# EXPERIMENTAL CULTIVATION OF THE SOUTH AFRICAN SCALLOP, *PECTEN SULCICOSTATUS*


**By**

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# DECLARATION

I declare that this thesis is my own work. This thesis has not been submitted in this or any other form to another university. Where use has been made of the research of others, it has been acknowledged by means of a complete reference. Experimental work discussed in this thesis was carried out under the supervision of Prof. C. L. Griffiths from the University of Cape Town and Dr. G. C. Pitcher from the Department of Agriculture, Forestry and Fisheries.

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# DEDICATION

This thesis is dedicated to my parents, the late Fredrick Norman and Elmeé Norman who nurtured my goals and instilled in me the importance of education. To my daughters, Sasha-Leigh and Tatum, who unknowingly motivated me, I hope this will help to pass on the scepter of the importance of education to the next generation. To my loving husband Irwin, my heartfelt thanks for all his support and motivation, but most of all his love and patience throughout this journey and our life together.

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# ABSTRACT

Although scallops have a high economic value, they have yet to be commercially harvested or farmed in South Africa. Of the 29 Pectinid species recorded on the southern African coastline, *Pecten sulcicostatus* is the only species considered suitable for aquaculture, due to its large size. An investigation of the suitability of *P. sulcicostatus* for farming in South Africa forms the basis of this study. The successful cultivation of *P. sulcicostatus* will depend on successful rearing from fertilized egg to market size, and achieving a growth rate comparable to that of other commercial scallop species. This study aimed to examine the reproductive life cycle, in order to establish whether this species can be artificially conditioned to produce ripe gametes throughout the year and to investigate the various stages of cultivation, in order to determine whether this species is viable for farming. This thesis therefore describes the reproductive cycle of *P. sulcicostatus* and also reports on the first assessment of broodstock conditioning, larval rearing and the grow-out of spat.

The reproductive study was undertaken by monthly collection of scallops in their natural habitat in False Bay from August 2004 - October 2005 and again from August 2010 - August 2011. The reproductive cycle was assessed by means of both gonadosomatic index (GSI) and qualitative and quantitative histological investigation. Environmental parameters were also monitored to determine any linkage to the reproductive cycle. The reproductive cycle was seasonal, with a peak spawning period in winter. A resting period appeared absent, as individuals started producing new gametes immediately after spawning, indicating a possible lack of synchronicity.

Spawning events were linked to environmental conditions, in that they were associated with a decline in bottom temperature and reduced food availability.

Owing to the seasonality of spawning, it was important to determine whether this species could be artificially conditioned to produce ripe gametes throughout the year. Laboratory held broodstock were artificially conditioned using three different feed concentrations. Following establishment of the most suitable feed concentration, further experiments were conducted to determine the rate of conditioning. Gonad status was assessed in all experiments by means of a gonadosomatic index (GSI), the GSI frequency, and by qualitative (oocyte stage) and quantitative (oocyte diameter) histological studies. Feed clearance and ingestion rates were also determined throughout the experiment. Clearance rates in medium and high food concentrations remained high for two weeks, before gradually declining. Gonad maturation was clearly influenced by feed concentration, both GSI and oocyte diameters increasing with higher food concentration. The study demonstrated successful artificial conditioning of broodstock. Although gamete development occurred in animals exposed to all three feed concentrations, the high feed concentration increased the rate of gamete development. To prevent uncontrolled spawning activity, it was shown that broodstock should not be conditioned for more than two weeks at a high feed concentration.

Spawning induction methods for *P. sulcicostatus*, including thermal shock, food deprivation, desiccation and hormones, were investigated. The hormone serotonin was the most effective for the induction of spawning. Following spawning induction the effect of temperature on larval growth and survival from D-larvae to pediveliger was also investigated. Of the four experimental temperatures 12.5, 15.5, 18 and 22 ºC,

larval growth and development was shown to be fastest at 22 ºC. The growth rate of 8 μm $day^{-1}$ compared favourably to that of other commercial species, but survival was low. As there was no correlation between temperature and survival other factors, such as food concentration and density, are considered to have contributed to the low survival of larvae.

The settlement of pediveligers on three substratums (poly-amide mesh, crushed oyster shells and polyethylene string) was investigated. A petri dish with no substratum served as a control. Settlement experiments were conducted in both light and dark environments. Pediveligers settled on poly-amide mesh and oyster shells and did not show a preference for light or dark environments.

The final investigation focussed on the grow-out of spat. Hatchery-reared juveniles were grown-out in suspended culture during three experimental periods between 2010 and 2012. Growth was assessed monthly through increments in shell height in relation to changing environmental conditions, as determined through continuous measures of temperature and chlorophyll *a* concentration. The mean growth rate of 0.10 mm $day^{-1}$ compared favourably with other commercially-cultured scallop species. Scallop growth was poorly correlated with both temperature and chlorophyll *a* concentration, but survival appeared to be influenced by temperature, exhibiting low survival during midsummer.

In conclusion, the study has shown that *P. sulcicostatus* can be conditioned and successfully spawned throughout the year. Although survival was low in both larval rearing and in the grow-out of spat, growth rates were comparable to those of other

commercial species. The study has thus demonstrated the potential for the farming *P.sulcicostatus* in South Africa, providing that the survival of all life cycle stages can be improved.

# CHAPTER 1:

# General Introduction

## Contents

## 1.1 Aquaculture

Fisheries and aquaculture make considerable contributions in terms of providing protein resources to ensure global food security. However, as the world population grows, the demand to supply food and nutritional needs increases, placing pressure on finite natural resources. Globally, production from capture fisheries has stabilised and most of the fishing areas and stocks have reached their maximum potential (FAO 2014). Aquaculture has therefore become an important sector in terms of providing additional protein resources and hence reducing the pressure on natural resources (Lee & Yoo 2014). Aquaculture also has the potential to alleviate poverty in rural areas.

FAO data for 2012 indicate a global yield of 154 million tons of fish from capture fisheries and aquaculture, of which aquaculture contributes 63.3 million tons (FAO 2014). The contribution of aquaculture to total fish production has increased steadily from 13.4 % in 1990, to 25.7 % in 2000 and to 42.2% in 2012, with China remaining by far the top producer in aquaculture, contributing 62% of the total (FAO 2014). The top three regions in terms of aquaculture production in 2012 were Asia (58 million tonnes or 88.38%), America (3 million tonnes or 4.79%) and Europe (2 million tonnes or 4.32%) (FAO 2014). Globally in 2012 about 38 599 250 tonnes of fresh water fish, 5 551 905 tonnes marine fish and 15 170 738 tonnes of molluscs were cultured (FAO 2014). However, the sustainability of the rapid global development of aquaculture remains challenging and three elements, namely environmental, economic and sociological, need to be addressed (Caffey *et al*. 1998, Naylor *et al*. 2000, Barrett 2002).

In South Africa the *Policy for the Development of a Sustainable Marine Aquaculture Sector* (Department of Environmental Affairs and Tourism 2007) outlines the importance of aquaculture and is aimed at promoting an economically sustainable and globally competitive industry. The main aim of the Policy is to encourage development of the industry and Government is required to facilitate and support this growth in order to address the main challenges facing South Africa today, which are poverty and unemployment. In terms of the Policy, there are a number of reasons for South Africa's poor performance and relatively low contribution to global aquaculture production. One of these is limited human resource capacity in aquaculture research, management, technical and advisory services.

## 1.2 Marine aquaculture in South Africa

In 2012 aquaculture production in South Africa (excluding seaweed, carp, ornamentals and koi carp) was 3 926 tons, of which the marine aquaculture industry contributed 2 262 tons (DAFF 2013). The only marine species cultured in South Africa are abalone (*Haliotis midae*), Pacific oyster (*Crassostrea gigas*), mussels (*Mytilus galloprovincialis* and *Choromytilus meridionalis*), dusky kob (*Argyrosomus japonicas*) and seaweeds (*Ulva* spp and *Gracilaria* spp) (Table 1.1) (DAFF 2013). Several other species are being investigated as potential aquaculture species in South Africa (Table 1.1). Abalone is the highest contributing sub-sector, with a production of 1 111 tons, followed by mussels (859 tons) and oysters (241 tons) (DAFF 2013).

