*Callista chione)*, pacific oyster (*Crassostrea gigas*) and the blue mussel (*Mytilus edulis)*. Conversely, egg development was slower, with a greater proportion of developing stages (VGI 3-5) for individuals in all treatment and initial groups, compared to the male counterparts. In addition, scallops within the initial group were all in the active development stages (VGI 5-6), with no matured or spawning individuals. In comparison, scallops within the feeding groups had a small proportion of matured individuals and a greater range of developing stages. This pattern suggests that scallops which were fed in the experiment matured well, spawned and started to regenerate oocytes. However, no apparent trends were observed with increasing food rations. Previous studies have shown increasing gonad development with increasing food concentration (Heasman et al., 1996; O'Connor et al., 2000). Heasman (1996) found that increasing food concentration from 12.5% to 100% (

$6x10^9$ cells per scallop) resulted in increasingly higher gonadal production and fecundity. The fact that in this study such patterns were not observed may indicate that the food rations may not have differed enough to result in significant effects on gonadal development.

Similar sperm and egg developmental patterns to those of the visual gonad index data were observed with the histological gonad index data, where the majority of male gonad portions of scallops in all groups were matured and the female portions were developing more slowly with a wide range of early development stages. However, histological examination of the initial group revealed a spread of four developmental stages (HGI 1, 5, 6, and 7) compared to the two stages observed with the VGI (VGI 5 and 6).

2.6.3 Histological assessment

Based on the histological analyses, the combined male and female spawning data show that some proportion of scallops spawned within the initial and feeding groups, while partially spawning only occurred within the feeding treatments. Partial spawning has been suggested to be a bet-hedging strategy to ensure greater reproductive success (Williams, 2005). Thus, a release of a small portion of gametes sporadically may improve the chances that some individuals will survive. Alternatively, this approach may be a way to synchronize spawning (Hardege & Bentley, 1997). By releasing a few gametes at a given time, scallops may sense the reproductive maturity of conspecifics in the vicinity. This may be achieved by chemically sensing the presence of gametes of the opposite sex in the water.

An advantage of the histological method is that it can provide superior information regarding tissue structure and development. Indeed, histological analyses have been conducted in reproductive studies of bivalves to obtain a detailed quantitative analysis of gonadal state, including egg size (Kim, Ashton-Alcox, & Powell, 2006; Williams, 2005), number of eggs and follicle area (Royer et al.,

2008). In this study, the acini data showed an increase in size with increasing food ration, but this trend was not significant. In addition, the mean acini size for the initial groups did not differ significantly from those in the feeding treatments. Similarly, the egg size data did not reveal an increase in egg size with increasing food ration, although scallops within these treatments had greater egg sizes than those in the initial group. These patterns may reflect the fact that eggs were spawned throughout the experiment in different proportions among the treatment groups, and new eggs were subsequently developed.

Finally, this broodstock conditioning experiment provided some indication that feeding increases gonad condition, especially for the female portion of *P. novaezelandiae* gonads. However, the study was not able to detect significant differences in gonad development as a response to food concentration. In addition, the different non-destructive and destructive methods used in this study were in general agreement, but clearly the histological analyses were more quantitative and informative that the external visual analyses. Nonetheless, external visual gonad indices and colour analyses may provide sufficient information for aquaculture purposes.

3 Chapter 3: To characterise larval development (morphological, physiological characteristics) of the New Zealand scallop *Pecten novaezelandiae* from D-larvae to pediveliger

3.1 Introduction

The New Zealand scallop, *Pecten novaezelandiae* is an important commercial, recreational and customary fishery. Although this fishery has been in existence from the 1840's the landings began to be officially documented in 1959 (Mincher, 2008). However, the fishery collapsed in the 1980's, and was then revived through scallop enhancement and a Quota Management System (Drummond, 2008). Now landings from the scallop fisheries are once again under pressure and showing a 30% decline in greenweight takes over the last decade (Food and Agriculture Organisation of the United Nations, 2011). Studies for *P.* novaezelandiae have mostly focused on fisheries management, scallop enhancement and environmental assessments (Bull, 1976; Lyon, 2002; Morrison, 1999; Williams, 2005). Few studies have concentrated on the life cycle of *Pecten novaezelandiae*, especially with regard to morphological and physiological descriptions of life stages.

Studies on bivalve larval development have highlighted similarities among planktonic larvae, especially regarding embryogenesis, egg development and early shell development. Often, recognition of the larval species can only be achieved when the larva has developed a shell (Booth, 1983; Brink, 2001; Rees, 1950). Morphological characteristics of the shell and the shell hinges have been used to differentiate lamellibranch families (Brink, 2001; Rees, 1950). Booth (1983) described 12 common planktonic species within Pectinidae, Veneridae, Macridae, Mesomatidae, Haetillidae, Pholadidiae and Teredinidae from New Zealand waters. However, this study did not include embryonic comparisons among these groups, which still require further investigation.

The developmental morphology of scallop species has been previously studied for the purposes of improving a potential fishery via scallop enhancement. Culliney (1974) reared the giant scallop, *Placopecten magellanicus* to describe the larval development, salinity tolerance and settling behaviour. In Australia, the Queen scallop *Equichlamys bifrons* (1976)*, Pecten* meridionali (1975) or now confirmed as the *Pecten fumatus,* (Dijkstra, 2013) was studied to identify the larvae in the plankton and fill the larval knowledge gaps. Rose studied the dough boy scallop*, Chlamy asperrimus,* (1984) and saucer scallop, *Amusium balloti*

(1988) to improve identification of larvae in the plankton. All the above mentioned studies were undertaken under laboratory conditions, but their descriptions covered only the early embryonic stages and not the D-larvae stage or beyond.

The introduction of photography (light microscopes [LM] and Scanning Electron Microscopes [SEM]) has provided a new approach to capturing and measuring the microscopic phases of larvae. Hodgeson & Burke (1988) successfully utilised Electron Microscopy (EM) and LM to characterise the spiny scallop, *Chlamys hastate,* from egg to settlement. Velasco, Barros and Acosta (2007) characterized the embryogenesis of the Caribbean scallops, nuclear scallops, *Argopecten nucleus* and lion paw scallop, *Nodipecten nodosus* using light microscopy (LM).

3.1.1 Cultivation systems

There are three types of seawater delivery systems used in the next phase of larvae culturing for scallops. A static system is where the water is exchanged every few days. A water recirculating systems [RAS] is a close circuit of seawater. Flow Through Seawater [FTS] involves pumping a constant flow of fresh seawater through the tank. Static and recirculating seawater systems have been used successfully in the past whilst culturing the *Pecten maximus* and *Pecten fumatus* (Andersen et al., 2011; Dix, 1976; Dix & Sjardin, 1975; Rose et al., 1988; Utting & Spencer, 1991). Larval culturing in aquaculture has moved to FTS, which may minimise the bacterial accumulation within the tanks and increases larval densities, regardless of whether the water comes indirectly from the ocean or a stored seawater RAS (Magnesen & Jacobsen, 2012; Ragg et al., 2010). However, regardless of the rearing system, antibiotics contribute significantly to lower larvae mortalities and combat bacteria such as *vibrio* sp. (Robert, Miner, & Nicolas, 1996; Torkildsen et al., 2002; Torkildsen, Lambert, Nylund, Magnesen, & Bergh, 2005; Torkildsen & Magnesen, 2004). The most successful antibiotic to date has proven to be chloramphenicol. However, this chemical has been banned in the European Union (Robert et al., 1996; Torkildsen et al., 2002)

3.1.2 Lifecycle

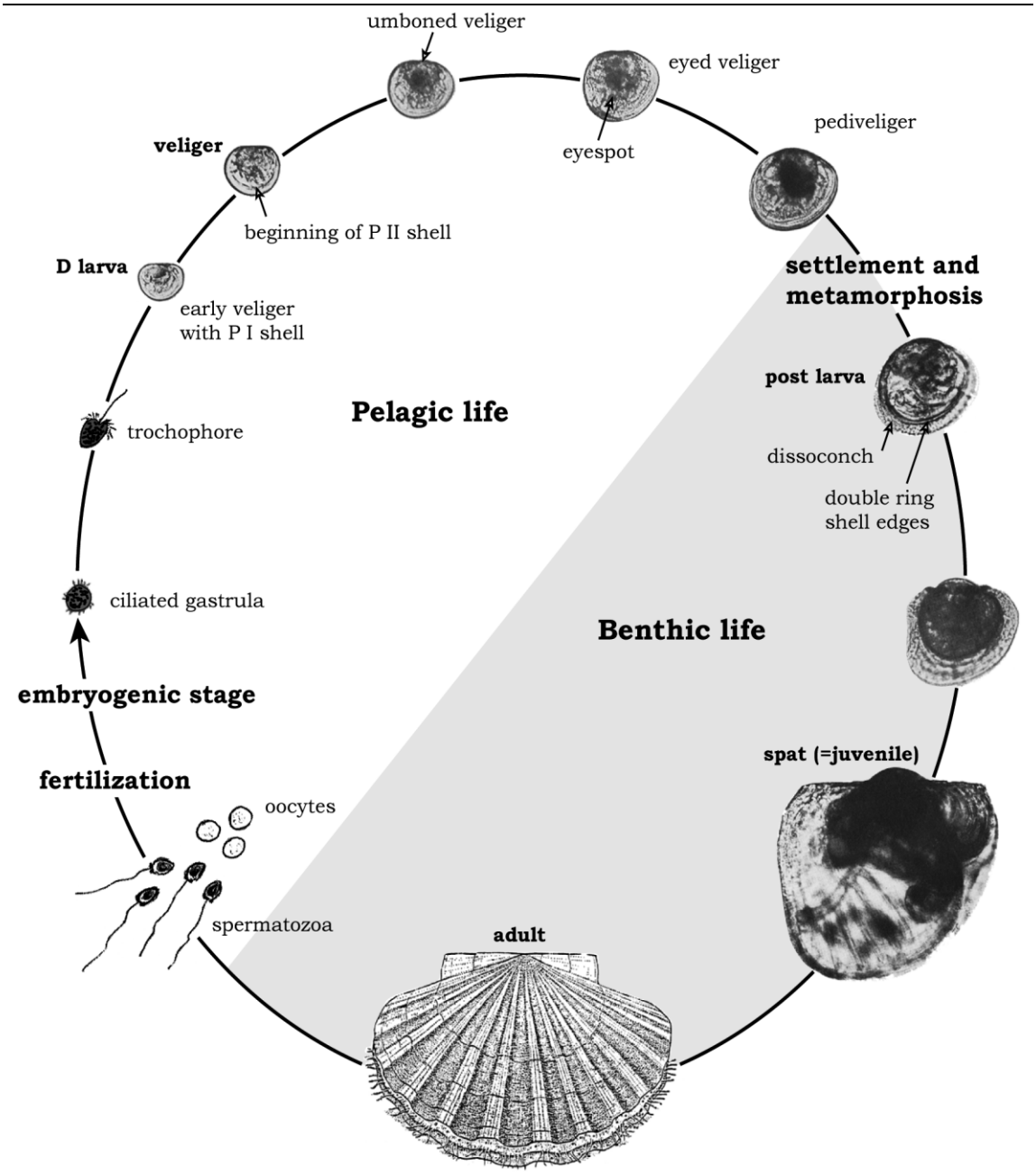


Figure 3.1. Life cycle of Pecten maximus (le Pennec, 2003).

An important prerequisite for successful aquaculture is a good understanding of the life cycle of the organism being cultured. A review by Le Pennec (2003) divided the scallop *Pecten maximus* into pelagic and benthic phases (Figure 3.1). Nine key developmental stages in the pelagic phase of *Pecten maximus* were

described by Le Penn (2003); fertilisation, ciliated gastrula, trochophore, veliger, umboned veliger, eyed veliger, pedivileger. Fertilisation is the process of sperm locating and inseminating an egg. Once this is achieved, the embryo develops into a ciliated gastrula. The ciliate gastrula is identifiable by fine hairs that have grown around the embryo, making the embryo motile. The trochophore has further cilia banding around the zygote and additional morphological and physiological features such as a shell gland. Two shells are secreted from the shell gland, enveloping the soft tissue of the larvae. This stage is represented by the D-larvae and the veliger. The veliger larval phase has a developing secondary prodissoconch emergent from the primary prodissoconch (first shell). The umbone is the shell feature located at the dorsal of the larvae protruding beyond the hinge. Later in the larval development, an eyespot emerges in the centre of the shell. A solitary foot then develops and this indicates that settlement is approaching (Heasman et al., 1994b; Helm et al., 2004; Le Pennec et al., 2003). Four stages were determined for the benthic life of a scallop, relating to the spat and settlement; post larvae; attached with byssal threads, juvenile and adult. However, embryonic stages of *P. maximus* were not covered in any detail by Le Pennec (2003). An intensive study of *Aequipecten irradians concentricus* by Sastry (1965) identified eleven embryonic phases (Figure 3.2) and fourteen shelled, morphological features. These embryonic and larval features identified by Sastry (1965) were used as a guide in this study.

Nicholson (1978) succeeded in recording the times taken from embryogenesis to veliger. The time taken for 1st veliger, 9 min; 1st cleavage 60min; 2nd cleavage, 72min; blastula 9h; trochophore 12h; veliger 28h. Temperatures for the experiment were conducted at 19°C. Nicholson (1978) observed the time it took each stage of embryogenesis to be completed, and found great variations among individuals, but not among populations.

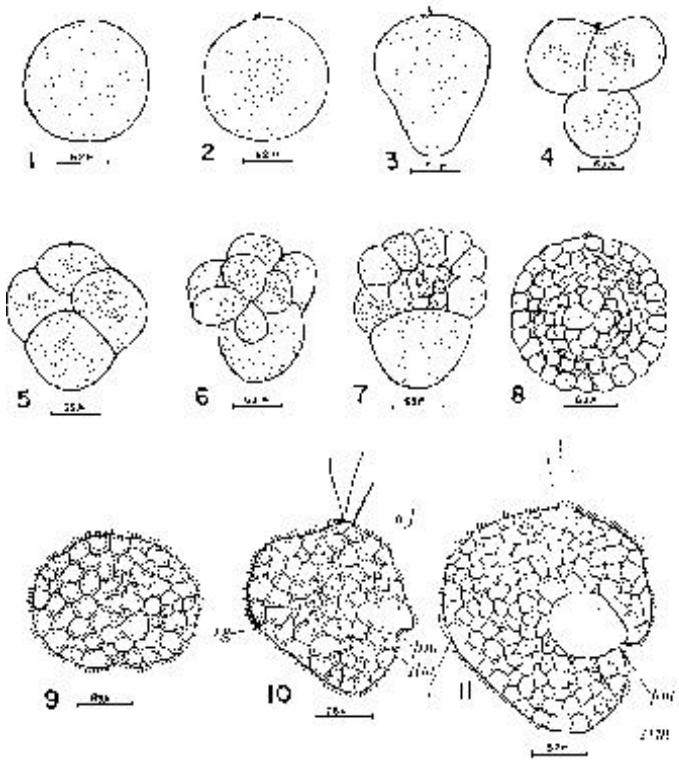


Figure 3.2: Embryogenesis of *Arquipecten irraidians*

1 - unfertilised egg,

2 - fertilised egg with polar bodies,

3 - polar lobed,

4 - first cleavage,

5 - second cleavage,

6 - third cleavage,

7 - fourth 8-cleavage,

9 - blastula,

10 - gastrula,

11 - early trochophore

12 - top shaped trochophore.

3.1.3 Spawning

Spawning the *Pecten novaezelandiae* has shown disappointing results. Nicholson (1978) attempted to spawn *P. Novaezelandiae,* but had limited success. Further experiments by Nesbit (1999) also were unsuccessful. Serotonin has successfully provoked sperm release in *P. Novaezelandiae*. However, this chemical did not induce egg release (Nesbit, 1999; Williams, 2005). Williams (2005) induced sperm release with serotonin, which coincided with egg

release, for his studies on the kinetics of fertilisation. Williams (2005) found that tickling or water jetting, could bring on spawning. The tickling technique is applied by placing an inlet seawater hose near an individual scallop, so a stream of seawater will rush into and over the scallop shell (Williams, 2005). A common method of inducing spawning in scallops and other bivalve is thermal shocking. Using temperature changes for short periods of time can stimulate scallops to spawn (Heasman et al., 1994b; Helm et al., 2004; Nicholson, 1978). Spawning may also be induced by adding microalgae, such as *Pavlov* sp., *Chaetoceros* sp. or *Isochrysis* sp. into the spawning tray (Pazos, Román, Acosta, Abad, & Sánchez, 1996).