Table 1.1: The operational scale of marine aquaculture species in South Africa during 2012 (DAFF 2013)

| **Common Name** | **Scientific Name** | **Operational Scale** |
|---|---|---|
| Abalone | *Haliotis midae* | Commercial |
| Pacific oyster | *Crassostrea gigas* | Commercial |
| Mediterranean mussel | *Mytilus galloprovincialis* | Commercial |
| Black mussel | *Choromytilus meridionallis* | Commercial |
| Seaweed | *Ulva* spp | Commercial |
| Seaweed | *Gracilaria* spp | Commercial |
| Dusky kob | *Argyrosomus japonicus* | Commercial |
| Yellowtail | *Seriola lalandi* | Research |
| White stumpnose | *Rhabdosargus globiceps* | Research |
| Spotted grunter | *Pomadasys commersonnii* | Research |
| Yellowbelly rockcod | *Epine marginatus* | Research |
| Mangrove snapper | *Lutjanus argentimaculatus* | Research |
| South Coast sea urchin | *Tripneustes gratilla* | Research |
| South African scallop | *Pecten sulcicostatus* | Research |
| Bloodworm | *Arenicola loveni* | Research |

A total of 34 marine aquaculture farms, of which five were new abalone farms, were operational at the end of 2012 (DAFF 2013). Twenty-three farms were situated in the

Western Cape Province, comprising four sub-sectors namely: abalone, finfish, oysters and mussels (DAFF 2013). The total value of aquaculture in South Africa in 2012 was estimated at R504 million, of which abalone was by far the largest contributor, estimated at R405 million (DAFF 2013).

## 1.3 Scallop aquaculture

Scallops are tasty, robust and not cannibalistic. Furthermore, scallops have a high economic value, rapid growth rate and show early maturity, all features which make them suitable for aquaculture. The adductor muscle, which is cream in colour, is the most sought after part of the scallop.

Worldwide about 40 scallop species are commercially exploited, of which 18 species account for the bulk of global production of 2.8 million tons of live weight from both capture fisheries and aquaculture (FAO 2014). China is the largest contributor, and contributes about 50% of scallop production (Guo *et al*. 1999), followed by Japan with a contribution of 25%. As the demand for scallops increases, scallop cultivation has to a large extent replaced captured fisheries in countries such as Japan, China and Chile (Ventilla 1982, Guo *et al*. 1999, Stotz 2000). Although scallops are successfully cultured in a number of geographic locations, the demand for scallops continues to increase.

## 1.4 Anatomy of scallops

The shell of *Pecten* species consists of two valves, with the right (lower) valve being convex and the left being flattened (Branch *et al*. 2010). The valves are composed of calcium carbonate and consist of three layers. These layers are known as the nacreous (inner) layer, the prismatic (middle) layer and the periostacum (outer) layer. The region where the shells are joined together is known as the umbo or hinge (Helm *et al*. 2004). The shell of a scallop can also be used to age the scallop and to provide information as to the environmental conditions experienced during its life (Kilburn & Rippey 1982).

The functions of the mantle of a scallop include to secrete the shell and ligament, to generate the swimming response via the velum, and to have a respiratory function (Fig. 1.1) (Beninger & Le Pennec 2006). In scallops, inhalant and exhalent regions control the inflow and outflow of water within the matle cavity directed by the beating of the gill cilia (Beninger & Le Pennec 2006).

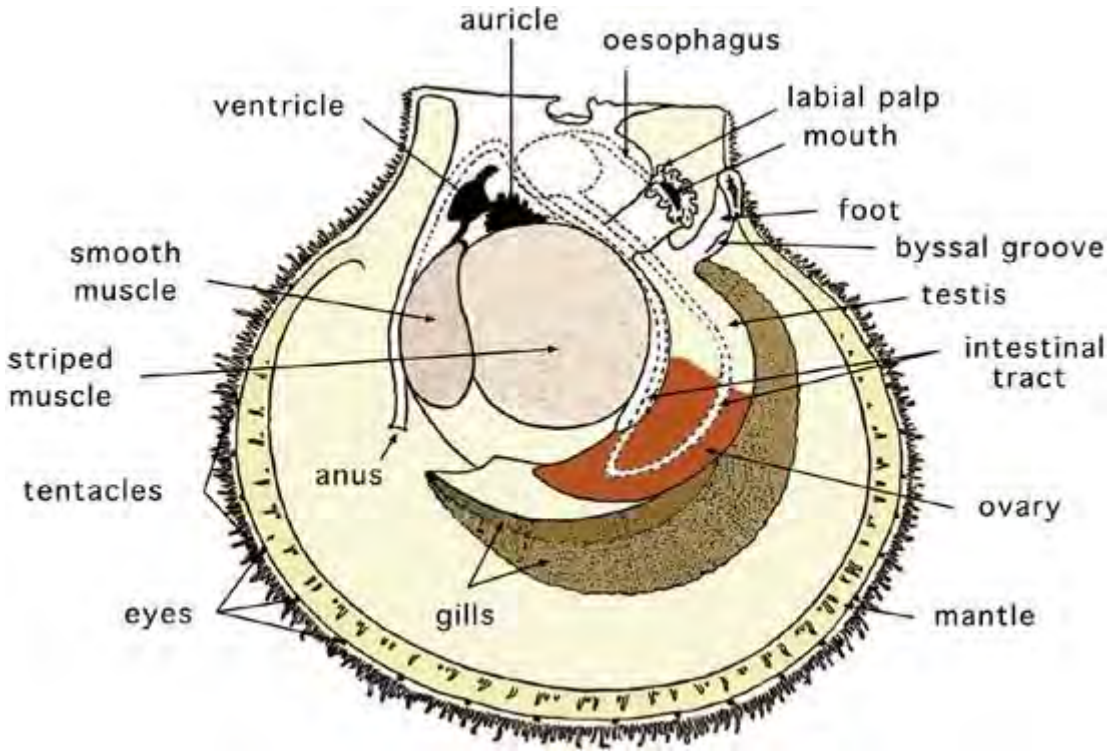


Figure 1.1: Internal anatomy of a typical scallop (from Helm *et al.* 2004)

There is usually a fold on the edge of the mantle, known as the velum (Cox 1957). Scallops have dozens of beautiful simple blue eyes arrayed along the edges of their mantle (Dakin 1910a, Land 1965, Speiser and Johnsen 2008, Speicer 2010). According to Dakin (1910a) the numbers of eyes varies from animal to animal and species to species and the size of the scallop does not affect the number of eyes present. Each eye can form a decent image using a combination of a lens, a focusing mirror, and two functionally distinct retinas back to back (Land 1965). The two retina types are very sensitive to any changes in light (Land 1965, Speiser and Johnsen 2008). Pectinids uses their eyes to sense potential predators and to find a good habitat (Hamilton & Koch 1996). Speiser and Johnsen (2008) have also nore that other functions of the eyes are to assess the concentration of suspended particles and water

flow rates for feeding purposes. Speicer (2010) notes that scallops which are highly mobile tent to have better vision than those species that are less mobile, or immobile.

*Pecten* species have a large adductor muscle that is centrally located in the scallop. This muscle is the most commercially valuable part of the scallop and is regarded as a delicacy in many parts of the world (Chantler 2006). The adductor muscle found in scallops is much more developed than that of other bivalve species, as they are active swimmers (Boyd & McNevin 2005). According to Helm e*t al.* (2004), the adductor muscle has the function of closing the valve of the scallop and consists of a set of rough fibres known as a quick muscle and a set of smooth fibres known as a catch muscle. The closing of the valves forces a strong jet of water on either side of the hinge. After each contraction, the muscle relaxes and the elastic energy stored in the hinge ligament opens the shells, ready for another contraction. In this way, the sequence of shell movements seen during swimming looks like the scallop is "biting" into the water. At the end of the swim, the shells slowly come closer together as the rapid shell movements cease and the animal sinks to the seabed (Joll 1989, Cheng *et al*. 1996). Larger scallops display limited swimming activity than scallops smaller in size (Winter & Jenkins 1985, Jenkins *et al*. 2003, Bailey and Johnson 2005). Winter & Jenkins (1985) has also described swimming activity to be effected by the height attained in the water column and the amount of rest allowed after a previous swim. Scallop larvae first become mobile at the gastrula stage (Cragg 2006) and have been described to have three phases of swimming behaviour before metamorphosis, namely: swimming upwards continuously in trochophores and early veliger stage; swimming intermittently in a vertically orientated spiral and then sinking during veliger and pediveliger stages and finally accumulating close to substratum just before

settlement (Cragg 1980). Young juveniles can be carried by water currents, aided by secreting a byssus thread (Beaumont & Barnes 1992).