3.1.4 Larvae rearing

Scallop gametes can be acquired from matured wild animals collected and maintained under favourable conditions in the laboratory until they spawn. With wild stock, additional feeding of microalgae may improve the probability of spawning (Heasman et al., 1994b; Williams, 2005). The induction of spawning in New Zealand scallops is poorly understood. The most common method used has been thermal shocking, involving alternating exposure to warm and cool water (generally 10°C approximate) for periods of 30-60 minutes. However, this method is unreliable and there is no guarantee that spawning will be induced. Injections of serotonin into the gonad to induce spawning was explored by Willliams (2005), Nesbit (1999), Heasman (1994b). Following injection, scallops would release viable sperm within half an hour, but the injections had no apparent effect upon the release of eggs. A common method with oysters is ‘strip-spawning’ in which the gonad is cut open and gametes transferred by pipette into spawning beakers (Helm et al., 2004). Upon achieving a successful spawning and fertilisation, the embryos are transferred into static tanks filled with fresh seawater and gently aerated until they mature into D-larvae (Heasman et al., 1996; Helm et al., 2004). Preliminary testing has shown strip spawning to be unsuccessful with *P. novaezelandiae* (Personal observation, N de Jong).

Temperature is indicated as a primary factor in the growth of embryo and larvae (Heasman, 2007; Sastry, 1965). Although temperature influences the growth of larvae, temperatures was not used as a factor in this study.

3.1.5 Larval feeding

Heasman (2007) suggests that a ration of 2,000 cells per larvae per day is the optimal initial algal ration for *P. fumatus* larvae. Thereafter, Heasman (2007) prescribed the following formula for calculating feeding ration in relation to larval size:

$$number\ of\ algae\ cells = 0.0765 \times shell\ length\ in\ \mu m$$

However, Heasman (2007) stressed that the amount of food in the gut is the primary analysis for increasing or decreasing the algal concentration. There must be microalgae in the majority of the larvae guts being sampled. However, the gut should not be excessively crammed with microalgae. This nutrient delivery model delivers cells based on the number of larvae and the size and will be referred to as dynamic delivery [DD].

Navvaro & Pascual (2001) fed 30,000 cells to the tehuelche scallop, *Aequipecten tehuelchusI* over 18 days from D-larvae to pediveliger without varying the ration. This delivery of nutrients will be referred to as static delivery [StD]. These microalgae feeding concentrations indicate a potential feeding regime for *P. novaezelandiae* larvae. A combination of DD and StD was employ during this study.

3.2 Aim

This research aims to characterise the prominent morphological and physiological changes in *Pecten novaezelandiae* from spawned gametes through fertilisation, embryogenesis, and larval development to pediveliger. This

information will inform comparative studies of larval development and will be fundamental to the establishment of a hatchery-reared spat program for re-seeding wild beds and/or aquaculture production.

3.3 Methods and Materials

3.3.1 Broodstock

Three separate scallop broodstocks (SN1, SN2 and SA3) were collected for test spawning to obtain scallop larvae for investigation of lifecycle stages. SN1 and SN2 were collected from Charlotte Sounds, Nelson, South Island and from Crail Bay Aquaculture Ltd, Marblorough, South Island, respectively. Both these broodstocks were spawned at the Cawthron Institute, Aquaculture Park, Nelson, New Zealand. Broodstock SA3 was collected from Jones's Bay Tawharanui, Hauraki Gulf North Island and these animals were spawned at the Auckland University of Technology (AUT), Auckland, New Zealand. Scallops greater than 65mm across and with ripe gonads, based on visual inspection, were cleaned of epifauna and transferred back to the laboratory where they were placed in either a free flowing or re-circulating seawater system. Animals were maintained at 17˚C for one week. SN1 and SN2 were fed *ad libitum* a diet of *Chaetoceros calcitrans*, *Pavlova lutherii*, *Chaetoceros muelleri* and *Isochrysis galbana*. SA3 was fed the commercial diet Reed Mariculture Shellfish Diet 1800.

Weekly observations were made to determine when the scallops were ripe for spawning. To corroborate the reproductive state, three animals were sacrificed to remove gonad tissue for histological analyses. These gonad samples were placed in Davidson's solution for a week until they could be processed. The samples were then passed through a gradient of ethanol baths before being embedded in paraffin wax. Five micrometer sections were then cut, mounted and stained using Haematoxylin and Eosin stains as described by Howard (1983).

3.3.2 Spawning procedure

Matured scallops were placed in spawning trays and exposed to air for 30-45 minutes or until the animals were gaping. The spawning tray was then filled with 17°C seawater and left until the individuals reverted to a relaxed state. The relaxed state was identified when the scallops opened their shell in the filtering position and the tentacles protruded out from the shell. Scallops were then put through a thermal shock regime of first hot (25˚C) water for 30 minutes and then cold (17˚C) water for 30 minutes. A temperature change of 10°C has been suggested to produce scallop spawnings (Heasman, 2007; Helm et al., 2004). This process was repeated for seven thermal cycles or until spawning was observed.

In some cases, additional spawning techniques were used when temperature shock was not successful. These included food, chemical inducers and water jetting. Microalgae were added into the spawning in an attempt to induce spawning. Another attempted methods used was natural chemical cues. The gonad was stripped of its gametes by slicing open the gonad sac and extracting the gametes or by adding fresh sperm. Fresh sperm was obtained by injecting 1.5ml of serotonin (Sigma H7752-1G), into the male gonad. Of the additional methods used adding food and fresh sperm assisted in spawning the scallops.

When a scallop was observed spawning, it was removed from the spawning tray and placed in a spawning bowl, filled with fresh seawater (Heasman, 2007; Helms, 2004). The scallop ware removed from the spawning bowl and placed in a fresh spawning bowl every 15-30 minutes. The more gametes expelled by the scallop, the more frequent bowl changes occurred. If the scallops spawning altered from male to female or *vice versa*, the contaminated gametes were discarded.

Once gametes were obtained eggs and sperm were counted under a microscope and placed in a 1L glass beaker filled with fresh filtered (1µm) seawater [FFSW], at ratio of 25:1, respectively. The gametes were exposed to gentle aeration at a temperature of 20°C until fertilisation took place. Zygotes were then transferred into 1L beakers, filled with FFSW, and gently aerated at the same temperature.

3.3.3 Larval rearing and sampling

The fertilised eggs of SN2 were placed in 170L conical tanks with 1µm filtered seawater. The zygotes were left for three days during which time random samples were taken for examination under a light microscope to determine the progress of embryogenesis. After three days, the majority of the larvae had reached D-larvae stage and were then transferred into the Cawthron Ultra-Density Larvae rearing System [CUDL] (Figure 3.3) (Ragg et al., 2010). The larvae were separated into six CUDLs at 200 larvae per ml. Seawater was kept at 19°C with a flow rate of 65ml/min per CUDL. Three CUDLs were treated with chloramphenicol (Calbiochem 220551) after cleaning the larvae. The larvae were place in a CUDL containing two litres of filtered seawater at $8mgl^{-1}$ for four hours, on and ten of the study, for four hours before resuming the flow through seawater (Robert et al., 1996; Torkildsen et al., 2005; Uriarte, Farías, & Castilla, 2001).

The larvae were fed a combination of *Chaetoceros calcitrans* (CC), *Pavlova lutherii* (PAV) and *Isochrysis galbana* (ISO). Initially the larvae were fed only CC at 20,000 cells per ml per day. The diet was then modified to a 7: CC: 2 ISO: 1% PAV ratio over the course of fourteen days and by measuring clearance the microalgal clearance rates per CUDL with a fluorometer. The fluorometer was designed by Cawthron Institute to detect chlorophyll [CHl A] at an excitation 440nm (blue) and detecting the light emitted at 680nm (red) ranges (N. King, personal communications, 30/03/2012). Concentrated suspensions of each microalgal species were pumped at constant flow rates into a header tank and were mixed by gentle aeration. The contents of the header tank were then pumped into the CUDLs. The clearance rate of microalgae from the CUDL water column at the time of sampling was determined fluorometrically. A measurement of Chl A from the inlet and outlet of the CUDL was taken daily.

$$Chl\ A_{clearance}\ (mV) =,\ Ch\ lA_{in}\ (mV) - Chl\ A_{out}\ (mV).$$

Increasing and decreasing the feeding rates was corroborated by the observation of food particles in the larval guts and the ambient algal density.

Once in the CUDL system, larvae were sampled every two days when the larvae were washed. This was accomplished by draining the CUDL onto a 45µm mesh,

then gently hosing the larvae with fresh filtered seawater. Poor performing individuals (i.e., small individuals that passed through the mesh) were removed from the tank with a selective sieving range from 45 µm to 120 µm over 30 days. Larvae sizes were calculated under the microscope and by using imaging software as discussed below.

Figure 3.3. Cawthron Ultra Dense Larval Rearing (CUDL) system

Eggs, zygotes, and larvae at different stages of development were sampled from the beakers or rearing tanks using a pipette and placed into 1.5ml polypropylene centrifuge tubes. The samples were spun at 500rpm for 30 seconds in a Labnet, Prism R centrifuge, to create a loose pellet of larvae. The excess seawater was then removed and replaced with either Davidsons Solution or Glutaralderhyde, and the two samples were used for light and SEM sampling, respectively.

3.3.4 Light microscopy

Photographs of live larvae were obtained using an Olympus CK2 light microscope with an Olympus C-7070 camera or Leica DM2000 light microscope and a Leica DFC290 camera. All images were scaled by comparison to an image of a haemocytometer grid under the same magnification.

3.3.5 Scanning Electron Microscopy

Samples for SEM were preserved with Glutaraldehyde and Davidson's solution (Appendix A). SEM samples were removed, with a pipette, from the 1.5ml eppindorfs and gently transferred to a modified 1.5ml eppendorf tube, which had 5mm holes drilled through the cone and cap, and washed to remove debris. A 20µm cloth mesh was inserted and held in place by the eppendorf cap. The samples were then placed in the modified eppendorf tube and washed in increasing gradient baths of ethanol before being placed on a SEM stud, with a transfer pipette, or by placing the filter onto a two sided adhesive tape attached to the stub, and air dried in a fume hood for 48 hours. The mounted sample was then coated with a Hitachi E-1045 Ion Sputter at 6 Pa, 25 mA for 40-60 seconds. The SEM images were captured with a Hitachi SU-70 FE-SEM with scale bars superimposed from the microscope's internal calibration.

3.3.6 Sample analyses

When sampling zygotes, the samples were taken from the beakers with a pipette and observed under a light microscope. Larva samples were removed from the CUDLs before washing, placed in a microscope well, where the larvae could be photographed and observed. Larvae were observed for how much food was in the gut and how much ambient microalgal concentration was in the surrounding seawater, Each larva was measured on the longest shell axis (posterior to anterior) using the ocular measurer and ImageJ software (Abramoff et al., 2004; Baron, Diter, & Bodoy, 1989; Dix & Sjardin, 1975). A T-test was performed to determine if there was any significant difference between the treated and untreated larvae.

3.4 Results

Sperm were released on most attempts to spawn the scallops. Approximately, one successful spawning in six attempts yielded both male and female gametes. In total, nine scallops were induced to release spermatozoa and four scallops released eggs.

Care was taken to limit self fertilisation. However, a small portion of self fertilised eggs took place with all fertilisations. A 100% mortality rate was experienced with spawning SN1 and SA3 by day four. SN2 released ~ 3,000,000 eggs which were fertilised. Of the eggs fertilised, 888,000 developed into D-larvae. The remainder of the larvae had been compromised through either through self fertilisation or antibiotics. These uncompromised larvae were grown out in the CUDLs over a period of 30 days. During this time they exhibited 80% mortality.

Spawnings SN1 and SN3 yielded results for the early phases of *P. novaezelandiae* for light microscopy and SEM. The SN2 produced 88,000 larvae spawned of which 20,000 survived to day 17 and 98% mortality was experienced by day 30. There was no significant difference between the untreated larvae and the larvae treated with chloramphenicol ($p>0.05$) when the CUDLs were pooled by treatment. However, there was not enough replication in this study for the results to be robust. The larvae were washed and sieved on nine occasions within the study. The sieves ranged between 62µm and 125µm. The inferior larvae were removed from the study and discarded. A constant growth rate of 6.59 + 74.337µm was recorded for the first 30 days of the larvae (Figure 3.5).

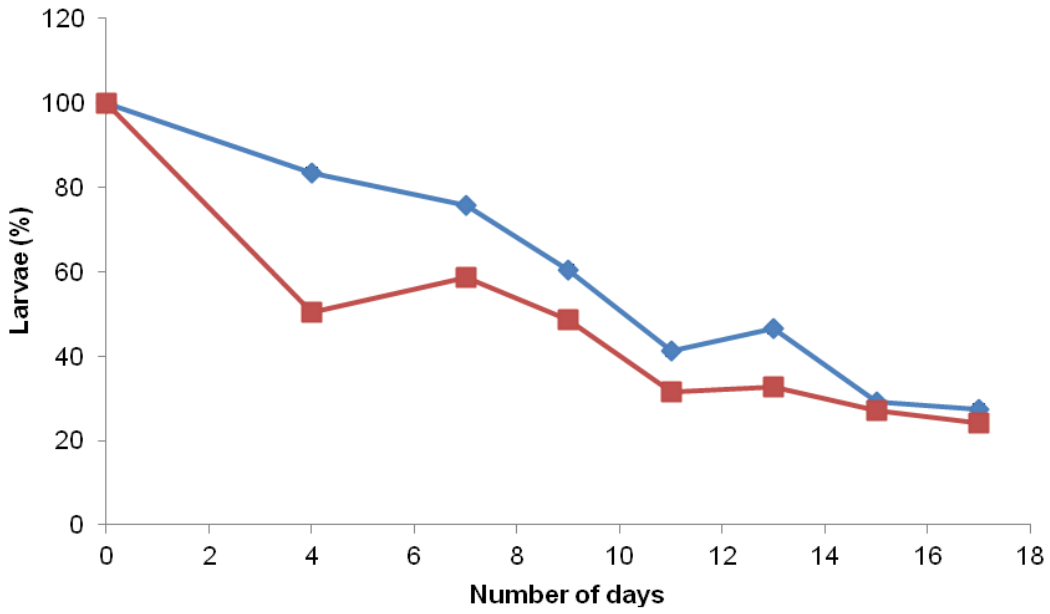
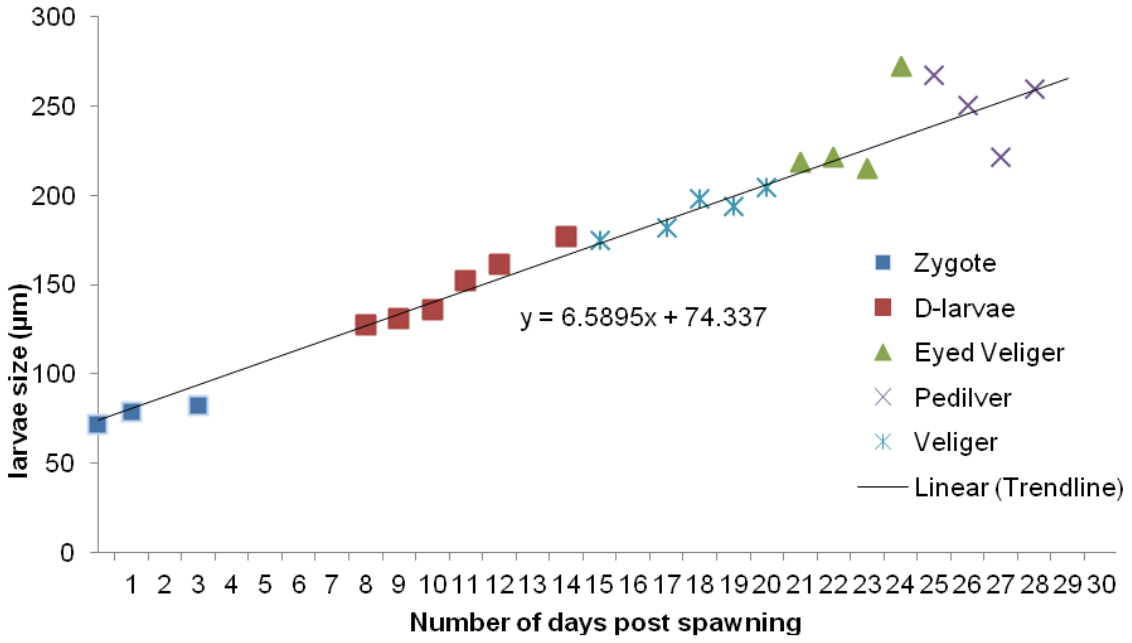


Figure 3.4. Survival (5) of larvae (diamonds- untreated, squares – treated)

3.4.1 Growth rates

There was a strong linear growth trend for *P. novaezelandiae* larvae. Regression analysis of larval diameter revealed a trend against time corresponding to 6.5895 × age in days + 74.337. The larva grew in diameter at an average rate of 5.92 µm per day over the 30 days of the study.



3.5. *Pecten novaezelandiae* size grouped by larval phase (zygote, D-larvae, veliger, pediveliger and eyed veliger).

3.4.2 Clearance rates

At four days post-spawning the SN2 larvae were presented with 2,000 algal cells per larvae per day. In terms of chlorophyll fluorescence, this was measured as 34.58±0.84 mV (mean±SE) Chl A (Figure 3.6). The Chl A concentration was increased to 124.4±1.33 mV on Day 8. The Chl A concentration was lowered to 80.05±1.54 mV Chl A on day 10. The decrease was attributed to declining microalgal densities in the rearing tanks. Increases in microalgae of 218mV of Chl A were delivered by day 20. An average of 192.22±18.68 mV of Chl A was maintained until settlement. The larvae cleared 65.4±2.59% of the Chl A concentration throughout the 30 days of culturing.

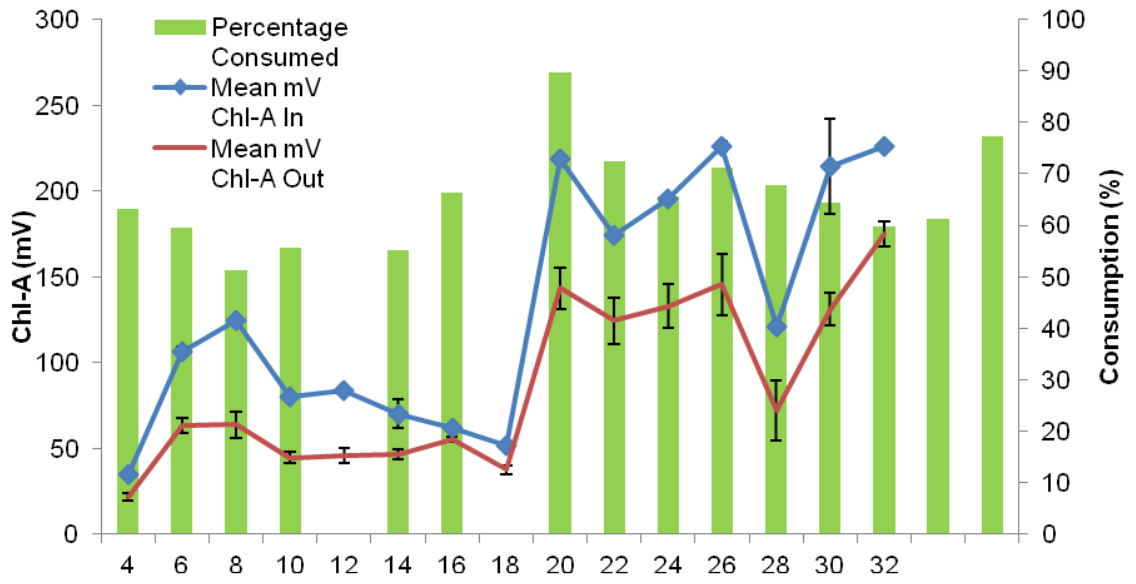


Figure 3.6. Larval clearance rates of microalgal Chl-A, measured as fluorescence in millivolts and consumption of microalgae (%) over 30 days.

3.5 Embryogenesis

The development of the embryos matched closely that of *Aequepecten irradians concentricus* (Sastry, 1965). Images of the various stages of embryonic and larval development identified by Sastry (1965) in *A. irradians concentricus* are presented for comparison alongside similar images acquired in this study of *Pecten novaezelandiae* (Figure 7-18).

3.5.1 Sperm

Scallop sperm were released in short puffs (T_0) and they had a white appearance. A constant release of the sperm was achieved once full spawning was underway. The sperm nucleus (n) from the tip of the acrosome to the base of the centrioles was 2.14 ±0.05 µm in length and 1.61 ±0.07 µm in breadth (n=7), the broadest central plane of the sperm. Three centrioles are visible (Figure 3.7 A). The length

and breadth of the centriole (c) was 0.44 ±0.04 µm and 0.51 ±0.05 µm (n=7), respectively. The aristole length was 0.39 ±0.02 µm (n=7), from the tip of the aristole to base of the aristole. The sperm tail (t) or flagellum diameter was 0.11 ±0.02 µm thick (n=5). Micropores emerge on the nuclei appear to be consistently located.

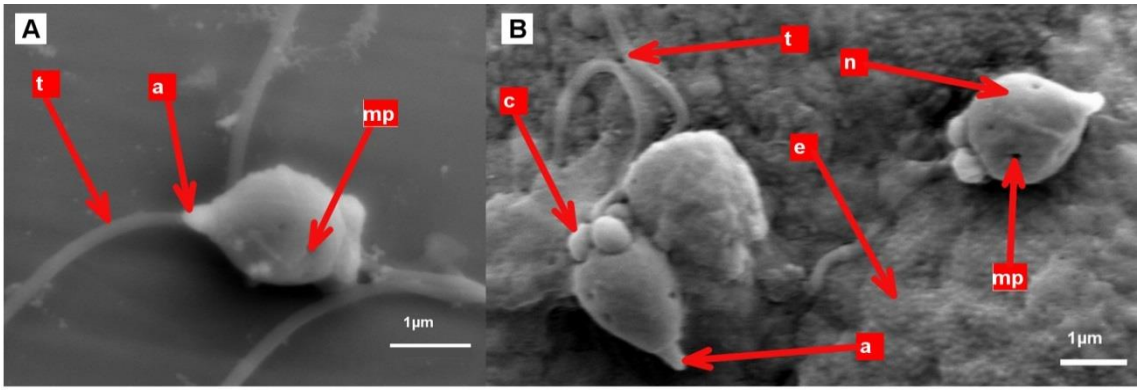


Figure 3.7. Sperm.

A. Spermatozoa of *P. novaezelandiae* (SEM).
B. Spermatozoa of *P.* novaezelandiae resting on an egg (SEM)

(a = acrosomes; c = centrioles; e = egg; t = sperm tail; mp = micropores).

3.5.2 Unfertilised egg

Histological slides of sperm and eggs show the sperm nuclei stained black (s) and the eggs pink with the dyes haemotoxylin and eosin (Figure 3.8 B). Spermatozoa develop in a centripetal manner with the sperm tails towards the cavity of the acinus (ac) within the gonadal sac. Before the eggs (oc) are released from the gonad they are compactly compressed within the acini adopting a berry-like shape (ac). The small black dots within the egg (e) the nuclei (en) of the cell and the darker pink vittelin (vit). When spawned the eggs are an irregular shape (Figure 3.8 A, C and D) and are orange in colour. However, the egg appears brownish under a light microscope. The irregular shape disappears within an hour.

After the first hour (T+1h) the egg completes meiosis. This process expands the oocytes into a spherical form (Figure 3.9). *P. novaezelandiae* eggs measured between 70µm - 90µm. A thin jelly coat (jc) (Figure 3.9 B) may be observed around some eggs. While thermal shocking a scallop a thick clump of jelly with

eggs trapped may be forcefully expelled out of the gonad. The jelly packet dissolved within 24 hours. The vittelin membrane, egg surface, had a sponge-like texture covering the entire surface (Figure 3.9. C and D).

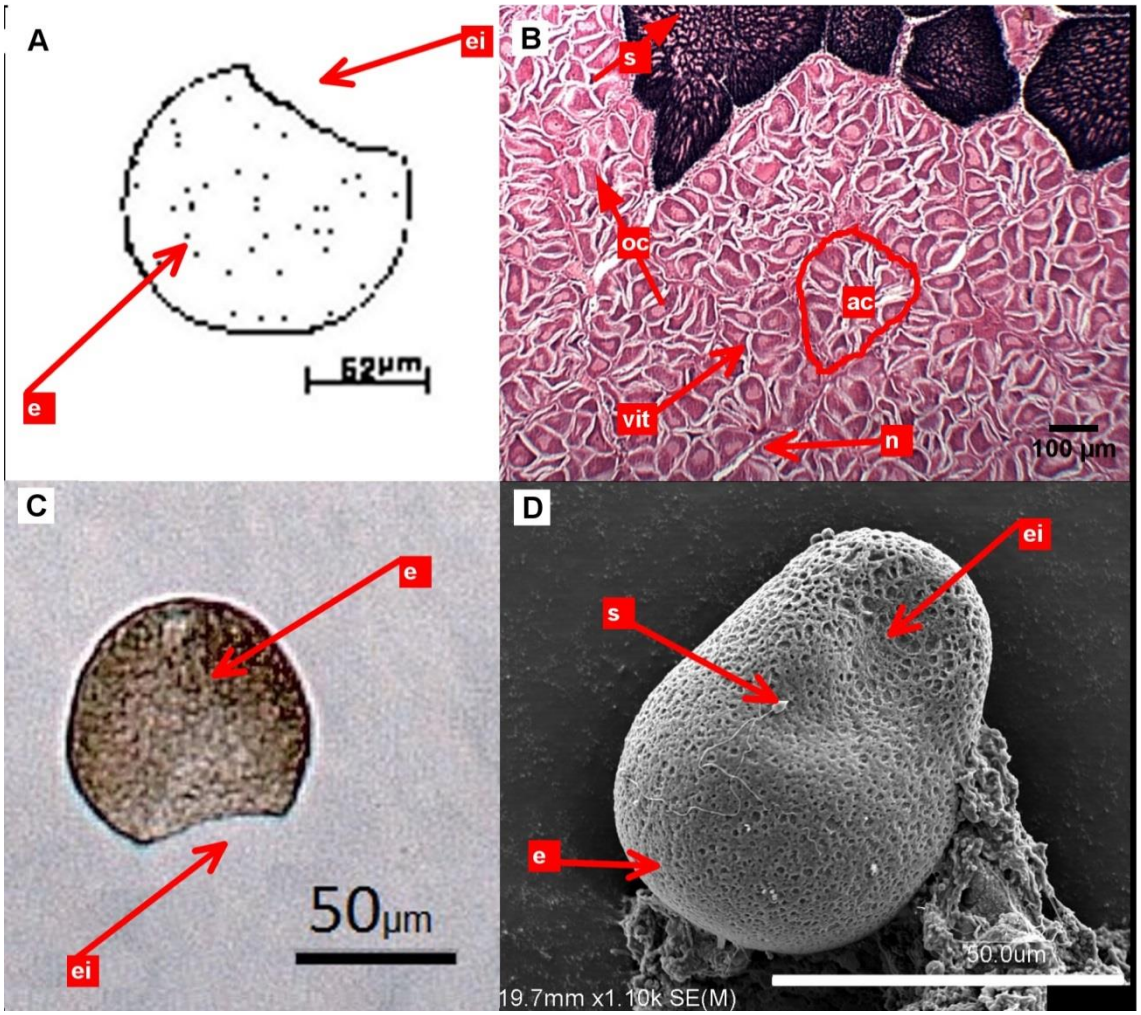


Figure 3.8. Unfertilised egg.

A. *A. irradians* recently spawned with the egg still indented.
B. Histological section of *P. novaezelandiae* gonad before spawning (LM).
C. An indented *P. novaezelandiae* egg before *meiosis* (LM).
D. Spawned egg, still depressed before meiosis (SEM).

(ac = acini; e = egg; ei = egg impression; en = egg nucleus; oc = oocyte; vit = vittilen)

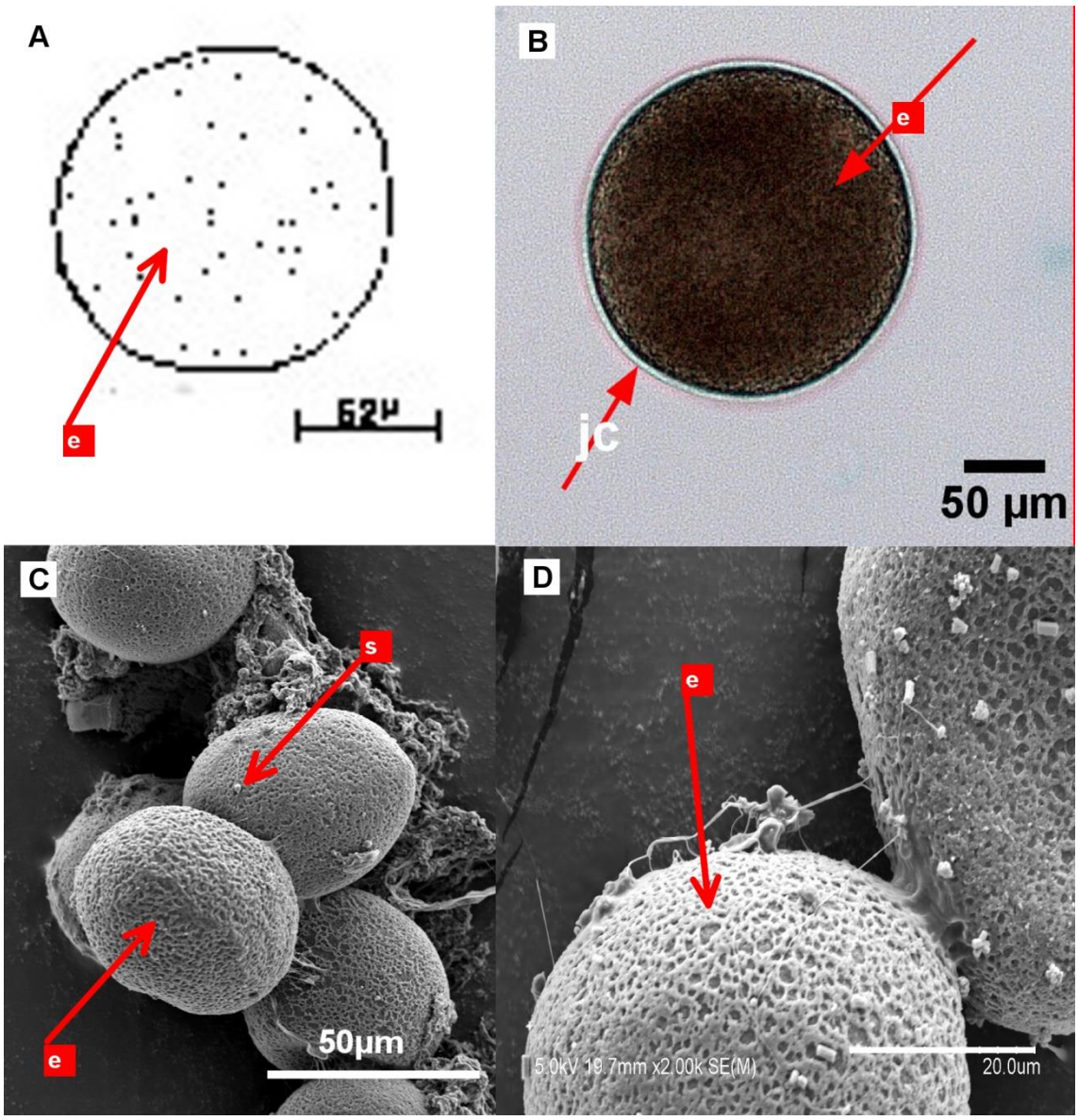


Figure 3.9. Healthy**,** unfertilized egg.

A. *A. irradians* unfertilized egg undergoing meiosis*.*

B. P*. novaezelandiae unfertilized egg with jelly coat surrounding the egg* (LM).

C. A group of *P. novaezelandiae* eggs unfertilized (SEM).

D. A healthy unfertilized egg with sperm and diatoms resting on the egg

(dia = diatom ; e = egg; jc = jelly coat; s = sperm; t = sperm tail; SEM)

3.5.3 Zygote

Once the sperm penetrates the egg, fusion occurs immediately with the mitochondria from the centriole being transferred instantly into the egg changing

the chemical composition. This stops any other sperm from penetrating the egg. Sperm remain visible upon oocyte surfaces up to three days post-fertilisation.

Following fertilisation a small polar body appears almost immediately (Figure 3.10B, C and D). The polar body is commonly a small cylindrical protrusion. However, the polar body can differ in shape from the normal shape previously described (Figure 3.10D).