Scallops possess a muscular foot that is specialized for burrowing into sand and mud. In adult scallops, the foot may be reduced. However in larvae and juveniles, the foot is important for locomotion (Helm *et al.* 2004). The gills are well developed and are responsible for respiration. Cilia presenton the surface of the gills serve to draw water into the mantle cavity. The water then circulates over the gills and other body surfaces, until it is expelled (Karleskint Jr. 1998). The gills are also important because they generate water currents that bring in food necessary for the survival of the scallop (Cox 1957). Once the gills have collected food particles from the water, the labial palps then sort and pass the food into the mouth. The food, which is in the form of a bolus (particles bound together by mucus), then travels through a short oesophagus to a hollow, chambered stomach with many openings. Leading from one of the openings of the stomach is a gland known as the diverticulum (Helm *et al.* 2004). Another opening leads to a sac-like tube known as a crystalline style. The style functions to mix the food in the stomach and to secrete enzymes that assist with digestion of food (Helm *et al.* 2004). Once the food has been digested it is then absorbed in the intestine and passes through the rectum, with any waste material then excreted through the anus.

## 1.5 Reproductive physiology

Understanding the reproduction cycle of a species contributes to the management of any commercial fishery (Barber & Blake 2006). Furthermore an understanding of

reproductive process and spawning periods is of importance to the production of commercial bivalve molluscs, because maximised spat collection and hatchery production is based on artificially-induced spawning (Yuan 2012). The reproductive cycle also indicates the best time to collect scallops, as in most cases the weight of the adductor muscle varies seasonally in relation to gametogenesis (Barber & Blake 1993, 2006). In sea ranching, specifically when stock-specific juveniles are used for recruitment, knowledge of the reproduction cycle and performance in the hatchery becomes imperative (Magnesen & Christophersen 2008). For example, in Norway a demand of spat for sea ranching sometimes occur outside the reproductive cycle and the Norwegian government regulations state that animals deployed for sea ranching must be from local origin in order to prevent risk of pathogens or parasites or genetics of conspecies (Anon 2006).

In pectinids the reproductive cycle includes activation, growth and gametogenesis, maturing of gametes, spawning and an inactive period (Sastry 1979). Sause *et al*. (1987) suggested that scallops from the southern hemisphere, spawn during the same calendar months as those from the northern hemisphere [June – October (winter – spring)], indicating that species from the southern hemisphere spawn when temperature is at its minimum. In the species *P. alba* from Victoria, Australia, the main spawning event is in winter/spring (Sause *et al*. 1987). This period is similar to that of *P. maximus* from Austevoll in Norway (Strand & Nylund 1991); *A. irradians irradians* from the USA (Sastry 1970a); *A. irradians concentricus* from Florida, USA (Sastry 1961); *Placopecten magellanicus* from Canada (Beninger 1987). However, in *P. zelandiae* from New Zealand the main spawning period in Wellington is May – January (Booth 1983). In *Amusium japonica balloti* from the Shark Bay population in

Australia the main spawning period is December - January (Heald & Caputi 1981). In *Aequipecten tehuelchus* from the Patagonia population in Argentina, the main spawning period is also in December – January with an increase in water temperature (Navarte & Kroeck 2002). Therefore, it appears that there is no consistent pectinid gametonic response to temperature and other factors like inter- and intra- specific variations, exogenous and endogenous factors influence gonad growth and gametogenesis (Sastry 1979, Barber & Blake 2006).

There are also inter- and intra-specific variations in frequency and timing of spawning activity (Barber & Blake 2006). Variations in timing of gametogenesis have been observed in several species. For example, in *P. maximus*, differences in timing in gametogenesis were observed between populations from south Norway and north Norway (Magnesen & Christophersen 2008). The gonads of scallop populations from southern Norway decrease during autumn and winter, whereas gonads of scallop populations from northern Norway increases during autumn. In *Placopecten magellanicus* collected from different sites in Passamaquoddy Bay, Canada, over a 13 year period the reproductive cycle of the giant scallop was consistent (Parsons *et al*. 1992). In *Argopecten irradians* gametogenesis were consistent over three years for the population in southern Florida, USA (Barber & Blake 1983). However, spawning occurred later in the southern population than to the northern population of *A. irradians in Florida* (Barber & Blake 1983).

Temperature and food are the exogenous factors most often cited as influencing gametogenesis in bivalves (Sastry 1979, Barber & Blake 1983, Rodman & Capuzzo

1983). For example, in *A. irradians*, the gametogenic cycle increased with an increase in food availability and water temperature followed by a spawning event after the temperature dropped tremendously (Barber & Blake 1983, Rodman & Capuzzo 1983). In *Patinopecten yessoensis* on the east coast of Korea, higher food concentrations increased the GSI levels and an increase in water temperature triggered spawning (Uddin *et al*. 2007). Endogenous regulation such as neurohormones, secreted in the ganglia regulates the physiological changes in the gonad (Barber & Blake 2006). For example, in *P. yessoensis* the levels of dopamine and noradrenaline in gonads was correlated to gametogenesis instead of water temperature and regulated by estrogen (Osada & Nomura 1989). Yuan *et al*. (2012) described that a novel neuronal protein, oocyte maturation arresting factor, occurred in *P. yessoensis* that inhibits seretonin induced oocytes to spawn. In *P. maximus* it was found that seasonal levels of serotonin and dopamine were associated with the gametogenic cycle (Paulet *et al*. 1993).

Several means of gamete assessment have been described, of which visual observation is most widely used (Mason 1958a). Visual observation may be used to assess the size, shape and colour of the gonads. Gonads that are more mature are rounded and fattened, and the female portion is either deep red or bright orange in colour. The development of gonads has been classified in various stages (4-9) (Mason 1958a, Naidu 1970, Davidson & Worms 1989, Williams & Babcock 2005). When a gonad is spent the entire gonad is colourless and has a watery appearance (Barber & Blake 2006). Although visual assessment is the simplest way, it only provides a rough estimate of gonadal development.

Greater information regarding gonadal development is provided by establishing either gonad weight or a gonad index. Gonad weight has been used to determine gametogenic cycles for several scallop species (Ursin 1956, Ansell 1974, Broom & Mason 1978, Dredge 1981, Moyer & Blake 1986). Dry weights are generally preferential to wet weights, as the water content in gonads varies seasonally. The mean gonad weight is determined throughout the year and declines in gonad weight provide a simple yet effective means of assessing the timing of gametogenesis (Barber & Blake 1981).

The gonad index (GI) or gonosomatic index (GSI) is another method to define gametogenic cycles in scallops. The GSI describes gonadal mass as a proportion of total body mass. The GSI has also been calculated by using remaining tissue weight instead of total tissue weight (Latrouite & Claude 1979). When using indexes, the allometric relationship between gonad and somatic tissue should not change over the size range of a population, the growth equations of the gonad and somatic tissue are the same and the weight of the somatic tissues minus the gonad weight does not change over time (Barber & Blake 2006).

In order to determine cytological changes within the gonad, histological investigation is required. Histological investigations are expensive and time-consuming, but provide a means to examine and assess gamete development more precisely. When the index mass decreases this may be due to either reabsorption of the oocytes, full-on

spawning activity, or a partial spawn (Barber & Blake 2006). Histological investigation is the only method that can describe the exact cause of a decrease in the index mass. Developmental stages of gametogenesis have been ascribed to certain cytological features (Mason 1958a, Sastry 1961, Bull 1976, Dredge 1981) and these descriptions may be used in a qualitative analysis. However, it has also been found that oocyte diameter decreases sharply after a spawning event, generating quantitative data.