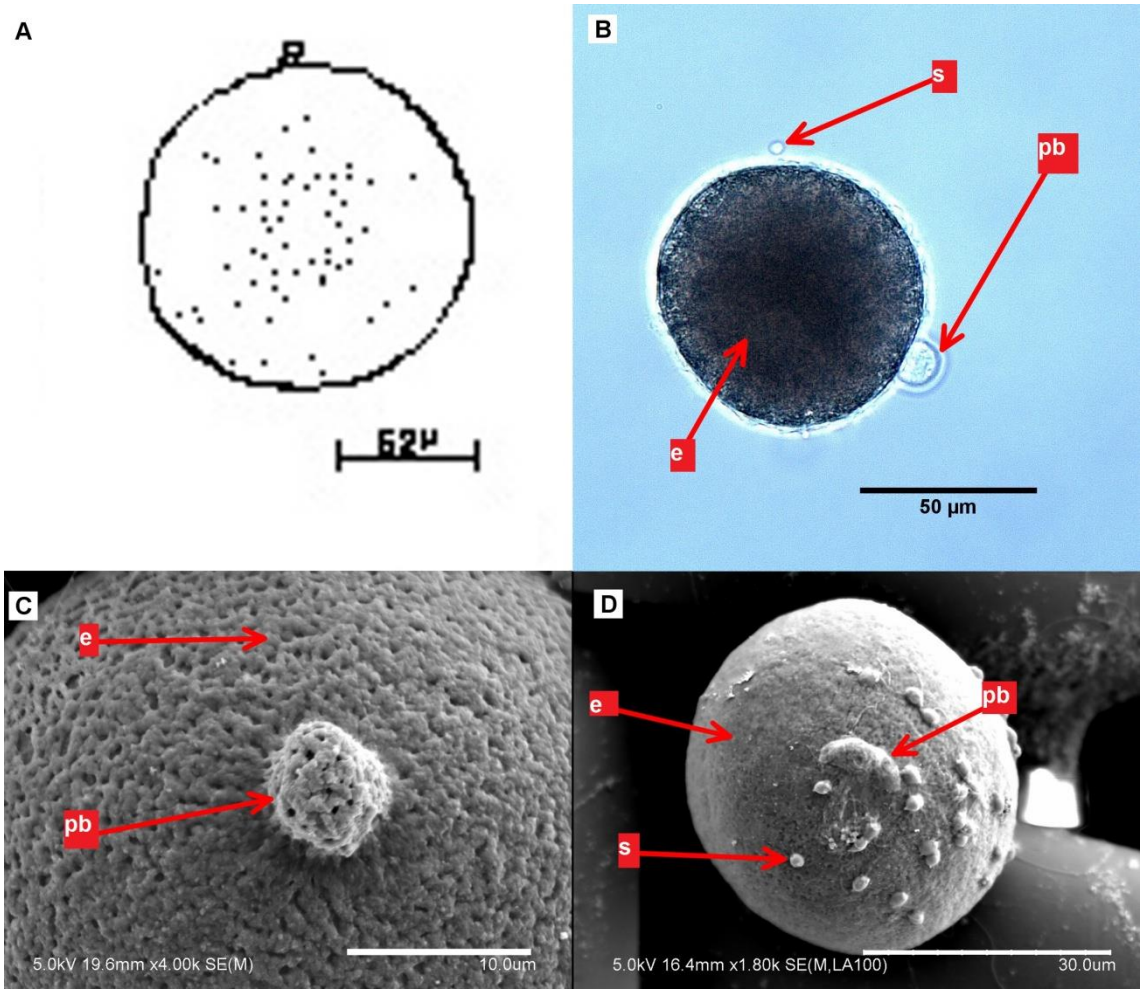


Figure 3.10. Polar bodied zygote.

A. *A. irradians zygote with a polar body protruding*.
B. Zygote of *P. novaezelandiae* (LM).
C. A sagital view of the polar body (SEM).
D. A sagitical view with the polar body protruding and sperm resting on the zygote (SEM).

(e=egg; s = sperm; pb = polar body)

3.5.4 Polar lobed zygote.

The zygote begins to expand prior to cleavage. The primary expansion of cytoplasm (ct) occurs on the vegetal pole to the primary polar body, named the animal pole for referencing (Figure 3.11A and B). Expansion of the cytoplasm creates a blastomere (bl). This division of the cells may also be referred as double cleavage (C). The polar lobed form is evident approximately 15 minutes post-fertilisation [PF].

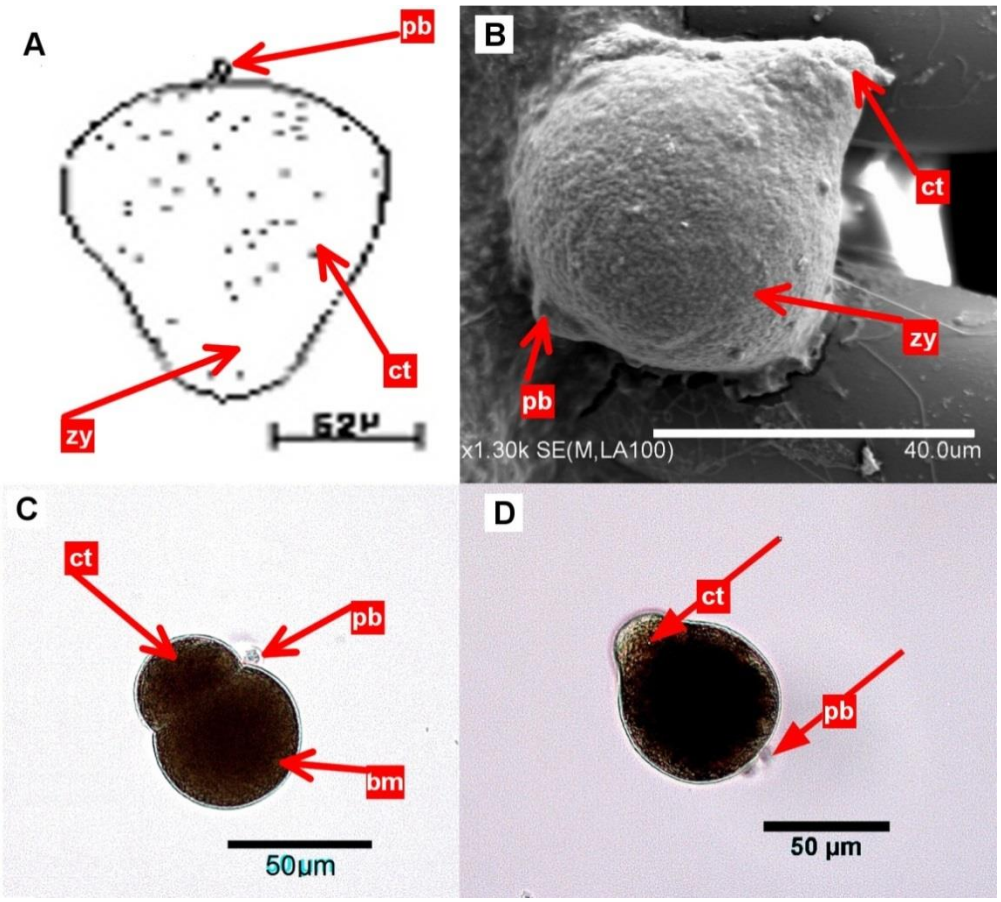


Figure 3.11**:** Polar lobed.

A. A polar lobed shaped *A. irradians*

B. Cytoplasm expanding from within the zygote creating a protruding lobe (SEM).

C. A zygote developing morphing into triple cleavage (LM).

D. Early cytoplasm growth (LM)

(pb = poloar body; ct = cytoplasm; zy = zygote).

3.5.5 Cleavage blastomeres and cell division

After two to four hours post-fertilisation, three prominent blastomeres were distinguishable (Figure 3.12 A, B and C). Cell division appears to have been irregular during the initial stages of embryogenesis, *circa* 4hrs PF. The cells divided exponentially to form a rounded gastrula of approximately 64 cells at 24 hrs PF (Figure 3.13). The cells or blastomere adopted a spherical form during the later part of embryogenesis at 48 hrs PF.

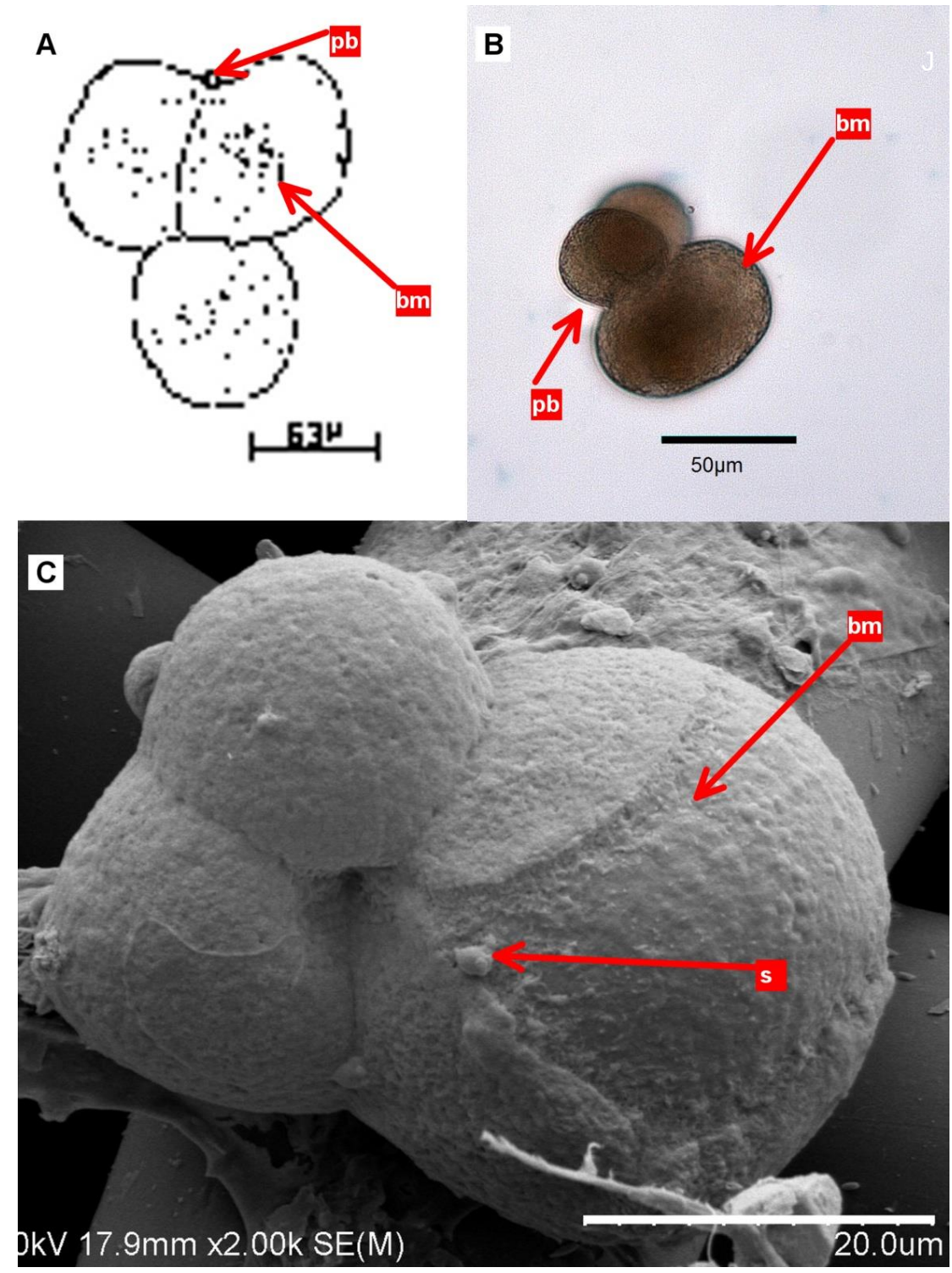


Figure 3.12. Cleavage blastomere's developing 2 hrs PF.

A. *A. irradians* blastomere.

B. Triple cleavage zygote experiencing cell division (77.83µm; bl = blastomere; pb = polar body; LM).

C. Sperm is still evident 3 days after fertilisation (46.42 µm;s = sperm; bl = blastomere; 20µm = 20µm mesh; SEM)

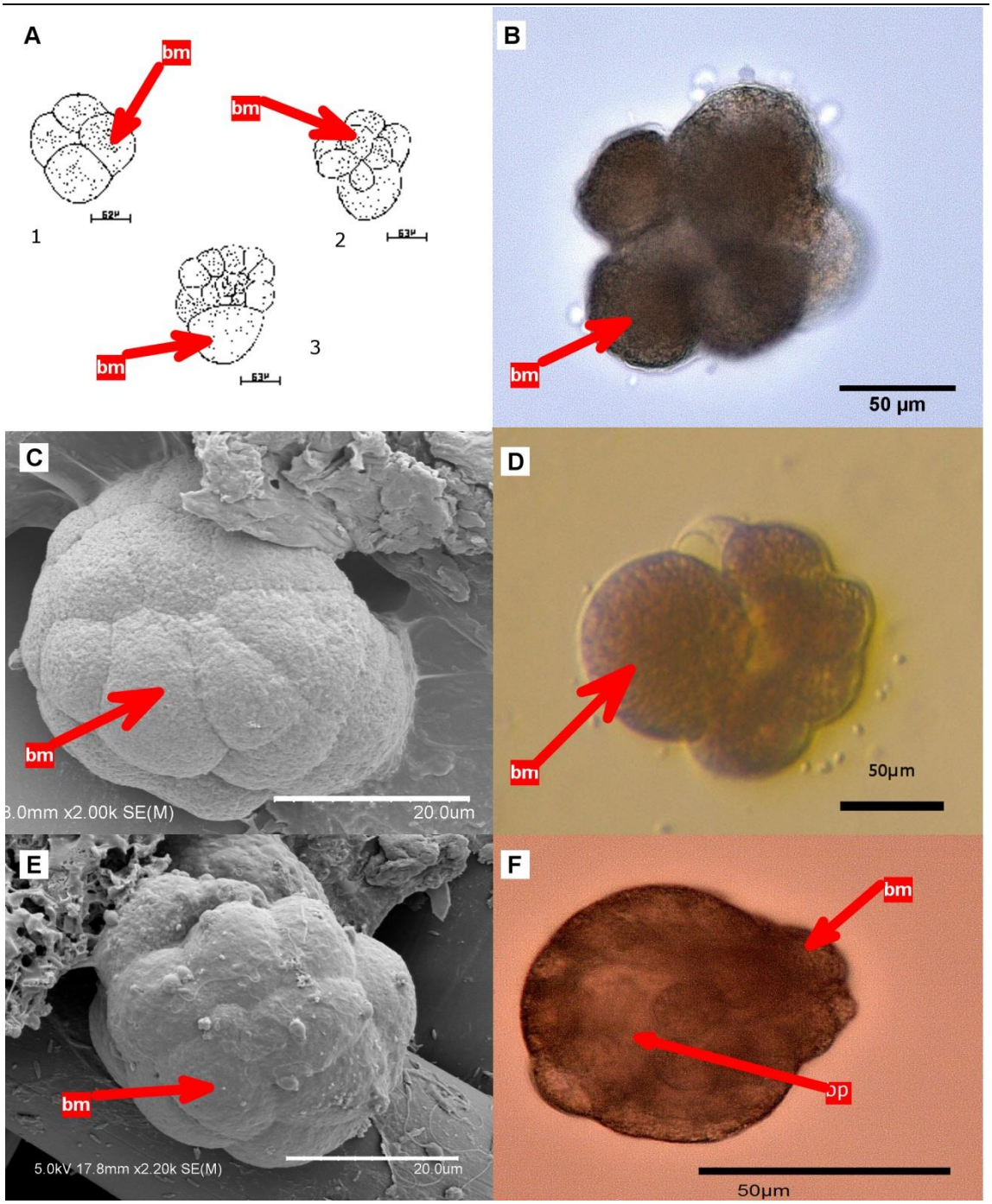


Figure 3.13**:** Cell division of the zygote over 48 hours PF:

A. *A. irradians* cellular divisions into blastomeres.

B. Six visible blastomeres

C. *P. novaezelandiae* blastomeres (SEM).

D. *P. novaezelandiae* blastomeres (LM)

E. *P. novaezelandiae* blastomeres (SEM).

F. The irregular shape of the embryo late into embryogenesis (LM)

3.5.6 Blastula and gastrula.

The blastula after 48 PF was composed of numerous micromeres. The overall embryo shape changed from spherical to elongate. Cilia at 58h PF were now apparent and the gastrula was motile within the water column. A blastopore (bp) was now evident (Figure 3.14 B. Cilia around the apical tuft were visible.