## 1.6 Scallop nutrition

Scallops are filter-feeders, and can ingest living and inert particles suspended in the water column (Vernet 1977, Shumway *et al*. 1985, Cranford & Grant 1990, Milke *et al*. 2006, Lu *et al*. 2015). Phytoplankton are the main nutrient source for scallops, due to their nutritional value which includes protein, essential fats, trace minerals and B complex vitamins (Cranford & Grant 1990, Coutteau & Sorgeloos 1992, Milke *et al*. 2006). Various research studies have been conducted to determine the nutritional requirements for scallop larvae, postlarvae and broodstock (Shumway *et al*. 1985, Cranford & Grant 1990, Whyte *et al*. 1990, Coutteau & Sorgeloos 1992, Milke *et al*. 2006, Gagné *et al*. 2010).

Diets of cultured phytoplankton (*Isochrysis* sp. and *Chaetocerous* sp.) and re-suspended sediments were fed to the sea scallop *P. magellanicus* (Cranford & Grant, 1990). The above study indicated that the sea scallop only utilized a small fraction of the total sediment organic matter. The results demonstrated that particulate matter

may play an important role in energy gain and nitrogen, however, phytoplankton contributed significantly to the diet. It was indicated that although phytoplankton is important to the diet of sea scallops, detrital particles can contribute to energy gain during periods when phytoplankton are less available to meet energy demands. Particle selection occurs in scallops; with negative particle selection having been previously demonstrated (Vernet 1977). Larvae and adult scallops have the ability to retain particles and the consumption of particles depends on food concentration, temperature, the nutritive value of food and water flow (Shumway *et al*. 1985, Wildish *et al*. 1987, Cranford & Grant 1990). However, sea scallops are incapable of selecting organic particles in preference to inorganic silt during ingestion (Cranford & Grant 1990). Shumway *et al*. (1985) demonstrated in a laboratory that the sea scallop has the ability to reject less palatable species in the form of pseudofeces and feces.

Traditional hatchery diets generally include *Isochrysis* sp., *Pavlova* sp. and *Chaetocerous* sp. and are considered to meet the nutritional requirements of bivalves (Coutteau & Sorgeloos 1992, Torkildsen & Magnesen 2004, Gagné *et al*. 2010). A study conducted on *Patinopecten yessoensis* demonstrated that a high level of diatoms contributed to the scallop's nutrition (Silina & Zhukova, 2007). Silina & Zhukova (2007) also showed that relatively high proportions of heterotrophic flagellates, ciliates and invertebrate larvae form part of this specie's diet and an insignificant contribution of detritus occurs. The study further described that scallops found on muddy bottoms were more reliant on diatom food sources. O'Connor *et al*. (2000) demonstrated that the fecundity in the scallop *Mimachlamys asperrima* is greatest when fed *C. muellerii*. Essential amino acids and fatty acids have been shown to be important in the tissue formation and growth of scallops (Webb & Chu 1983, Chu &

Webb 1984). Chu & Greave (1991) has demonstrated that *P. maximus* has limited ability to elongate and desaturate fatty acid and require specifically eicosapentaenoic and docosahexaenoic acid for gonad maturity. *Chaetocerous* and *Isochrysis* have been shown to have high levels of eicosapentaenoic and docosahexaenoic acid respectively (Utting & Millican 1998). In the scallop *Argopecten purpuratus* from Chile, individuals were conditioned with a high, normal and low protein diet (Farías & Uriarte 2001). The diet consisted of *Isochrysis galbana* and *Chaetocerous neogracile* pre-conditioned at different nitrogen levels (high, normal and low). The study demonstrated that gonads matured in the high and normal levels during conditioning (Farías & Uriarte 2001). Pectinids fed a mixture of microalgae in culture have shown positive results (Farías & Uriarte 2001). Mixed diets generally contain the diversity of biochemical needed to satisfy most nutritional requirements for larval growth (Whyte *et. al*. 1990). In a hatchery environment, recirculation systems can be used (Heasman *et al*. 1996), however a flow-through system may be more suitable, as the phytoplankton in the incoming seawater is an important compenent in the diet of broodstock, specifically during conditioning (Millican & Helm 1994).

Milke *et al*. (2006) identified the dietary requirements for post-larvae and juveniles of *Argopecten irradians*. Several diet combinations were made up of one of three diatoms (*Chaetocerous muellerii, Thalassosira weissflogii, Fragilaria familica*) grouped with one or more flagellates (*Pavlova lutherii, Pavlova sp., Tetraselmis striata, Rhodomonas lens* (Milke *et al*. 2006). *C. muellerii* were also grouped with the four flagellates and *Pavlova sp.* were also used as a unialgal diet. The study demonstrated that a combination diet of *Chaetocerous muellerii* and *Pavlova sp.* was the best diet for post-larvae and juveniles of *Argopecten irradians* (Milke *et al*. 2006).

In *P. maximus* it was demonstrated that the addition of *R. salina* to a diet improved the growth, survival during larval and post-larval development (Gagné *et al*. 2010). Larval growth and metamorphosis are to a large extent dependent on the acquisition of energy reserves (Gagné *et al*. 2010). A sufficient lipid reserve is required for bivalves to complete metamorphosis, as their ability to feed on exogenous particulates is limited during that stage (Baker & Mann 1994). Growth and survival of marine bivalves also rely on obtaining essential fatty acids and sterols (Soudant *et al*. 1998a, Soudant *et al*. 1998b, Pernet & Tremblay 2004). In the larvae of *Crassodoma gigantea* carbohydrates are important in a diet, to convert the macronutrients to energy and tissue reserves (Whyte *et al.* 1990).

## 1.7 Broodstock conditioning

Broodstock conditioning is essential for the provision of larvae throughout the year in the operation of a hatchery. It has been shown that scallops exposed to adequate temperature and feeding regimes can be successfully conditioned to undergo gametogenesis outside of their normal seasonal cycle (Turner & Hanks 1960, Sastry 1961). Adequate nutrition has been described as essential in producing a high number of good quality gametes (Monsalvo-Spencer *et al*. 1997, Racotta *et al*. 1998, Navarro *et al*. 2000). A microalgal diet consisting of high protein levels has been shown to decrease gamete development, improve the energy balance and increase the fecundity of females (Farías & Uriarte 2001).

In *Argopecten purpuratus*, a diet consisting of *Isochrysis galbana*, *Chaetoceros gracilis* and *C. calcitrans* for 48 days did not produce mature gametes; however individuals in the wild during the same period became fully matured and spawned (Martinez *et al*. 1992). Navarro *et al*. (2000) in turn fed *A. purpuratus* a microalgal diet with a lipid emulsion supplement and the gametes became fully mature. Another possible impact on broodstock conditioning is the delivery of the diet, as conditioning in some species is successful with continuous feeding, while others do better in batch feeding systems (Racotta *et al*. 1998). Although overfeeding of broodstock may not influence the gametogenic cycle, it has a financial impact on commercial hatcheries due to cost of phytoplankton production (Barber & Blake 2006). It is therefore important to find the proper diet, method of delivery, and the right quantity and quality of algae for a specific scallop species in order to successfully condition broodstock.

## 1.8 Spawning and fertilisation

Scallops spawn during different times of the year depending on the species and geographic location. Several environmental factors trigger spawning events, such as temperature, chemical and physical stimuli, water currents, phytoplankton biomass or a combination of these or other factors in the natural environment (Vélez *et al*. 1990, Martinez *et al*. 1996).