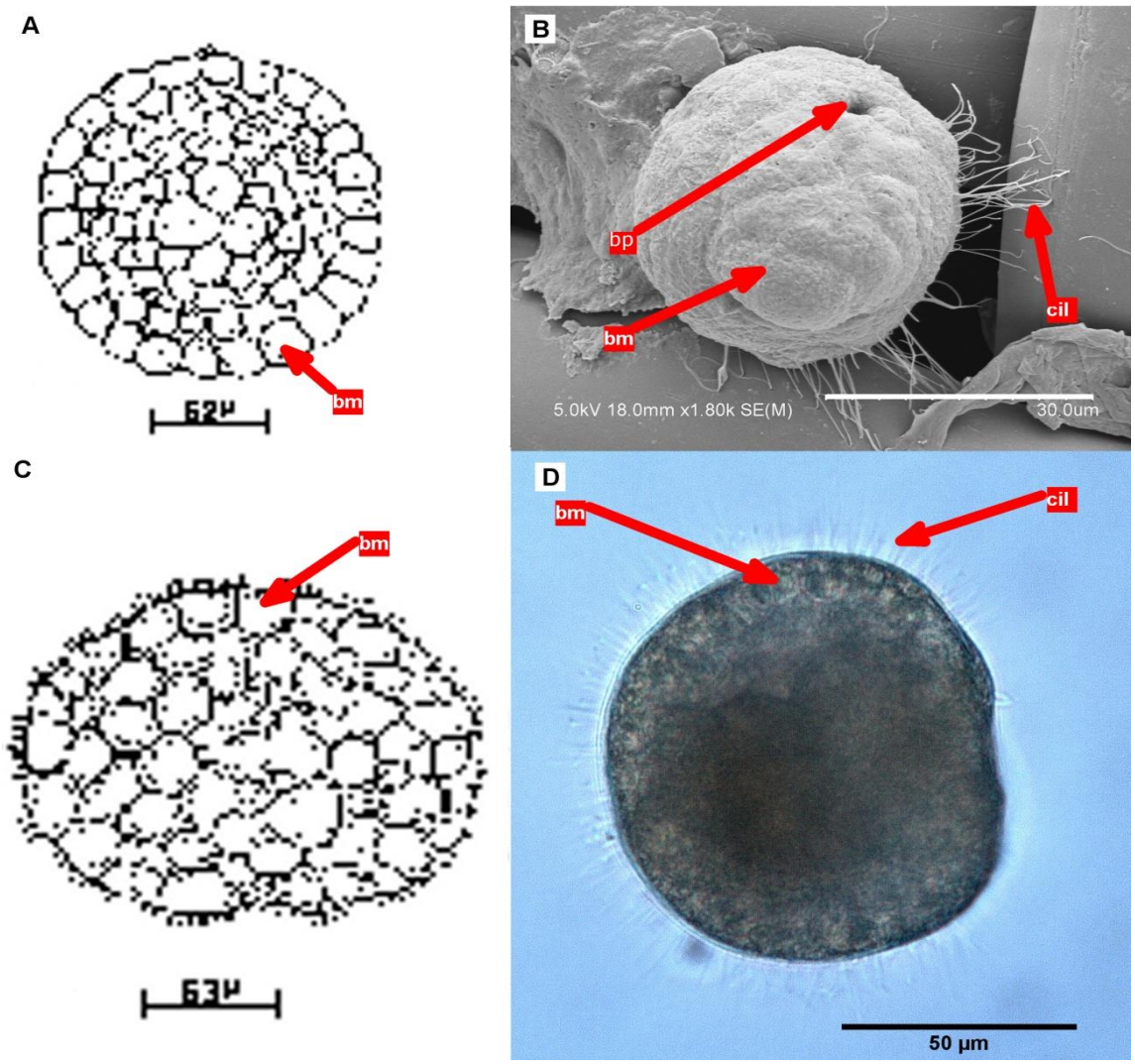


Figure 3.14. Blastula and gastrula ;

A. *A. irradians* blastula composed of micromeres or small blastomeres.

B. *P. novaezelandiae* cilia around the apical tuft with a blastopore.

C. A. irradians gastrula altering its shape to become more elongated.

D. P. novaezelandiae gastrula with the cilia and micromeres.

(cil = cilia; bl – blastomeres)

3.5.7 Trochophore

By ~48 hrs PF the larvae had grown into a pear-shaped trochophore (Figure 14). A long flagellum extended from the apical pole. The flagellum was made up of banded cilia (Figure 3.15D). A blastopore was visible. Within the glastopore a gland excreted a shell (Figure 3.15 A and B). Cilia were visible around the prototroch and teleotroch (Figure 3.15 B).

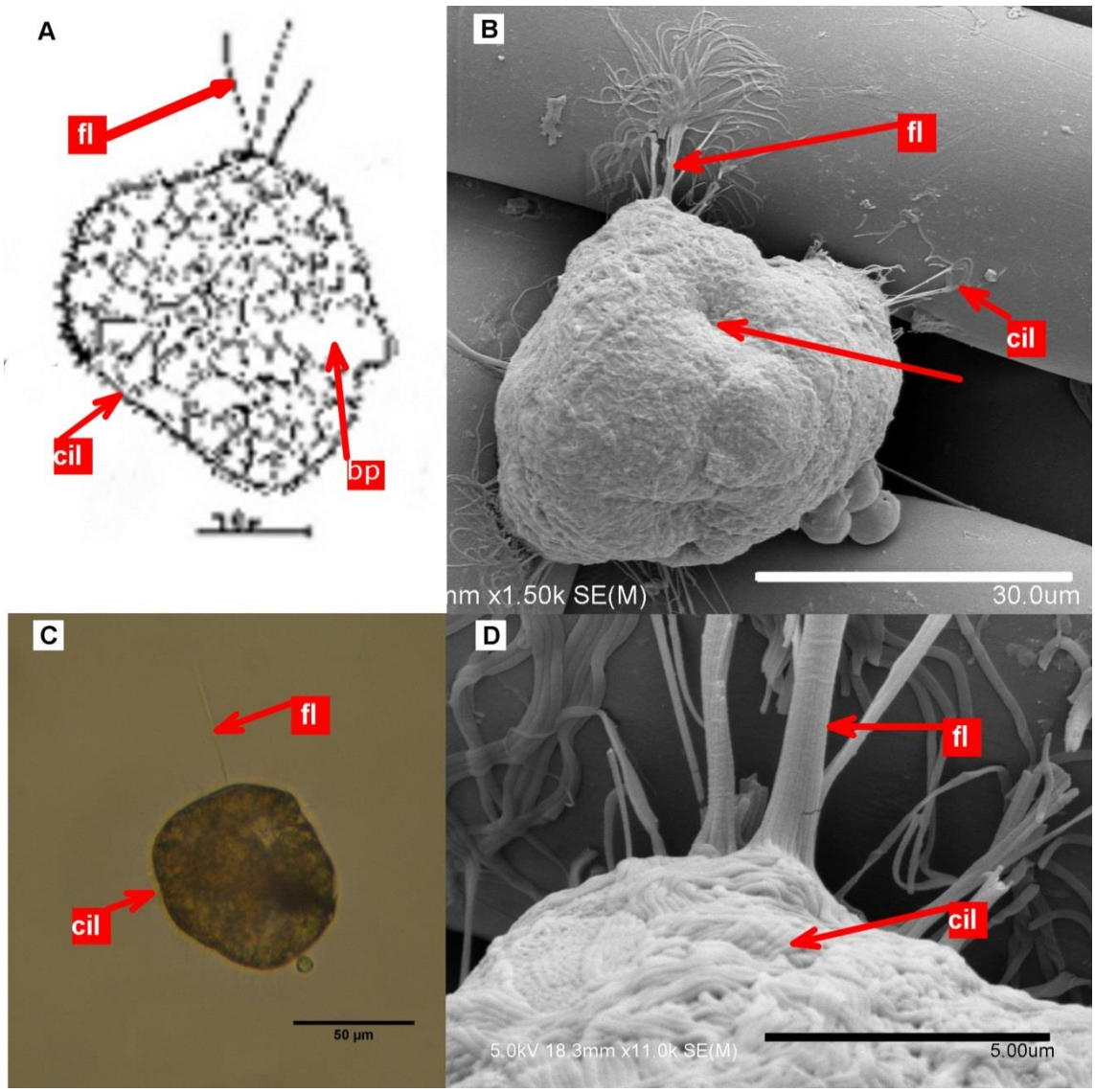


Figure 3.15. Trochophore.

A. *A. irradians* trochophore with the bastapore present.

B. *P. novaezelandiae* trochpore residing on a 20µm mesh.

C. A flagellum extending from *P. novezelandiea*. Ceillia surround the entire torchopore.

D *An expanded view of P. novaezelandiae* flagellum in figure B. On the apex of the trochophore a band of cilia produce the flagellum. A cilia layer covers the trocohophore's surface (SEM).

(pb = blastopore; tt = teleotroch; pr = prototroch; cl = cillia; fl = flaggelum)

3.5.8 D-larvae

By three to four days PF, the larvae had grown to D-larvae and had become semi-translucent. Organs were distinguishable by light microscopy. The early D-larvae still displayed globules of lipids. The larvae were now able to catch microalgae and a digestive gland was developing. A velum (ve) extended from the valves with cilia aiding motility and the acquisition of microalgae (Figure 3.16 A). With the velum extended beyond the shell mantle, the mantle cavity within the bivalve (mc) was clearly visible. The shells were strong enough to endure “washing” process”. A stellate (st) and radial zone would be seen on the primary prodissoconch (PI) and the developing secondary prodissoconch (PII) (Figure 3.16 B). *P. novaezelandiae* displays a straight hinge on the early developed D-larvae.

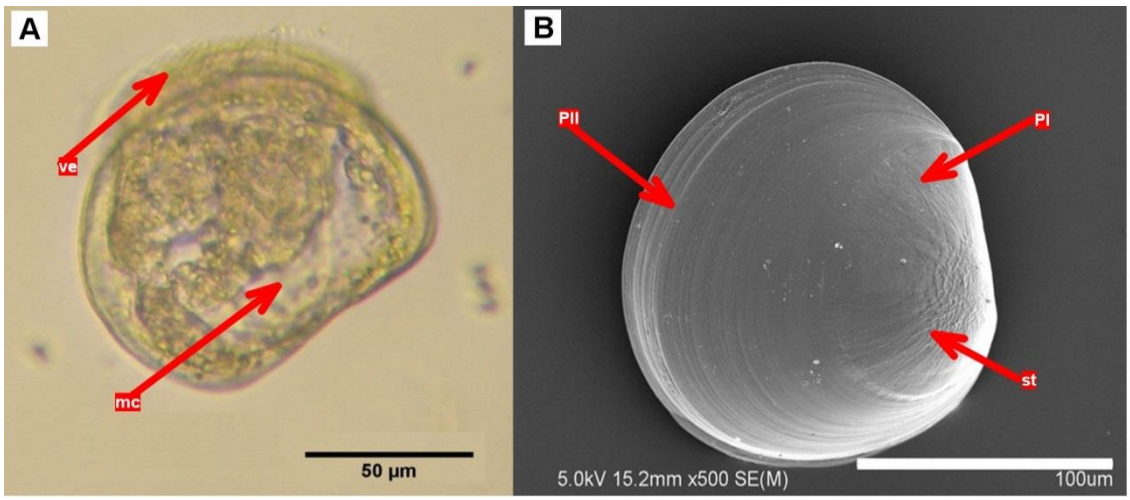


Figure 3.16. Three day old *P. novaezelandiae* D-larvae.

A- *P. novaezelandiae D-larvae at 3 days* (LM).

B- *P. novaezelandiae* D-larvae at 3 days (SEM).

(ve = velum; mc=mantle cavity; = prodissoconch; prodissoconch II; st = stellate / radial).

3.5.9 Veliger

At six days PF, a digestive gland (dg) was fully formed and the anterior adductor muscle was visible. The light brown digestive gland was visible next to the hinge (Figure 3.17- B). The gland visibly darkened with feeding. The veliger was developing a prominent prodissoconch PII (Figure 3.17 A).

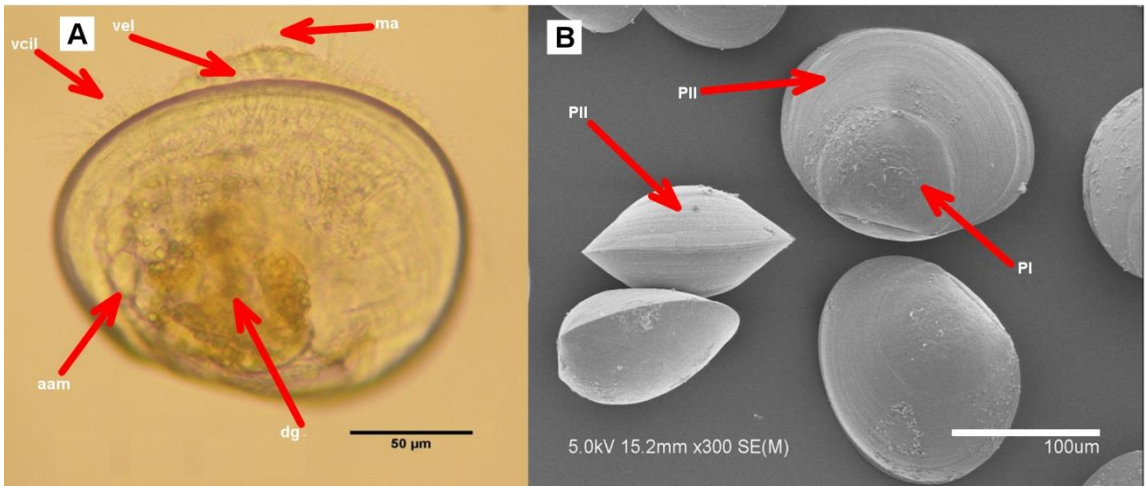


Figure 3.17**:** *P. novaezelandiae* veligers six days PF.

A- *P. novaezelandiae* extended the velum. The adductor muscle is visible (LM).

B- *P. novaezelandiae* transaction and sagital view (SEM).

(ve = velum; vcil= velum cilia; aam = anterior adductor muscle; dg = digestive gland; ma = micro algae; PI = prodissoconch I; PII prodissoconch II)

Eyed veliger

An eye spot developed 23 days PF, roughly in the center of the larval shell (Figure 3.18). Gill filaments (gl) were clearly visible. A foot (ft) was forming though it did not extend beyond the shell mantle. A pronounced prodissoconch II was visible (Figure 3.18 B).

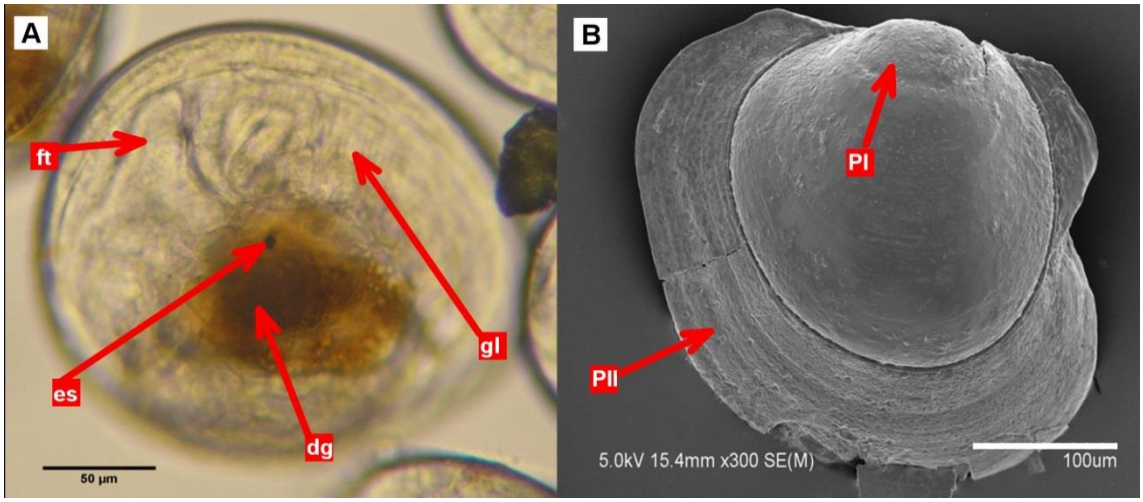


Figure 3.18. Eyed veliger.

A. *P. novaezelandiae* eyed veliger with an eye spot and developing foot (LM).

B. A *P. novaezelandiae shell at 23 days.* (ft = foot; es = eyed spot; dg = digestive gland; PI =prodissoconch ; PII = second prodissoconch)

3.5.10 Pediveliger

After thirty days post-fertilisation the foot was clearly evident. The larvae were seen crawling on the microscope slide. The larvae were ready to settle and many of the larvae were searching for attachment substrates. Foot cilia (fc) extend from the foot (ft). The gills were now more pronounced. A dissoconch was clearly visible on the thirty day old shell. An umbo was visible, protruding slightly above the hinge.

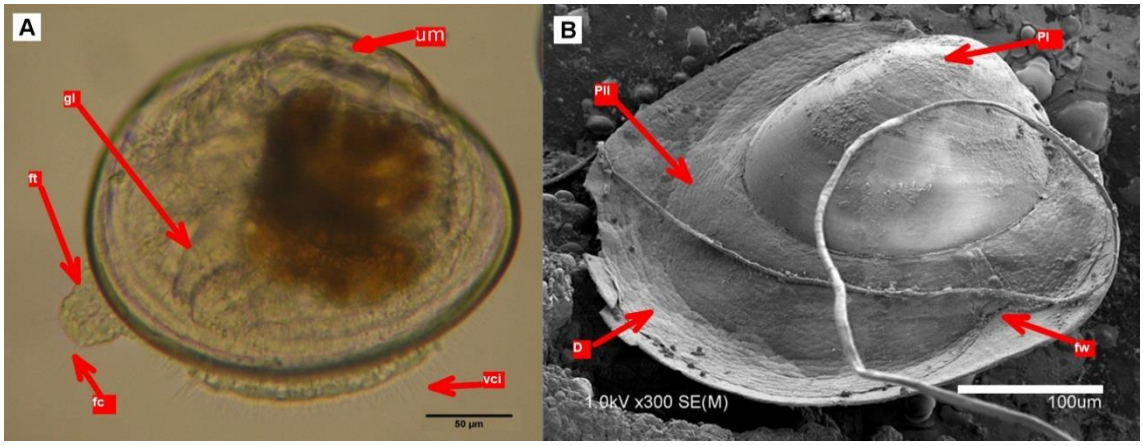


Figure 3.19. Pediveliger.