In a hatchery environment spawning is usually induced by mimicking chemical, physical and sexual stimulation experienced in nature. Introgonadal injection of

serotonin and seawater irradiated by ultraviolet light (UV) were both successful methods used to induce spawning activity in *Placuna placenta* (Madrones-Ladja 1997). In *Patinopecten yessoensis*, the number of eggs released increased significantly when ovarian pieces of the scallop were reared in media containing serotonin (Matsutani & Nomura 1987). ). However, the use of serotonin injected into the gonad of *A. pleunorectes* induced spawning activity in the males only and eggs were obtained by stripping gametes of ovaries (Belda & Del Norte 1988). Vélez *et al*. (1990) showed that spawning induction using serotonin, thermal stimulation or serotonin and thermal stimulation was successful in releasing spermazoa only in *P. ziczac*. In *P. maximus* spawning induction is successful by thermal stimulation (Cochard & Devauchelle 1993, Millican 1997). However, *P. maximus* injected with serotonin has been described to only release sperm and the treatment is usually followed by mortality (Utting & Millican 1998). Thermal stimulation was also the only method to successfully release eggs in *P. ziczac* (Vélez *et al*. 1990). However, thermal stimulation was unsuccessful to induce spawning activity in *Amusium pleunorectes* (Belda & Del Norte 1988).

In hermaphroditic bivalves it is essential to ensure that cross fertilisation takes place in order to prevent stagnation in cell division, or the development of abnormal larvae. Once sperm have been added to the eggs, it takes approximately ± 60 - 90 min for fertilisation to occur, depending on the species and temperature of incubation. The first sign of successful fertilisation is extrusion of a transparent dome-like structure above the egg, called a polar body (Fig. 1.2) (Helm *et al*. 2004). However, in some species the polar body forms within a couple of minutes, e.g. in *Placuna placenta* the polar body formed within 15 minutes (Madrones-Ladja 1997). Following fertilisation

the egg will begin to divide into two equal cells, followed by four unequal cells and then an eight cell stage (Fig. 1.2) (Helm *et al*. 2004). The time from fertilised egg to eight cell stage is dependent on the species and specifically the temperature of incubation. In *A. pleunorectes* the cleavage of zygotes was obtained 1 hour after fertilization (Belda & Del Norte 1988). In *Placuna placenta* the entire embryonic stage took less than 6 hours to be completed ((Madrones-Ladja 1997).

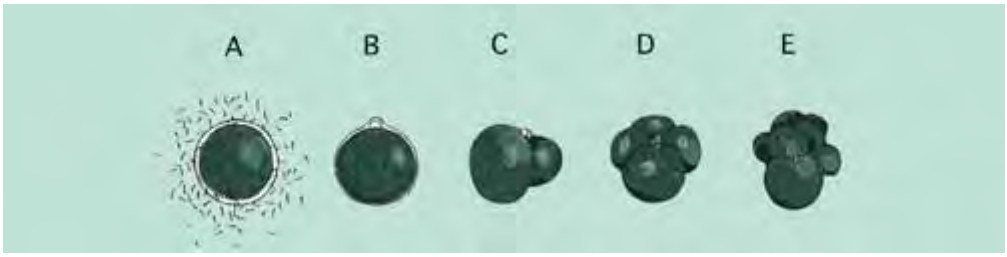


Figure 1.2: Stages in the early development of eggs; (A) sperm swarming around a rounded-off egg, (B) extrusion of the first polar body following fertilization, (C) two-cell stage also showing the second polar body (D) four-cell stage and (E) eight-cell stage. The eggs of most oviparous bivalves range in size from about 60 - 80 μm, depending on species. The time from fertilization to the various developmental stages is species and temperature dependent (from Helm *et al*. 2004).

## 1.9 Larval rearing and settlement

Larvae can be reared in either flow-through systems, or in high density static systems (Helm *et al*. 2004), with the latter systems presently the more common option. It is important that the rate of feed ingestion by larvae is known as it is better to overfeed than underfeed larvae (Helm *et al*. 2004).

Environmental parameters are very important in larval rearing. Light intensity is not often investigated in larval rearing experiments, however, Krassoi *et al.* (1997) demonstrated that high light intensities correlate with high abnormal development. Temperature is the most important parameter for larval development and growth and is often critical in achieving the best results during the first developmental stages of scallop larvae (Roman *et al*. 1996, Le Pennec *et al*. 2003, Helm et al. *2004,* Parsons & Robinson 2006).

Growth of larvae can be described in terms of growth stages, cleavage rate and shell growth (Cragg 2006). Generally scallop larvae have a growth rate of 3 - 5 μm $d^{-1}$ (Cragg 2006), but it is unknown whether larvae develop faster in the laboratory than in the sea. The rate of shell growth in larvae is generally an indication of the effectiveness of feed and feeding regimes (Cary *et al*. 1981). In some studies egg size has been shown to be a predictor of shell growth, with larger eggs producing larger veligers (Paulet *et al*. 1988). Self-fertilisation of eggs can occur during spawning induction and even in the natural environment (Winkler & Estévez 2003). An increase of malformations and the reduction of sustainability and fecundity are some of the first indicators of inbreeding depression in animals (Concha *et al*. 2011). It has been shown that larvae from cross-fertilised eggs of *A. circularis* develop much faster than self-fertilised eggs (Ibarra *et al*. 1995). In *P. maximus* reduced growth rates of larvae were related to inbreeding (Beaumont 1986). In *Aequipecten tehuelchus* less larvae were obtained in self-fertilized crossings than from cross-matings (Navarte & Pascual 2003). A study between self-fertilization and a mass-spawned group on *Argopecten irradians concentricus* from China, showed that survival, shell length, and live weight were lower in the larvae, juveniles and adults from self-fertilized eggs (Lui *et al*.

2011). However, Winkler & Estévez (2003), found that in *A. purpuratus* no difference occurred in the survival and growth of larvae and juvenile between self-fertilized and cross fertilized individuals. Also in the species *Euvola ziczac*, self-ferlization also had no effect on growth and survivorship of larvae (Betancourt *et al*. 1994). Once larvae become pediveligers, they are ready for settlement and then search for a suitable substrate to settle on. High mortalities usually occur during settlement, as larvae undergo behavioural changes associated with their search for an appropriate substrate. High mortalities also occur if settlement is induced too early and the larvae are not ready to settle (Culliney 1974). Other factors, such as larval origin, rearing density, microalgae quality and quantity and water conditions have been previously described to cause high mortalities in scallop larvae (Liu *et al*. 2006, Magnesen *et al*. 2006). Massive mortalities of commercial hatchery reared larvae of *A. purpuratus* were caused by a pathogenic strain of *Vibrio splendidus* (Rojas *et al*. 2015). Pathogenic bacteria have also been described to be one of the main factors affecting larval survival in *P. maximus* (Marine 2015). The type of culture system used can also affect the rate of survival in larvae, with a higher percentage of larval survival obtained in *A. purpuratus* larvae cultured in a closed aquaculture system than in recirculating aquaculture system (Merino *et al*. 2009).

Several types of settlement substrates have been used to settle larvae successfully, including fragments of glass, shell, pebbles, Nitex$^{TM}$, fibre glass tanks, acrylic plastic, polyethylene and onion bags (Culliney 1974, Fournier & Marsot 1986, Dabinett 1989, Tremblay 1988, Pearce & Bourget 1996). Chemicals such as chitin have also been successfully used to induce larval settlement (Harvey *et al*. 1997). Harvey *et al*. (1997) described that over 66% more bivalves and 35% more giant scallops were

obtained on Netron spat collectors coated with chitinous film, compared to those without chitinous film. Pearce & Bourget (1996) promoted settlement of *Placopecten magellanicus* larvae on various artificial and natural substrata. The substrata used were various diameters of nylon monofilament mesh with and without marine microbial film, polyethylene onion bag mesh, polyethelene Astroturf, smooth and roughened clear acrylic plastic, polyester aquarium filter-wool and adult giant scallop shells with and without marine microbial film (Pearce & Bourget 1996). Although settlement occurred on all the substrata used, polyester filter-wool was by far the best substratum for spat collection. Cropp (1993) obtained a 39.3% settlement of *Chlamys australis* larvae in a hatchery environment using red mesh bags filled with hardened monofilament shark netting. It has become a common practice to promote the larval settlement of *Argopecten purpuratus* using collectors covered with a coat of microbial biofilm in hatcheries (Leyton & Riquelme 2008). Avendaño-Herrera *et al*. (2002), described that microbial film formed on benthic surfaces may serve as food for newly settled scallop larval–postlarval stages. Microfouling of setting substrates under uncontrolled conditions has been found to be a highly variable and ill-defined process due to natural environmental variation (Leyton & Riquelme 2008).

In Tasmania, 4 mm onion bags and 7-8 mm Netlon bags were used as artificial substrates to collect spat of *P. fumatus* (Hortle & Cropp 1987). Hortle & Cropp (1987) found that the onion bags collected and retained a significant amount more spat than Netlon bags. Shrub branches (50g) included in two polyethylene onion bags of 6 mm in mesh size and Japanese Netlon (120 g) inside polyethylene bags 2 mm in mesh size was used as artificial substrata to collect spat of *Aequipecten tehuelchus* in San Matías Gulf (Navarte 2001). Significantly more spat settled on the Japanese Netlon included

in polyethylon onion bags the shrub branches in the polyethylene onion bags (Navarte 2001). Onion bags (40 x 70 cm, 6 mm mesh size) and polyethylene bags (30 x 30 x 30 cm, 4.5 mm mesh size) packed with nylon monofilament gill netting were used to collect *Chlamys islandica* spat in west Iceland and the polyethylene bags collected much more spat than the onion bags (Thorarinsdóttir 1991). Studies conducted in Norway showed that *Chlamys islandica* prefer thin (0.15 mm) monofilament to thicker (0.8 mm) monofilament (Wallace 1982). Naidu *et al*. (1981) showed that *Placopecten magellanicus* (Gmelin) prefers monofilament gill netting to flat polyethylene strips.

## 1.10 Grow-out of scallops

Spat are typically first grown in an intermediate culture to about 15 mm, before being transferred to another form of culture for final grow-out to market size (Hardy 1991). Pearl nets are most commonly used for intermediate culture (Ventialla 1982, Naidu & Cahill 1986). Important for intermediate culture is the cost of the gear and the requirement for minimal handling, as repeated handling increases mortality (Cole *et al*. 1996). Stocking density also needs to be taken into consideration in balancing the requirement for low densities to reduce mortality versus the requirement to use fewer nets to reduce costs (Parsons & Dadswell 1992).

There are two methods for final grow-out of juvenile scallops, namely suspension culture (also known as hanging culture) and bottom culture. Grow-out in suspension culture uses either cages (such as multi-tiered lantern nets and stackable plastic trays) or ear hanging (Bourne *et al*. 1989, Parsons *et al*. 1998). Suspension culture relies on

a longline or raft system. Rafts are more expensive than longline systems and are restricted to sheltered bays; however an advantage of raft systems is that less handling time is required, relative to longline systems. The ropes in longline systems are completely submerged, therefore reducing visual pollution.

Ear hanging is a cheaper method compared to that of lantern nets. Ear hanging involves drilling a hole in the ear, or in the byssal notch, and then threading a cord through this hole, tying it into a loop and attaching it to a submerged rope. This process is very labour-intensive, although semi-automation equipment is available (Anonymous 1991a). Mortalities can occur in the drilling process, particularly if scallops are small. Therefore ear hanging is not recommended for smaller scallop species. However, scallops grown by ear hanging methods have faster growth rates than scallops grown in cages (Wildish *et al*. 1988, Grant *et al*. 2003).

Bottom culture is the seeding of scallops at the bottom of the ocean, and therefore requires a leased area or property rights. An advantage of bottom culture is that it is much cheaper than suspension culture (Wildish *et al*. 1988). However, the scallops are more exposed to predators and may escape from the leased area (Barbeau *et al*. 1998). Another disadvantage is that growth rates are typically slower due to the inaccessibility of mid-water plankton (Hardy 1991).

Scallops grow faster in suspension culture than in bottom culture (Naidu & Cahill 1978, MacDonald 1986) because they have access to mid-water algal populations

(Hardy 1991). Further, the meat yield of scallops grown in suspension has been shown to be greater than that of scallops grown in bottom culture (Chandler *et al*. 1989).

## 1.11 Classification of *Pecten sulcicostatus*

**Kingdom:** Animalia

**Phylum:** Mollusca

**Class:** Bivalvia

**Order:** Ostreoida

**Family:** Pectinidae

**Genus:** *Pecten*

**Species:** ***Pecten sulcicostatus***

The scallop *Pecten sulcicostatus* G.B. Sowerby (1842) is one of 29 species of Pectinidae recorded off the southern African coast (Dijkstra & Kilburn 2001). Only 24% (7 species) of the 29 species recorded are largely or wholly endemic to southern Africa. The endemic species are: *Delectopecten vitreus, Psedamassium gilchristi, Tatochlamys multistriata, Pecten sulcicostatus, Aequipecten commutatus, Veprichlamys africana* and *Talochlamys humilis* (Dijkstra & Kilburn 2001). *P. sulcicostatus* is the only endemic species of South Africa large enough to be considered for marketing (Table 1.2).

Table 1.2 Distribution, size, depth and description of the seven scallop species endemic to South Africa

| **Species** | **Distribution in South Africa** | **Size and depth** | **Description** | References |
|---|---|---|---|---|
| *Delectopecten vitreus*<br><br>Gmelin (1791) | West Coast off Saldanha bay | 25 mm<br><br>200 - 310 m depth | 3-5 ridges, Semi-transparent, grayish in colour. | Gmelin J F 1791, Gofas *et al*. 2001, Dijkstra & Kilburn 2001 |
| *Psedamassium gilchristi*<br><br>Sowerby (1904) | Off Cape Columbine, Port Nolloth | 35 mm<br><br>130 - 420 m depth | 8 rounded ridges, orange or pinkish in colour | Dijkstra & Kilburn 2001 |
| *Talochlamys multistriata*<br><br>Poli (1795) | South Coast up to Kwa-Zulu Natal | 37mm<br><br>23 – 420 m depth | 7 – 15 spinose ridges, colour very variable, orange, purple, white, brown or yellow with pale lines. | Dijkstra & Kilburn 2001, Branch *et al*. 2010 |
| *Pecten sulcicostatus*<br><br>Sowerby (1842) | False Bay to East London | 106 mm<br><br>22 – 70 m depth | 12 – 15 ridges, pink to brownish in colour, equal ears, brightly coloured simple eyes at mantle edges | Dijkstra & Kilburn 2001, Branch *et al*. 2010 |
| *Aequipecten commutatus*<br><br>Monterosato (1875) | Eastern Cape Kwa-Zulu Natal | 20 mm<br><br>200 – 500 m depth | Creamy with white and orange spots and streaks | Dijkstra & Kilburn 2001 |
| *Veprichlamys Africana*<br><br>Iredale (1929) | Eastern Cape Kwa-Zulu Natal | 36 mm<br><br>275 – 420 m depth | 2 ridges, greyish in colour | Dijkstra & Kilburn 2001 |
| *Talochlamys humilis*<br><br>Sowerby (1904 | Agulhas bank up to Eastern Cape | 25 mm<br><br>70 – 140 m depth | 8 – 10 squamous ridges, colour strongly variable, uniform cream, yellow, orange, reddish, pinkish, purplish, brownish | Dijkstra & Kilburn 2001 |

## 1.12 The South African scallop *Pecten sulcicostatus*

*P. sulcicostatus* is endemic to the inner continental shelf of South Africa occurring on sand or muddy sand (Branch *et al*. 2010). The valves of *P. sulcicostatus* are unequal in that the left valve is flat and the right valve is convex. Both valves are sculptured with 12 - 15 radial costae and their colour is off-white, with pink, salmon or brown markings (Fig. 1.3 A). *P. sulsicostatus* is a functional hermaphrodite (Fig. 1.3 B).