A. *P. novaezelandiae* pediveliger with the foot extend (LM).

B. *P. novaezelandiae* 30 day old shell with the foot well (fw) exposed (SEM).

(fc = foot cilia; ft = foot; ve = velum; g = gills; LM: d= dissoconch; fw = foot well; um = umbo)

3.6 Discussion

There is a dearth of empirical research on the embryonic or larval growth for *P. novaezelandiae*, with a handful of studies in the grey literature (Booth, 1983; Bull, 1976; Nicholson, 1978). This is the first study to characterize the development of *P. novaezelandiae* from embryo to pediveliger. Bull (1976) first identified that *P. novaezelandiae* remained in the plankton for 3-4 weeks before settling, but he was not able to identify all the developmental stages. Then Nicholson (1978) described some aspects of the embryonic process for this species. Until now, it has been assumed that larval development and growth patterns for *P. novaezelandiae* are similar to temperate counterparts (i.e., *P. fumatus* and *P. maximus*). However, this inference has been based purely on the fact that these species have similar adult morphologies. Based on the results of the present study, P. novaezelandiae undergoes fertilisation, cell division, trochophore, D-larvae, veliger, and pedivilger and these embryonic and larval development stages are indeed similar to those of *A. irriadians*, *P. maximus, Equichlamys bifron* and *P. fumatus* (Dix, 1976; Dix & Sjardin, 1975; Le Pennec et al., 2003; Sastry, 1965).

3.6.1 Survival rates

Results from the present study indicate that 20% of the larvae survived to pediveliger. These results are comparable to those by Magnesen (2006), who cultured *P. maximus* in a flow through seawater system and achieved a 23% larval survival rate. In the present study, larval survivability did not differ significantly between tanks that were treated with antibiotics and those that were not treated without antibiotics. These results suggest that *P. novaezelandiae* larvae may be resilient enough to withstand aquaculture rearing conditions. However, further studies would need to be carried out with greater replication to clearly elucidate the potential effects of using antibiotics to rear larvae *P. novaezelandiae* larvae. Chloramphenicol has been suggested to be carcinogenic and weakly genotoxic which may have implications for human health (Hanakamp

& Calabrese, 2007). Chloramphenicol remains the best performing antibiotic in scallop rearing (Torkildsen et al., 2002; Torkildsen et al., 2005).

3.6.2 Larval growth

Superior *P. novaezelandiae* larvae displayed linear growth from the zygote to the pedivileger state. In the present study, inferior larvae were removed from the larval rearing process by sieving and washing the larvae every two days to produce a more homogenous population. Therefore, the average sizes recorded here are a reflection of a “normally developing” larval batch, and these values may serve as a baseline for further larval studies within an aquaculture environment. *P. novaezelandiae* larvae grew at an average of 6µm a day. This growth rate was 1.2µm greater than that reported for hatchery-reared *P. maximus* (Magnesen et al., 2006), but considerably slower than that of *P. fumatus*, which grew at an average of 8µm per day (Heasman et al., 1994b). Heasman (1995) found that temperature played a significant role in the growth of *P. fumatus* larvae, and that a temperature of 18°C was optimal for that species. In the present study, temperature was not investigated as a factor in the rearing *P. novaezelandiae* larvae, but it was assumed that a temperature of 19°C was comparable and appropriate for culture of this species.

During this study, it was noted that there were significant differences in shell size between the samples used for light microscopy and for SEM, with the latter being consistently smaller. Thus, it appears that shrinkage may have occurred during the air drying process of the SEM samples. However, the reported larval sizes in this study are based on the light microscopy samples, which are comparable to those reported in the literature for other species.

3.6.3 Clearance rates

Results of the microalgal clearance rates indicate that on day 18, a 90% clearance rate was achieved. It seems that microalgal demand was high during this period and that the amount provided may not have been sufficient for optimal larval growth. This period also coincided with the developmental transition from veliger to pedivileger stage (between day 16 and 22), which likely required a greater energy requirement to achieve metamorphosis. However, there are no relevant larval energetic studies to corroborate this increased energy requirement. Future studies on energetics of larvae throughout different larval stages would be useful to identify more specifically the feeding requirements and optimal feeding rations for *P. novaezelandiae*.

In this study, the optimal feeding dose appeared to be 25,000 cells per larvae per day. MacDonald (1988) also determined that 30,000 cells per larvae per day resulted in the best growth rates and hypothesised that scallop larvae were more efficient at clearing microalgae at low concentrations, although 15,000 cells per larvae per day was the minimum ration for successful *P. yessoensis* culture. In addition, Lu & Blake (1997) established that 20,000 cells per larvae per day was optimum for growth of *Argopecten irradians concentricus*, and Magnesen (2006) determined that 25,000 cells per larvae per day increased survival and yields for *P. maximus*. However, Magnesen (2006) also indicated that there was an inverse relationship between survival and yield to microalgal concentrations in a flow through system. This suggests that the optimal feeding ration differs from one culture system to another and so the optimal ration needs to be established experimentally for each system.

3.6.4 Spawning

Spawning broodstock synchronously whilst avoiding self fertilisation is a continuous impediment in hatcheries when dealing with hermaphroditic bivalve species. The lack of spawning success will ultimately undermine opportunities for consistent hatchery production. In this study, self fertilisation was minimized by placing a spawning scallop in separate spawning bowl, filled with fresh sea water,

every 15 minutes. The spawning protocol used in this study (thermal shocking, microalgae and tickling/water jetting) resulted in successful spawning of *P. novaezelandiae* adults. Induction with serotonin was not achieved in this study. Similarly, Heasman et.al. (1994b), Nesbit (1999) and Williams (2005) were unsuccessful in releasing eggs with serotonin for *P. novaezelandiae* and *P. fumatus*. Stripping eggs from *P. novaezelandiae* gonads, and using serotonin to induced sperm did not create any viable D-larvae (Helm et al., 2004).

3.6.5 Sperm

The sperm SEM images of *P. novaezelandiae* show micropores on the sperm nuclei spread in a distinctive pattern. These have not been described in the literature before. The micropores may be caused as an artefact of the air drying or preservation process. However, the strategic placing of the microspores may infer a structure for alternative purposes .*P. novaezelandiae* sperm sizes recorded in this study were identical to the sperm sizes reported by Benninger and Le Pennec (2006) for *P. maximus*. Categorical descriptions of *Pecten* sperm described by Waller (1991) classified sperm based on their shape or pyriform, either rounded (category one) or elongated (category two). Morphologically, the sperm of *P. novaezelandiae* fits in the rounded shape category (category one), along with the sperm of *P. fumatus* and *P. maximus* are included.

3.6.6 Embryogenesis

When *P. novaezelandiae* eggs are released from the gonad, they emerge as irregular in shape, but they soon expand into spheres. This is a common occurrence with mollusc eggs (Helm et al., 2004; Sastry, 1965). Once the egg of *P. novaezelandiae* is fertilised, the resulting zygote follows the classic larval cell division observed in other bivalve larvae (Mouëza, Gros, & Frenkiel, 1999; Sastry, 1965; Wassnig & Southgate, 2011). A small polar body becomes visible on the egg within the first 15 minutes after the sperm penetrates the egg. The next phase of embryogenesis includes the appearance of a polar lobed body form where the

cytoplasm expands from the embryo. A double polar body emerges and a second polar body erupts 30min later, making a triple cleavage within one hour of fertilisation. Four blastomeres or cells form within four hours post-fertilisation. Further cell division occurs over the following 48 hours. The embryo key life milestones do not correspond to the times described by Nicholson (1978) for *P. novaezelandiae* with 55% veligers emerged with 33 hours. Seasonal differences and stock locations (i.e. South Island and North Island) are the most likely explanation for the differences in growth. This may also imply that growth occurs quicker in the North Island than on the South Island (Sastry, 1966).

Although the process of embryogenesis in bivalves has been reported in many studies (Da Costa, Darriba, & Martínez-Patiño, 2008; Desrosiers, Désilets, & Dubé, 1996; Sastry, 1965), few have traced individual cell or blastomeres throughout embryogenesis to determine their functions. The purple mussel, *Septifer virgatus* was used to trace each blastomere to ascertain the shell-producing cell (Kurita, Deguchi, & Wada, 2009). However, in the present study, it was not possible to identify any key cells responsible for these functions.

3.6.7 Larval development

P. novaezelandiae D-larvae emerges as a straight hinged D-larvae. An umbo is not present in the early stages of *P. novaezelandiae.* This result is in agreement with previous observations on this species by (Bull, 1976). However, observations from the present study indicate that the umbo appears at the late veliger and early pediveliger phase for *P. novaezelandiae*. These shell characteristics are consistent with those of *P. fumatus*, and differ slightly from those of *P. maximus* (Dix & Sjardin, 1975; Le Pennec et al., 2003).The umboned character on *P. maximus* is prominent enough to be characterised as a unique phase in *P. maximus* development, but this characteristic cannot be used as a developmental milestone in *P. novaezelandiae.* An alternative measure of milestone development around the umbo phase for *P. novaezelandiae* may be shell height and length as proposed by Salaun (Salaun & Le Pennec, 1991). This measurement is the Dim_{90} index, based on length and curvature of the shell, at 90µm. Salaun (1991) use the Dim90 as a growth potential for *P. maximus* larvae.

However, easily accessible SEM imagery would be required to take advantage of the Dim_{90} index as light microscopy images would not provide the detailed morphological features needed for this analysis.

3.6.8 Larvae rearing

Although this was the first production attempt for *P. novaezelandiae,* the results indicate that this species requires 27-33 days to settlement. This time may vary based on environmental conditions, such as temperature, stocking densities and diet. However, the duration for larval production of this species is within the range reported for other species (*P. maximus,* 25 days; *A. Purpuratus,* 20-24 days; *Argopeceten ventricosus,* 20-26 days*; Nodipecten nodosus*, 25-27 days and *M. yessoensis*, 30-40 days) to settlement (Le Pennec et al., 2003; Magnesen et al., 2006).

The Cawthron Ultra-Density Larvae rearing System (CUDL) uses a freshly filtered seawater flow through systems, which has been successfully used to culture green-lipped mussels (*Perna canaliculus*) at 200larvae/ml (Ragg et al., 2010). In the present study, it is clear that this system also can be used successfully to culture *P. novaezelandiae* to pediveligers. However, it is important to note that further studies will need to be conducted to determine optimal stocking densities for this species. MacDonald (1988) showed that *Patinopecten* yessoensis had low survival rates if reared at densities greater than 2 larvae/ml. The stocking densities of *P. novaezelandiae* D-larvae were reared at 200 larvae/ml. This density was considerably higher than the 4–30 larvae/ml used for *P. maximus* and *A. pupuratus* (Martinez, Mettifogo, Perez, & Callejas, 2007; Torkildsen & Magnesen, 2004). These results suggest that the CUDL's potentially may provide the appropriate environment for higher stocking densities for this species.

3.6.9 Future studies

The New Zealand scallop (*P. novaezelandiae*) supports an important fishery that could benefit with hatchery supplied spat. However, there are many knowledge

gaps pertaining to the spawning and culturing of the larvae. Future studies should be conducted to ascertain consistent productive outcomes in spawning, optimum clearance rates and appropriate diets. Further studies also should be instigated to determine the effects of antibiotics in the culturing environment and to identify optimum stocking densities with the CUDLs or static larval rearing systems for *P. novaezelandiae*.

4 Chapter Four: General Discussion

The majority of studies conducted for the New Zealand scallop, *P*. novaezelandiae have been ecologically focused (Bull, 1976; Morrison, 1999; Nicholson, 1978; Williams & Babcock, 2004, 2005). Additional studies directly focused on scallop enhancement or fisheries (Bartrom, 1990; Lyon, 2002; Morrison & Cryer, 2003). Few studies have been conducted on scallops for aquaculture other than in grey research (Nesbit, 1999).

4.1.1 Lifecycle

The research within this study contributes significantly to the lifecycle of the *P. novaezelandiae.* Bull (1976) hypothesised *P. novaezelandiae* spend 3 weeks in the water column before settling on filamentous substrates. This study confirms that settlement occurs around 3 weeks. Further observation of the embryo, D-larvae, veliger and pedivileger demonstrated the similarity between *P. novaezelandiae* and other Pectinidae (Desrosiers et al., 1996; Le Pennec et al., 2003; Sastry, 1965). However, the umbo phase in *P. novaezelandiae* is not prominent like *P. maximus (Le Pennec et al., 2003)*. Alternative methods, such as the Dim_{90} Index, may be beneficial for larvae identification (Salaun & Le Pennec, 1991).

4.1.2 Broodstock

The results in this broodstock study may establish a base line for optimising the broodstock diet for *P. novaezelandiae.* Further studies pertaining to broodstock are relevant to establish the optimum dietary requirements for gametogenesis. Delaunay (1993) identified three specific fatty acids that contribute to the conditioning of *P. maximus*: eicosapentaenoic acid (20:5n−3) [EPA], docosahexaenoic acid (22:6n−3)[DHA] and arachadonic acid (20:4n−6) [AA]. More studies have concentrated on lipids, polypeptides and supplements with highly polyunsaturated [HUFA] and polyunsaturated [PUFA] fatty acids to optimise broodstock (Caers et al., 1999; Nevejan, Saez, Gajardo, & Sorgeloos, 2003; Sühnel, Lagreze, Zanette, Magalhães, & Ferreira, 2012). Dry weight [DW]

and Gonadalsomatic Index [GSI] are good indicators of scallop conditioning (Barber & Blake, 1981; Jasim Uddin, Park, Kang, Park, & Choi, 2007). However, further studies measuring glycogen, proteins in the digestive gland and adductor mussel are required (Barber & Blake, 1981; Epp, Bricelj, & Malouf, 1988; Racotta, Ramirez, Avila, & Ibarra, 1998). The results of such studies may indicated whether spawning scallops more than once a year in the laboratory is feasible and improve insight into the energetic requirements for gametogenesis and larvae survival (Caers et al., 1999; Nevejan, Courtens, Hauva, Gajardo, & Sorgeloos, 2003; Utting & Millican, 1997).

4.1.3 Colour

Colour used as an indicator of broodstock condition was novel in this study. Tang (1941) used “Colour Standards and Colour Nomenclatures” by R. Ridgeway (1912). Although Tang (1941) was able to assign colours to the reductive state of *P. maximus* gonad however, this methodology is subjective. Hand held colorimeters may be advantageous in measuring gonad in *situ* or hatcheries, removing the need for destructive sampling, and allowing individual gonad monitoring. However, the number of samples used in this study, was not sufficient to determine all the reproductive stages of the gonad, as described by Williams (2005). Further studies are required to correlate the reproductive categories, carotenoid, lipid content to colour (Li et al., 2010). This would introduce a simple, efficient and inexpensive methodology in assessing gonad condition.

4.1.4 Experimental measures

Three replicates of nine scallops placed in tank, within a recirculating sea water system, were fed a high, medium or low diet in this study. Success was measured by net tissue dry weight [DW], wet tissue weight [WW], gonad wet weight, colour and gonadalsomatic index [GSI], Histological Index [HI] and Visual Gonad Index [VGI].

The measures of microalgae clearance rate [CR] from the water column was excluded from this study (Bacon, MacDonald, & Ward, 1998). One empty tank

per feeding regime should have been included in the design to measure microalgae lost from suspension in the water column. Microalgae settlement from the delivery feed, settlement on the substrates and walls of the tank, may have potentially decreased the amount of microalgae available to the scallops. CR is not analogous to scallops' algal ingestion rate. The CR of microalgae for the adult *P. novaezelandiae* in class size categories has yet to be explored. Knowing the CR would determine the optimum and minimum feeding rates and durations in turn increasing broodstock condition and benefits larvae survival.

4.1.5 Diet

In this study, four microalgae were utilised in feeding this broodstock study: *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., and *Thalassiosira weissflogii*. Heasman (1996) found *P. fumatus* had problems ingesting microalgal *Tetraselmis suecica* and much of the microalgal was expelled through pseudofaeces. Although no preferential ingestion was observed in this study, little is known about the microalgae ingestion preference for *P. novaezelandiae*

4.1.6 Histology and Image Analysis

Histology is commonly used to examine the gonad reproductive status in scallops (Bower & Meyer, 1990; Duinker & Nylund, 2002; Howard & Smiith, 1983). A number of potential defects become apparent in histology that manifest itself in image analysis: Distortion of tissues sample, consistent colour stains and light microscopy (Howard & Smiith, 1983). Vitellin was not analysed in this study due to inconsistent light distribution and staining consistency. The inconsistencies between the colours made it impossible to develop colour specific categories.to identify Vitellin using automated image analysis.

4.1.7 Visual Grading Index

Williams (Williams & Babcock, 2004) determined eight categories to recognise the reproductive status of *P. novaezelandiae* (Table 2.1). These categories were based upon Heasman's (1996) interpretation of the gonad categories for *P. fumatus.* However, observations of VGI category eight, "Acini are not apparent and intestinal loop is not visible" (Williams, 2004), was never observed during this study. A portion of the intestinal loop was always visible during broodstock and spawning scallop studies. Should this state exist, it is likely for a short period of time, no more than a day or two. A recommendation of decreasing the number of VGI categories from eight to seven, excluding the eighth category would decrease the number of categories making it easier implement the grading index. Alternatively the eight category could be adapted for trickle spawning's. However, trickle spawning's often hard to detect without the use of histology.

4.1.8 Spawning

Studies attempted spawning *P. novaezelandiae* were met with limited success (Nesbit, 1999; Nicholson, 1978; Williams & Babcock, 2005). The ability to control spawning has considerable implications for the success or failure in scientific and commercial undertakings. Two key issues are evident: the lack of spawning success and self fertilisation of scallop embryos (Heasman et al., 1994b; Nicholson, 1978; Williams & Babcock, 2005). An alternative methodology to thermal shocking has been successfully employed on *Argopecten purpuratus* by means of stripping the embryos and exposing them to serotonin, inducing meiosis and then fertilising the eggs (Martinez et al., 2007). However, this methodology has not been tried on *P. novaezelandiae.* Should the described procedure be successfully employed on *P. novaezelandiae*, this would counteract self fertilisation, resolve spawning and the synchronised spawning problem. Although this method has not been tested, pilot experiments attempting to release eggs using serotonin from *P. novaezelandiae* and *P. fumatus* have proven unsuccessful (Heasman, O'Connor, & Frazer, 1994a).

Spawnings conducted at A.U.T., North Island were more successful than spawnings attempted in Cawthron, South Island. The times in which the spawning conducted (A.U.T., Nov-Dec/2012 and Cawthron Feb-March/2013) may suggest

a reason for the success or failure. The spawning period for the Hauraki Gulf stock may potentially be longer than that in the Tasman, Golden Bay and Marlborough sounds due to the warmer Northern climate (Barber & Blake, 1983; Bull, 1976; Williams & Babcock, 2004).

4.1.9 Larval rearing

Larvae rearing has traditionally suffered from high mortalities and stocking densities in static rearing systems. Newer technologies such as flow through systems [FTS] and recirculating sea water systems [RAS] have resulted in lower bacterial accumulation and enabled higher larval stocking densities (Torkildsen & Magnesen, 2004). However, antibiotics utilised in the rearing of larvae, such as chloramphenicol, improve larvae yields. In this study chloramphenicol was used in the rearing of larvae. No significant chloramphenicol results were determined from the study. Future studies with a focus on larvae density may possible prove FTS and the may not require antibiotics for larvae rearing as with mussels (Ragg et al., 2010)

4.1.10 Challenges

There are many challenges to overcome before hatchery production of scallop larvae and spat becomes robust and economical. Currently the main obstacle is obtaining sufficient quantities of viable gametes for fertilisation. Improvement in our understanding of broodstock conditioning is anticipated to provide control over the development of the gametogenesis and result in better larvae survival and condition. Best temperature rearing and diet regimes for *P. novaezelandiae* larvae have yet to been established. Comparison between currently cultivated species, such as *P. fumatus* and *P. maximus,* may inform best culturing practices for the *P. novaezelandiae* (Andersen, Burnell, & Bergh, 2000; Heasman et al., 1994b, 1996; Helm et al., 2004). However, intra specifics between Pectinidae may sufficiently differ that these methodologies are not transferab

References

Abramoff, M. D., Magelhaes, P. J., & Ram, S. J. (2004). Image Processing with ImageJ. *Biophotonics International, 11*(7), 36–42.

Andersen, S., Burnell, G., & Bergh, Ø. (2000). Flow-through systems for culturing great scallop larvae. *Aquaculture International, 8*(2-3), 249–257. doi:10.1023/a:1009271220868

Andersen, S., Christophersen, G., & Magnesen, T. (2011). Spat production of the great scallop (*Pecten maximus*): a roller coaster. *Canadian Journal of Zoology, 89*, 579–598. doi:doi:10.1139/Z11-035

Andersen, S., & Ringvold, H. (2000). Seasonal differences in effect of broodstock diet on spawning success in the great scallop. *Aquaculture International, 8*(2), 259–265. doi:10.1023/a:1009279422686

Auffret, M., Braille, L., Basenard-Cachennec, N., Blanc, F., Le Pennec, M., Lubet, P., ... Thielley, M. (2003). *An atlas of histology and cytology of marine bivalve molluscs*. Plouzané, France: Infremer.

Bacon, G. S., MacDonald, B. A., & Ward, J. E. (1998). Physiological responses of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles: I. Feeding activity and selection. *Journal of Experimental Marine Biology and Ecology, 219*(1–2), 105–125. doi:10.1016/S0022-0981(97)00177-9

Barber, B. J., & Blake, N. J. (1981). Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (say). *Journal of Experimental Marine Biology and Ecology, 52*(2–3), 121–134. doi:10.1016/0022-0981(81)90031-9

Barber, B. J., & Blake, N. J. (1983). Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) at its southern distributional limit. *Journal of Experimental Marine Biology and Ecology, 66*(3), 247–256. doi:10.1016/0022-0981(83)90163-6

Barber, B. J., & Blake, N. J. (2006). Chapter 6 Reproductive Physiology. In S. E. Shumway & G.J. Parsons (Eds.), *Developments in Aquaculture and Fisheries Science* (Vol. Volume 35, pp. 357–416): Elsevier. doi:10.1016/s0167-9309(06)80033-5

Baron, J., Diter, A., & Bodoy, A. (1989). Triploidy induction in the black scallop (Chlamys varia L.) and its effect on larval growth and survival. *Aquaculture, 77*(2–3), 103–111. doi:10.1016/0044-8486(89)90194-4

Bartrom, A. A. (1990). *The Coromandel scallop enhancement project 1987 to 1989*. MAF Fisheries North. (Unpublished report held at NIWA, Auckland).

Beninger, P. G., & Le Pennec, M. (2006). Chapter 3 Structure and function in scallops. In E. S. Sandra & G. J. Parsons (Eds.), *Developments in Aquaculture and Fisheries Science* (Vol. Volume 35, pp. 123–227): Elsevier.

Booth, J. (1983). Studies on twelve common bivalve larvae, with notes on bivalve spawning seasons in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 17*, 231–265.

Bower, S. M., & Meyer, G. R. (1990). *Atlas of anatomy and histology of larvae and early juveniile stages of the Japanese scallp (Patiopecten yessoensis)*. Otowa, Canada: Fisheries and Oceans.

Brand, A. R. (2006). Chapter 12 Scallop Ecology: Distribution and Behaviour. In S. E. Shumway & G.J. Parsons (Eds.), *Scallops: Biology, Ecology and Aquaculture* (2 ed., pp. 651–753): Elsevier.

Brink, L. A. (2001). Molluscs: Bivalvia. In A. L. Shanks (Ed.), *Identification guide to larval marine intvertebrates of the Pacific Northwest* (pp. 314): Oregon State University Press.

Bull, M. F. (1976). *Aspects of the biology of the New Zealand scallop, Pecten novaezelandiae Reeve 1853, in the Marlborough Sounds.* (PhD). Victoria University, Wellington.

Caers, M., Coutteau, P., Cure, K., Morales, V., Gajardo, G., & Sorgeloos, P. (1999). The Chilean scallop *Argopecten purpuratus* (Lamarck, 1819): II. manipulation of the fatty acid composition and lipid content of the eggs via lipid supplementation of the broodstock diet. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 123*(1), 97–103. doi:10.1016/s0305-0491(99)00047-4

Cameron, M. L. (1983). *A preliminary investigation of the economic vaiablity of scallop farming in New Zealand* (Discusion paper 15/83). Wellington: Ministry of Agriculture & Fisheries.

Cochard, J. C., & Devauchelle, N. (1993). Spawning, fecundity and larval survival and growth in relation to controlled conditioning in native and transplanted

populations of *Pecten maximus* (L.): evidence for the existence of separate stocks. *Journal of Experimental Marine Biology and Ecology, 169*(1), 41–56. doi:10.1016/0022-0981(93)90042-m

Cryer, M. (2002). *Northland and Corromandel scallop stock assesment for 2001*: New Zealand Fisheries Assessment Report 2002/60.

Culliney, J. L. (1974). Larval development of the giant scallop *Placopecten magellanicus* (GMELIN). *Biological Bulletin, 147*, 321–332.

Da Costa, F., Darriba, S., & Martínez-Patiño, D. (2008). Embryonic and larval development of Ensis arcuatus (Jeffreys, 1865) (Bivalvia: Pharidae). *Journal of Molluscan Studies, 74*(2), 103–109. doi:10.1093/mollus/eym051

Delaunay, F., Marty, Y., Moal, J., & Samain, J. F. (1993). The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) larvae. *Journal of Experimental Marine Biology and Ecology, 173*(2), 163–179. doi:10.1016/0022-0981(93)90051-o

Desrosiers, R. R., Désilets, J., & Dubé, F. (1996). Early developmental events following fertilization in the giant scallopPlacopecten magellanicus. *Canadian Journal of Fisheries and Aquatic Sciences, 53*(6), 1382–1392. doi:10.1139/f96-071

Dijkstra, H. (2013). *Pecten meridionalis Tate*, 1887. from World Register of Marine Species retrieved 2013-05-29 http://www.marinespecies.org/aphia.php?p=taxdetails&id=393906

Dix, T. G. (1976). Larval development of the queen scallop, *Equichlamys bifrons*. *Marine and Freshwater Research, 27*(3), 399–403. doi:dx.doi.org/10.1071/MF9760399

Dix, T. G., & Sjardin, M. J. (1975). Larvae of the commercial scallop, *Pecten meridionalis* from Tasmania, Australia. *Marine and Freshwater Research, 26*(1), 109–112. doi:dx.doi.org/10.1071/MF9750109

Drummond, K. L. (2008). The Role of Stock Enhancement in the Management Framework for New Zealand's Southern Scallop Fishery. In S. K. K.M. Lebere, H.L. Blankenship,and T. Svasand (Ed.), *Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities* (2 ed., pp. 397–412). Oxford: Blackwell Publishing Ltd. doi:10.1002/9780470751329.ch29

Duinker, A., & Nylund, A. (2002). Seasonal variations in the ovaries of the great scallop (*Pecten maximus*) from western Norway. *Journal of the Marine*

Biological Association of the United Kingdom, 82(03), 477–482. doi:10.1017/S002531540200574X

Duinker, A., Saout, C., & Paulet, Y. M. (2000). Effect of photoperiod on conditioning of the great scallop. *Aquaculture International, 7*(6), 449–457. doi:10.1023/a:1009298024046

Epp, J., Bricelj, V. M., & Malouf, R. E. (1988). Seasonal partitioning and utilization of energy reserves in two age classes of the bay scallop Argopecten irradians irradians (Lamarck). *Journal of Experimental Marine Biology and Ecology, 121*(2), 113–136. doi:10.1016/0022-0981(88)90250-x

Food and Agriculture Organisation of the United Nations. (1987). *Status of scallop farming: A review of techniques*. Retrieved 1 June, 2013, from http://www.fao.org/docrep/field/003/ab714e/AB714E01.htm#ch1.

Food and Agriculture Organisation of the United Nations. (2011). *Global Production Statistics 1950-2009*. Retrieved 31 May, 2013, from http://www.fao.org/fishery/statistics/global-aquaculture-production/en

Food and Agriculture Organisation of the United Nations. (2013). *Fisheries and Aquaculture Department: Global Aquaculture Production*. Retrieved 31 May, 2013, from http://www.fao.org/fishery/statistics/global-aquaculture-production/en

Hanakamp, J., & Calabrese, E. J. (2007). Chloramphenicol, European Legislation and Hormesis - Commentary. *Dose Response, 5*(2), 91-93. doi:10.2203/dose-response.06-012.Hanekamp

Hardege, J. D., & Bentley, M. G. (1997). Spawning synchrony in *Arenicola marina*: evidence for sex pheromonal control. *Proc Biol Sci., 264*(1384), 1041–1047. doi:10.1098/rspb.1997.0144

Hardy, D. (2006). *Scallop Farming* (2 ed.). Oxford; Ames, Iowa: Blackwell Publishing.

Heasman, M. P. (2007). *A practical guide of hatchery and nursery rearing techniques for the New Zealand king scallop*. Cawthron Institute. Nelson, New Zealand.

Heasman, M. P., O'Connor, W. A., & Frazer, A. W. (1994a). Detachment of commercial scallop Pecten fumatus, spat from settlement substrates. *Aquaculture, 123*(3–4), 401–407. doi:10.1016/0044-8486(94)90074-4

Heasman, M. P., O'Connor, W. A., & Frazer, A. W. (1994b). Improved hatchery and nursery rearing techiques for *Pecten fumates* Reeve. *Memoirs of the Queensland Museum, 36*(2), 351–356.

Heasman, M. P., O'Connor, W. A., & Frazer, A. W. (1995). *Evaluation of Hatchery production of scallops P. fumatus.* Deacon, ACT, Australia: Final Report to Fisheries Research and Development Corporation.

Heasman, M. P., O'Connor, W. A., & Frazer, A. W. (1996). Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. *Aquaculture, 143*(1), 75–90. doi:10.1016/0044-8486(95)01231-1

Helm, M. M., Bourne, N., & Lovatelli, A. (2004). *Hatchery culture of bivalves. A practicle manual.* Rome: F.A.O.

Hodgson, C. A., & Burke, R. D. (1988). Development and Larval Morphology of the Spiny Scallop, Chlamys hastata. *Biological Bulletin, 174*(3), 303–318.

Howard, D. W., & Smiith, C. S. (1983). *Histological Techniques for Marine Bivalve Mollusks*. Oxford: NOAA.

Jasim Uddin, M., Park, K.-I., Kang, D.-H., Park, Y.-J., & Choi, K.-S. (2007). Comparative reproductive biology of Yezo scallop, Patinopecten yessoensis, under two different culture systems on the east coast of Korea. *Aquaculture, 265*(1–4), 139–147. doi:10.1016/j.aquaculture.2007.01.047

Kim, Y., Ashton-Alcox, K. A., & Powell, E. N. (2006). *Histological Techniques for Marine Bivalve Molluscs: Update* (Technical Memorandum NOS NCCOS 27). Silver Spring, MD.

Kleinman, S., Hatcher, B. G., Scheibling, R. E., Taylor, L. H., & Hennigar, A. W. (1996). Shell and tissue growth of juvenile sea scallops (*Placopecten magellanicus*) in suspended and bottom culture in Lunenburg Bay, Nova Scotia. *Aquaculture, 142*(1–2), 75–97. doi:10.1016/0044-8486(95)01165-x

Kurita, Y., Deguchi, R., & Wada, H. (2009). Early development and cleavage pattern of the Japanese Purple Mussel, *Septifer virgatus*. *Zoological Sciences, 26*(12), 814–820.

Laurén, D. J. (1982). Oogenesis and protandry in the purple-hinge rock scallop, *Hinnites giganteus*, in upper Puget Sound, Washington, U.S.A. *Canadian Journal of Zoology, 60*(10), 2333–2336. doi:10.1139/z82-300

Le Pennec, M., Paugam, A., & Le Pennec, G. (2003). The pelagic life of the pectinid *Pecten maximus*—a review. *ICES Journal of Marine Science: Journal du Conseil, 60*(2), 211–233. doi:10.1016/s1054-3139(02)00270-9

Li, N., Hu, J., Wang, S., Cheng, J., Hu, X., Lu, Z., ... Bao, Z. (2010). Isolation and identification of the main carotenoid pigment from the rare orange muscle of the Yesso scallop. *Food Chemistry, 118*(3), 616–619. doi:10.1016/j.foodchem.2009.05.043

Logan, M. (2010). *Biostatistical Design and Anaylysis Using R: A Practicle Guide*. Singapore: Wiley-Blackwell. doi:10.1002/9781444319620

Lu, Y. T., & Blake, N. J. (1997). Clearance and ingestion rates of *Isochrysi galbana* by larval and jevenile bay scallops, *Argopecten irradians concentricus* (Say). *Journal of Shellfish Research, 16*(1), 47–54.

Lyon, R. (2002). *Determining the optimal scallop spat length and transport conditions for Pecten novaezelandiae during the seeding enhancement programme in Nelson, New Zealand*. University of Canterbury, Canterbury.