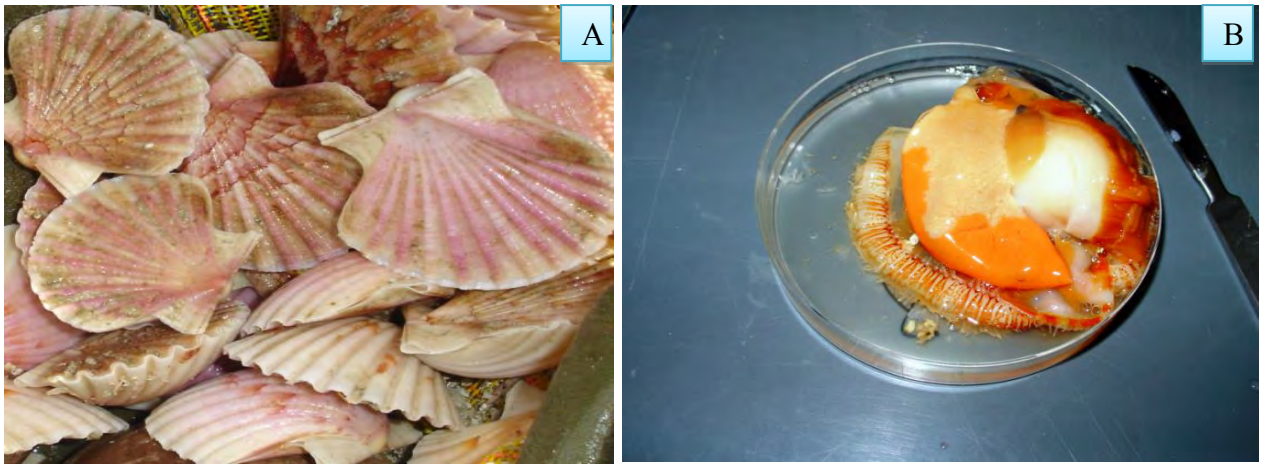


Figure 1.3(A) The South African scallop *Pecten sulcicostatus* and (B) gonads consisting of both male and female sexes (functional hermaphrodite)

Although Dijkstra & Kilburn (2001) treat *P. sulcicostatus* as a species, morphological similarity to *Pecten maximus* is acknowledged and both Fleming (1957) and Kilburn & Rippey (1982), who treated *P. sulcicostatus* as a subspecies of *P. maximus*. Previously *P. sulcicostatus* was known as *P. maximus sulcicostatus*, Sowerby 1842, but the status is currently stated as rejected (WoRMS). Saavedra & Peña (2005) have described *P. sulcicostatus* to be the first species to develop from *P. maximus*.

Exploratory fishing for *P. sulcicostatus* in 1972 revealed exploitable concentrations in the centre of False Bay, although the population size was regarded as insufficient to support a viable fishery (De Villiers 1976). Scallops were found at depths between 22 and 70 m, but the highest catches were at approximately 40 m depth. The average shell length of the scallops caught in False Bay was 94 mm, with a small number of animals exceeding 120 mm. The meat was considered to attain an adequate size for marketing, in that animals of 90 mm shell length yielded 15 g of meat. De Villiers (1976) attempted to determine the growth rate of *P. sulcicostatus* by analysis of size frequency distributions and measurements of annular growth rings. Growth curves presented for scallops from both False Bay and Mossel Bay indicated higher growth rates in False Bay. De Villiers (1976) suggested that commercial farming of *P. sulcicostatus* be considered, but advised that a period of 4 - 5 years may be required to achieve marketable size. All the above factors, including being a sub-species to a current commercial species, availability of broodstock, size, weight of adductor muscle, hermaphrodite, attractive colours and the increasing demand and the associated high price of existing commercial scallops, suggest that the South African scallop *P. sulcicostatus* is a viable candidate for culture. This has thus motivated this study, which aims to investigate the mechanisms suitable for commercial culture of this species.

## 1.13 Aims and objectives of this study

Interest has been raised in South Africa in developing culture technology for indigenous species. The South African scallop *P. sulcicostatus* is one of the species that has been identified as a candidate for aquaculture. This study aimed to investigate the viability of *P. sulcicostatus* for commercial culture in South Africa by addressing the following objectives:

1. To describe the reproductive cycle of *P. sulcicostatus*. An understanding of the reproductive cycle is important, as information is provided on the occurrence of distinct spawning periods, thereby indicating the best time of the year to collect broodstock for conditioning purposes. Important information relating to the conditioning and artificial spawning of broodstock is also provided by this understanding. This study is described in Chapter 2.

2. To determine whether *P. sulcicostatus* can be artificially conditioned to produce ripe gametes. The ability to condition broodstock throughout the year is essential for farming. Also determination of the duration of conditioning is important in preventing uncontrolled spawning events. This study is described in Chapter 3.

3. To determine the optimal temperature for larval rearing and the best substrate for settlement. Temperature is one of the key aspects in larval rearing with optimal temperatures favouring rapid growth, whereas suboptimal temperatures can stunt growth or cause high mortalities. Settlement of spat on a particular substrate during the right morphological stage is also vital for survival of scallop spat. This study is described in Chapter 4.

4. To determine the growth and survival rates of scallops during grow-out to market size. Successful scallop aquaculture depends on reliable growth and survival of spat during grow-out, as the cost of production is largely determined by the length of the grow-out period. The effect of environmental parameters on growth and survival is also important in determine the suitability of sites for grow-out purposes. This study is described in Chapter 5.

# CHAPTER 2:

## Reproductive cycle of the scallop *Pecten sulcicostatus* from the southern Benguela upwelling system

## Contents

## 2.1 Abstract

The reproductive cycle of *Pecten sulcicostatus* is described as part of an investigation into potential commercial culture of this species in South Africa. Scallops were collected monthly from False Bay, from August 2004 - October 2005 and again from August 2010 - August 2011, to determine seasonal variations in gonadosomatic index (GSI) and associated histological changes within the gonads. Investigations were also undertaken into the linkage of the reproductive cycle of *P. sulcicostatus* to environmental condition within False Bay, and of the parameters important to gonad maturation and that might trigger spawning. The reproductive cycle of *P. sulcicostatus* demonstrated clear seasonality. The mean GSI was highest from June - September (winter - early spring), and lowest from October - January (late spring - summer). The GSI findings were corroborated by quantitative and qualitative histological analysis of the gonads. Seasonal stratification and corresponding changes in phytoplankton biomass are considered to control the reproductive cycle of *P. sulcicostatus* in False Bay, as winter spawning and subsequent decline in GSI coincide with transition to spring upwelling conditions and decline in bottom temperature and food availability.

Key words: gonadosomatic index, *P. sulcicostatus*, reproductive cycle, histology, False Bay

## 2.2 Introduction

An understanding of reproductive processes is central to the management of any commercial fishery (Barber & Blake 2006), as information is provided on the recruitment and population dynamics of the species on which the fishery is based (Williams & Babcock 2004). Understanding the reproductive cycle of a species is also a requirement for successful cultivation, providing information essential to the collection, conditioning and spawning of broodstock. The reproductive cycles of many scallop species have been investigated (Sastry 1979, Barber & Blake 1983, MacDonald & Bourne 1987, Strand & Nylund 1991, Narvarte & Kroeck 2002) and in most cases have demonstrated distinct peaks in spawning activity. These peaks may differ temporally in association with the latitude of the population (Barber & Blake 1983, Strand & Nylund 1991) and in most temperate species the reproductive cycle has been linked to water temperature and food availability (Barber & Blake 2006).

*Pecten sulcicostatus* has been poorly studied and its biology and life history are unknown. This study therefore undertook to describe the reproductive life cycle of the *P. sulcicostatus* population in False Bay, South Africa, thereby providing knowledge fundamental to the future cultivation of this species. Investigations into the linkage of the reproductive cycle of *P. sulcicostatus* to the environment within False Bay, included assessment of the parameters important to gonad maturation and those that trigger spawning were also undertaken.

## 2.3 Materials and methods

### 2.3.1 Collection of *Pecten sulcicostatus*

To assess the reproductive cycle of *P. sulcicostatus* scallops were collected monthly from August 2004 - October 2005 and again from August 2010 - November 2011. Scallops were collected by scuba divers off Miller's Point in False Bay at a depth of approximately 20 - 30 m (Fig. 2.1). Sample size for the 2004 - 05 period varied from 13 - 46 individuals per month and for the 2010 - 11 period was maintained at 30 individuals per month.