MacDonald, B. A. (1988). Physiological energetics of Japanese scallop *Patinopecten yessoensis* larvae. *Journal of Experimental Marine Biology and Ecology, 120*(2), 155–170. doi:10.1016/0022-0981(88)90086-X

Magnesen, T., Bergh, Ø., & Christophersen, G. (2006). Yields of great scallop, *Pecten maximus*, larvae in a commercial flow-through rearing system in Norway. *Aquaculture International, 14*(4), 377–394. doi:10.1007/s10499-005-9039-5

Magnesen, T., & Jacobsen, A. (2012). Effect of water recirculation on seawater quality and production of scallop (*Pecten maximus*) larvae. *Aquacultural Engineering, 47*(0), 1–6. doi:10.1016/j.aquaeng.2011.12.005

Mallet, A. L., & Carver, C. E. (2009). The effect of photoperiod on the reproductive development of the northern bay scallop, *Argopecten irradians irradians*. *Journal of Experimental Marine Biology and Ecology, 374*(2), 128–133. doi:10.1016/j.jembe.2009.04.015

Marsden, I. D., & Bull, M. F. (2006). Chapter 31 New Zealand. In S. E. Shumway & G. J. Parsons (Eds.), *Scallops: Biology, Ecology and Aquaculture* (2 ed., Vol. 35, pp. 1413–1426): Elsevier.

Martinez, G., Mettifogo, L., Perez, M. A., & Callejas, C. (2007). A method to eliminate self-fertilization in a simultaneous hermaphrodite scallop. 1.

Effects on growth and survival of larvae and juveniles. *Aquaculture, 273*(4), 459–469. doi:10.1016/j.aquaculture.2007.10.033

Mason, J. (1958). The breeding of the scallop, Pecten maximus (L.), in Manx waters. *Journal of the Marine Biological Association of the United Kingdom, 37*(03), 653–671. doi:doi:10.1017/S0025315400005701

Miller, G. C., Allen, D. M., & Costello, T. J. (1981). Spawning of the calico scallop *Argopecten gibbus* in relation to season and temperature. *Journal of Shellfish Research, 1*, 17–21.

Mincher, R. (2008). New Zealand's Challenger Scallop Enhancement Company: from reseeding to self-governance. In R. Townsend, R. Shotton, & H. Uchida (Eds.), *Case studies in fisheries self-governance: FAO Fisheries Technical Paper. No. 504* (pp. 307–321). Rome: FAO.

Ministry of Fisheries Science Group. (2011). *Report from the Mid-Year Fisheries Assessment Plenary, November 2011:stock assessments and yield estimates*. (Unpublished report held in NIWA Greta Point library, Wellington.): NIWA.

Moore, B. (2012, Sept 29). Where have all the scallops gone? *The Nelson Mail,* pp. 13–14.

Morrison, M. A. (1999). *Population dynamics of the scallop Pecten novaezelandiae in the Hauraki Gulf* (PhD). University of Auckland, Auckland.

Morrison, M. A., & Cryer, M. (2003). *Potential for artificial enhancement of scallops in Northland* (AKL2003- 038). Auckland: National Institute of Water and Atmospheric Research.

Mouëza, M., Gros, O., & Frenkiel, L. (1999). Embryonic, Larval and Postlarval Development of the Tropical Clam, *Anomalocardia brasiliana* (Bivalvia, Veneridae). *Journal Molluscan Studies, 65*(1), 73–88. doi:10.1093/mollus/65.1.73

Naidu, K. S. (1970). Reproduction and breeding cycle of the giant scallop *Patinopecten magellanicus* (Gmelin) in Port au Port Bay, Newfoundland. *Canadian. Journal of Zoology., 48*(1003–1012).

Narvarte, M. A., & Pascual, M. S. (2001). Diet trials on tehuelche scallop Aequipecten tehuelchus (d'Orb) larvae. *Aquaculture International, 9*(2), 127–131. doi:10.1023/a:1014257008017

Nesbit, G. J. (1999). *Reseeding and Hatchery Potential of Pecten novaezelandiae and effects of recreational harvesting* (Master of Science in Environmental and Marine Science). University of Auckland, Auckland.

Nevejan, N., Courtens, V., Hauva, M., Gajardo, G., & Sorgeloos, P. (2003). Effect of lipid emulsions on production and fatty acid composition of eggs of the scallop *Argopecten purpuratus*. *Marine Biology, 143*(2), 327–338. doi:10.1007/s00227-003-1076-x

Nevejan, N., Saez, I., Gajardo, G., & Sorgeloos, P. (2003). Supplementation of EPA and DHA emulsions to a *Dunaliella tertiolecta* diet: effect on growth and lipid composition of scallop larvae, *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture, 217*(1), 613–632. doi:10.1016/s0044-8486(02)00585-9

New Zealand Aquaculture. (n.d.). *National Aquaculture Strategy and Action Plan*. Retrieved October, 2011, from http://www.aquaculture.govt.nz/strategy.php

Nicholson, J. (1978). *Feeding and reproduction in the New Zealand Scallop Pecten novaezelandiae*. University of Auckland, Auckland.

O'Connor, W. A., Heasman, M. P., & O'Connor, S. J. (2000). Algal diets for broodstock maintenance of the doughboy scallop *Mimachlamys asperrima* (Lamarck). *Aquaculture Research, 31*(8-9), 627–635. doi:10.1046/j.1365-2109.2000.318484.x

Pazos, A. J., Román, G., Acosta, C. P., Abad, M., & Sánchez, J. (1996). Stereological studies on the gametogenic cycle of the scallop, *Pecten maximus*, in suspended culture in Ria de Arousa (Galicia, NW Spain). *Aquaculture, 142*(1–2), 119–135. doi:10.1016/0044-8486(95)01247-8

Racotta, I., S., Ramirez, J. L., Avila, S., & Ibarra, A. M. (1998). Biochemical composition of gonad and muscle in the catarina scallop, *Argopecten ventricosus*, after reproductive conditioning under two feeding systems. *Aquaculture, 163*(1–2), 111–122. doi:10.1016/s0044-8486(98)00226-9

Ragg, N. L. C., King, N., Watts, E., & Morrish, J. (2010). Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture, 306*(1–4), 270–280. doi:10.1016/j.aquaculture.2010.05.010

Rees, C. B. (1950). *The identification and classification of Lamellibrach larvae.* (Vol. 3, no.19.): Hull.

Robert, R., Miner, P., & Nicolas, J. L. (1996). Mortality control of scallop larvae in the hatchery. *Aquaculture International, 4*(4), 305–313. doi:10.1007/bf00120947

Roe, R. B., Cummins, R., & Bullis, H. R. (1971). Calico scallop distribution, abundance, and yield off eastern florida, 1967-1968. *Fishery Bulletin, 69*(2), 399–409.

Rose, R. A., Campbell, G. R., & Sanders, S. G. (1988). Larval development of the saucer scallop *Amusium balloti* (Bernardi) (Mollusca : Pectinidae). *Marine and Freshwater Research, 39*(2), 153–160. doi:10.1071/MF9880153

Rose, R. A., & Dix, T. G. (1984). Larval and juvenile development of the doughboy scallop, *Chlamys (Chlamys) asperrimus* (Lamarck) (Mollusca : Pectinidae). *Marine and Freshwater Research, 35*(3), 315–323. doi:10.1071/MF9840315

Royer, J., Seguineau, C., Park, K., Pouvreau, S., Choi, K., & Costil, K. (2008). Gametogenetic cycle and reproductive effort assessed by two methods in 3 age classes of Pacific oysters, *Crassostrea gigas*, reared in Normandy. *Aquaculture, 277*(3–4), 313–320. doi:10.1016/j.aquaculture.2008.02.033

Saavedra, C., & Peña, J. B. (2004). Phylogenetic relationships of commercial European and Australasian king scallops (*Pecten* spp.) based on partial 16S ribosomal RNA gene sequences. *Aquaculture, 235*(1-4), 153–166. doi:10.1016/S0044-8486(03)00442-3

Salaun, M., & Le Pennec, M. (1991). Prodissoconch shell characteristics as indicators of larval growth and viability in *Pecten maximus* (Linnaeus, 1758). *Journal of Shellfish Research, 10*(1), 37–46.

Sastry, A. N. (1963). Reproduction of the bay scallop, *Aequipecten irradians* Lamark. Influence of temperature on maturation and spawning. *Marine Biological Laboratory, 125*, 146–143.

Sastry, A. N. (1965). The Development and External Morphology of Pelagic Larval and Post-Larval Stages of the Bay Scallop, *Aequipecten irradians concentricus* Say, Reared in the Laboratory. *Bulletin of Marine Science, 15*(2), 417–435.

Sastry, A. N. (1966). Temperature effects in reproduction of the Bay scallop, *Aequipecten irradians* Lamark. *Biology Bulletin, 130*(1), 118–134.

Sastry, A. N., & Blake, N. J. (1971). Regulation of gonad development in the bay scallop *Aequipecten irradians* Lamark. *Biological Bulletin, 140*(2), 274–283.

Slaughter, C., McCartney, M. A., & Yund, P. (2008). Comparison of Gamete Compatibility Between Two Blue Mussel Species in Sympatry and in Allopatry. *Biology Bulletin, 214*(1), 57–66.

Sühnel, S., Lagreze, F., Bercht, M., Ferreira, J. F., Carneiro-Schaefer, A. L., Magalhães, A. R. M., & Maraschin, M. (2010). Sexual stages of the female portion in the scallop *Nodipecten nodosus* (Linné, 1758) and astaxanthin quantity in each stage. *Brazilian Journal of Biology, 70*, 651–658.

Sühnel, S., Lagreze, F., Zanette, G., Magalhães, A. R. M., & Ferreira, J. F. (2012). Effect of the fatty acid EPA and DHA in the conditioning of the scallop *Nodipecten nodosus* (Linné, 1758). *Aquaculture, 330–333*(0), 167–171. doi:10.1016/j.aquaculture.2011.12.013

Talman, S. G., Norkko, A., Thrush, S. F., & Hewitt, J. E. (2004). Habitat structure and the survival of juvenile scallops *Pecten novaezelandiae*: comparing predation in habitats with varying complexity. *Marine Ecology Progress Series, 269*, 197–207.

Tang, S. F. (1941). The breeding of the scallop (*Pecten maximus* L.*)* with a note on growth rate. *Proceedings and transactions of the Liverpool Biological Society*(54), 9–28.

Torkildsen, L., Coyne, R., Samuelsen, O. B., Magnesen, T., & Bergh, Ø. (2002). Treatment of the early life stages of scallop (*Pecten maximus*) with antimicrobial agents; searching for an alternative to chloramphenicol. *Aquaculture International, 10*(5), 399–409. doi:10.1023/a:1023347020675

Torkildsen, L., Lambert, C., Nylund, A., Magnesen, T., & Bergh, Ø. (2005). Bacteria associated with early life stages of the great scallop, *Pecten maximus*: impact on larval survival. *Aquaculture International, 13*(6), 575–592. doi:10.1007/s10499-005-9002-5

Torkildsen, L., & Magnesen, T. (2004). Hatchery Production of Scallop Larvae (*Pecten maximus*) – Survival in Different Rearing Systems. *Aquaculture International, 12*(4), 489–507. doi:10.1023/b:aqui.0000042143.53903.21

Uriarte, I., Farías, A., & Castilla, J. C. (2001). Effect of antibiotic treatment during larval development of the Chilean scallop *Argopecten purpuratus*.

Aquacultural Engineering, 25(3), 139-147. doi:10.1016/S0144-8609(01)00078-4

Uriarte, I., Farías, A., Hernandez, J., Schäfer, C., & Sorgeloos, P. (2004). Reproductive conditioning of Chilean scallop (*Argopecten purpuratus*) and the Pacific oyster (*Crassostrea gigas*): effects of enriched diets. *Aquaculture, 230*(1–4), 349–357. doi:10.1016/s0044-8486(03)00419-8

Utting, S. D., & Millican, P. F. (1997). Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability. *Aquaculture, 155*(1–4), 45–54. doi:10.1016/s0044-8486(97)00108-7

Utting, S. D., & Millican, P. F. (1998). The role of diet in hatchery conditioning of *Pecten maximus* L.: a review. *Aquaculture, 165*(3-4), 167–178. doi:10.1016/s0044-8486(98)00268-3

Utting, S. D., & Spencer, B. E. (1991). *The hatchery culture of bivalve mollusc larvae and juveniles* (Laboratory Leaflet 68): Lowestoft.

Velasco, L. A., Barros, J., & Acosta, E. (2007). Spawning induction and early development of the Caribbean scallops *Argopecten nucleus* and Nodipecten nodosus. *Aquaculture, 266*(1–4), 153–165. doi:10.1016/j.aquaculture.2007.02.015

von Brand, E., Merino, G. E., Abarca, A., & Stotz, W. (2006). Chapter 27 Scallop fishery and aquaculture in Chile. In E. S. Sandra & G. J. Parsons (Eds.), *Scallops: Biology, Ecology and Aquaculture* (Vol. 35, pp. 1293–1314): Elsevier.

Wassnig, M., & Southgate, P. C. (2011). Embryonic and larval development of *Ptgeria penguin* (RÖDING, 1798) (Bivalvia: Pteriidae). *Journal of Molluscan Studies*. doi:10.1093/mollus/eyr051

Williams, J. R. (2005). *Reproductive ecology of the scallop Pecten novaezelandiae* (PhD). University of Auckland, Auckland.

Williams, J. R., & Babcock, R. C. (2004). Comparison of multiple techniques to evaluate reproductive variability in a marine bivalve: application to the scallop *Pecten novaezelandiae*. *Marine and Freshwater Research, 55*(5), 457–468. doi:dx.doi.org/10.1071/MF03200

Williams, J. R., & Babcock, R. C. (2005). Assessment of size at maturity and gonad index methods for the scallop *Pecten novaezelandiae*. *New*

Zealand Journal of Marine and Freshwater Research, 39, 851–864. doi:0028–8330/05/3904–0851

Woodburn, L. (1998). Genetic variation in the southern Australian *Pecten.* In M. Dredge, L., C., W. Zacharine, F., & L. Joll, M. (Eds.), *Proceedings of the Australian Scallop Workshop.* (pp. 226–240). Hobart: Tasmanian Goverment Printer.

Young, K. W., & Whittle, K. J. (1985). Colour measurement of fish minces using hunter L, a, b values. *Journal of the Science of Food and Agriculture, 36*(5), 383–392. doi:10.1002/jsfa.2740360511

Zhongguo, s. c. k. x. y. j. y., & Huang Hai shui chan yan jiu suo. (1991). *Training manual on breeding and culture of scallop and sea cucumber in China*. Qingdao: People's Republic of China.

5 Appendix

5.1 A
Formulae for solutions

Davidson's Solution

To make 3600ml of Davidson's Solution add the following chemicals in this order:

400ml Glycerin

800ml 30-40% Formaldehyde

1200ml 95% ETOH

1200ml of ambient filtered sea water

Harris's Haematoxylin

5g Haematoxylin (CL 75290)

50 ml absolute alcohol

100g Potassium alum (Aluminium potassium phosphate)

1000ml distilled water

40ml Glacial acetic acid

Eosin

1g Eosin Yellow

100ml distilled water

1 or two crystals thymol

Acid alcohol

99ml 70% ETOH

1ml Concentrated hydrochloric Acid (HCL sp.gr. 1.9)

Glutaraldehyde (3%)

4ml 3% glutaradldehyde

96ml filtered ambient sea water

5.2 B

Table 5.1. The embedding schedule for scallop's tissues using Shandon Citadel 2000 tissue processor.

Step	Chemical	Time	Amount of solution
1	70% ETOH	To be calculated. Choose number of hours to ensure schedule is completed during normal hours	1L (best fit all samples).
2	75%ETOH	1h	1L
3	80%ETOH	2h	1L
4	90%ETOH	2h	1L
5	95%ETOH	2h	1L
6	100%ETOH	2h	1L
7	100%ETOH	2h	1L
8	100%ETOH	1h	1L
9	Xylene	1h	1L
10	Xylene	2h	1L
11	Paraffin	2h	1L
12	Paraffin	2h	1L

5.3 C

Table 5.2. Staining methodology for scallop specimens using progressive staining.

Step	Chemical	Duration	Comments		Volume/ml
1	Xylol	5 min	Tank 1	Hydration	250
2	Xylol	5 min	Tank 2	Hydration	250
3	ETOH100%	3 min		Hydration	250
4	ETOH 100%	3min		Hydration	250
5	ETOH 95%	2min		Hydration	250
6	ETOH 80%	2MIN		Hydration	250
7	ETOH 75%	2MIN		Hydration	250
9	Distilled WATER	2MIN		Hydration	250
10	HAEMOLTOXYLIN	10MIN		Staining	250
11	Distilled WATER	5MIN	Wash	Staining	250
12	ACID ALCOHOL	10 SEC		Staining	250
13	Running tap WATER	2MIN		Staining	250
15	EOSIN	4MIN	May need to increase for better staining	Staining	250
16	WATER	2MIN	Wash	Staining	250
18	ETOH 95%	10 DIPS		Dehydration	250
19	ETOH 100%	10 DIPS		Dehydration	250
20	ETOH 100%	10 DIPS		Dehydration	250
21	XYLOL	10 DIPS		Dehydration	250
22	XYLOL	10 DIPS		Dehydration	250