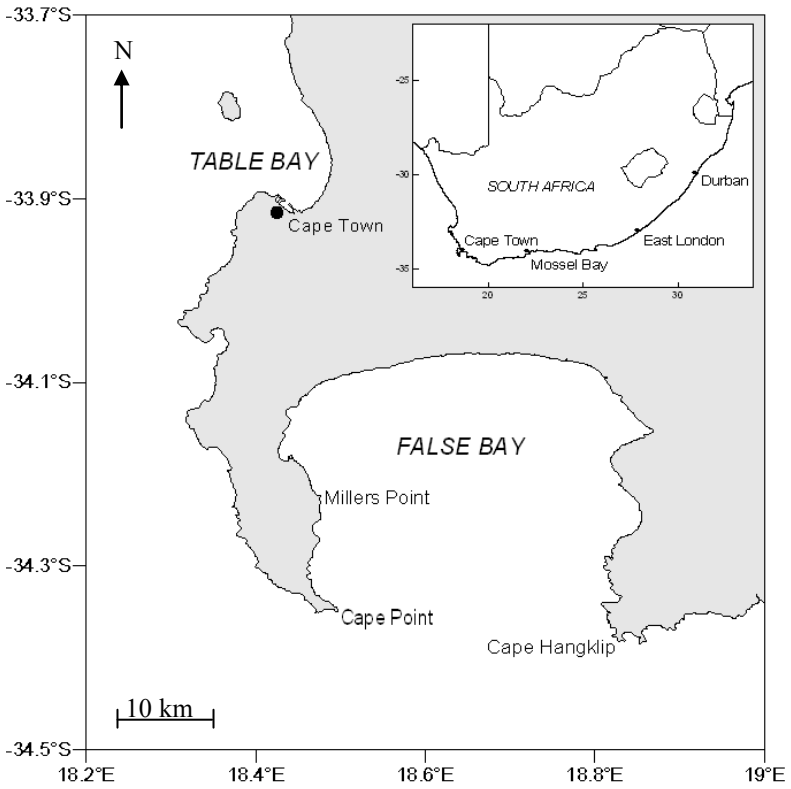


Figure 2.1: Map showing the site of collection of *P. sulcicostatus* off Miller's Point in False Bay.

The scallops were transported to the laboratory in Sea Point, Cape Town, wrapped in moist paper towels and packed into Styrofoam boxes held at 14 - 16 °C and were processed on the same day. Shell height was measured to the nearest 0.5 mm using a digital calliper and for the purpose of analysis scallops were divided into two size ranges: those < 70 mm in shell height, and those ≥ 70 mm for the 2004 - 05 collection. Scallops collected in 2010 - 11 were all between70 – 90 mm.

### 2.3.2 Gonadosomatic index (GSI)

The GSI was used to define the gametogenic cycle in the scallops. The whole scallop, shell, soft tissue, gonad and abductor muscle were each individually weighed (wet weight, ww) using a Denver Instrument APX-602 scale. All scallops were dissected in 2004 - 05 for GSI purposes. In 2010 - 11 only 15 of the 30 scallops collected each month were dissected for GSI purposes, as the remaining scallops were used for spawning induction. The GSI was calculated as follows (MacDonald & Bourne 1987):

GSI = (Gonad weight (ww) * 100) / Somatic tissue (ww)

The percentage of scallops undergoing various cycles of gametogenesis, such as vegetative stage, differentiation, cytoplasmic growth and vitellogenesis stage, were calculated for each month, in order to determine the GSI frequency. The following ranges of percentage GSI were used for each stage: vegetative 4 - 8%, differentiation 9 - 13%, cytoplasmic growth 14 - 18% and vitellogenesis 19 - 22%. The GSI and GSI frequency is a fast, inexpensive method to describe the gametogenic cycle of a

species, however, cytological details cannot be examined. However, histological investigations, although expensive and time-consuming, provides a more accurate description of gamete development.

### 2.3.3 Histology

Gonads collected were prepared for histological studies by placing them individually in Dietrich's fixative (Yevich & Barszcz 1977) for 1 h. To further expose the gonads to the preservative they were then removed from the fixative, cut into sagittal sections, wrapped in cheesecloth, and returned to the fixative for a further 24 h to ensure preservation of the interior tissues. The sections were then washed in seawater and stored in 70% ethanol. Further processing included six changes of tissue dehydrant comrising 70%, 80%, 95% and then three steps of absolute alcohol (99.9%). Time duration for each step was 1 h**.** Following three changes of the clearing agent toluene, the tissues were embedded in Paraplast®, following the procedures of Barber & Blake (1983). Tissues were sectioned (5 - 7 µm thick), placed on glass slides, stained with Harris' hematoxylin-eosin, and covered with a glass cover slip (Yevich & Barszcz 1977).

Oocyte areas, rather than diameters, were measured for quantitative analysis, as they are less influenced by sectioning techniques, and therefore provide more reliable data from which the oocyte diameter may be calculated (Dukeman *et al*. 2005). Oocyte areas in 2004 - 05 were measured using a Zeiss Photomicroscope and Boeckeler video imaging system, and in 2010 - 11 using a Nikon imaging system, basic research package version 3.2. Fifty oocytes were measured from each gonad. Oocytes prior to

maturation were targeted and included only those in which the nucleolus was clearly visible in the nucleus, as (Dukeman *et al*. 2005) assumed that a higher probability of sectioning through the centre of the oocyte occurs when the nucleoli is clearly visible. The oocyte areas were converted to diameters as follows (Griffin *et al*. 2006)

$$r^2 = \text{oocyte diameter average} \times \pi$$

$$r = \text{sqrt } r^2$$

$$d = r \times 2$$

where r = radius and d = diameter

Gonadal stages for specimens from 2010 - 11 were determined by qualitative histological analysis and classified into six different categories: early - maturation, mid - maturation, mature, partial spawn, spawn and recovery and spent (Navarte & Kroeck 2002).

### 2.3.4 Environmental assessment

During the monthly collection of scallops in 2004 - 05 seawater temperature was recorded to the nearest 0.5 ºC at a depth of 20 - 22m using a Santo D3 diver's watch. In 2010 - 11 environmental conditions were assessed for the duration of collection through continuous measurements of temperature and chlorophyll *a* throughout the experimental period. Measurements were set to record every 10 min. An Eco-fluorometer provided measures of fluorescence at 10 m depth and a Starman temperature recorder was deployed at 30 m depth. Measurements of *in situ* fluorescence were calibrated through comparison with extracted chlorophyll

concentrations as detailed by Parsons *et al*. (1984).

### 2.3.5 Spawning induction

Induced spawning took place for scallops only collected in 2010 - 2011. The remaining 15 scallops collected each month were acclimated over a 2 - 3 d period in filtered seawater prior to spawning induction. Before the scallops were induced to spawn the gonads of each scallop were visually assessed according to Williams & Babcock (2004) into the following stages (Table 2.1):

Table: 2.1 Visual grading system for measuring gonad condition in *P. novaezelandiae*. Visual grades are grouped into classes of general gonad condition (Williams & Babcock 2004).

| Visual Grade | Gonad appearance | Gonad condition |
|---|---|---|
| 1 | Gametes are absent or largely so. Gonad is small, thin, flacid, and translucent. Ovarian and testicular tissues are difficult to differentiate. Intestinal loop is clearly visible. | Immature or spent |
| 2 | Gonad is much reduced in size compared to visual grade 3 and has lost tugor. Ovary appears mottled or lattice-like, presumably owing to the majority of acini that have been vioded. 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. | |
| 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. Inestinal loop is partially obscured. | |
| 6 | Ovarian tissue appears uniform in colour and texture as acini fill. Very little of intestinal loop is visible (usually only a small portion of the ascending limb at the distal extremity of the gonad). | |
| 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 visble. | |

Following the visual assessment of gonads, the scallops were exposed to four spawning induction techniques: temperature shock, feeding after initial starvation, desiccation, and hormone injection. For the temperature shock treatment all 15 scallops were placed into a large tank where the temperature was raised from 12 - 17$^0$C over a period of 1 h. Thereafter a mixture of *Chaetocerous, Pavlova* and

*Isochrysis* at a 1:1:1 ratio, totaling 15 million cells $L^{-1}$ was added to the tank and scallops were left for a further hour, as part of the shock - feeding treatment. Scallops were then placed individually in glass bowls, from which they were removed every 10 min and returned following a 5 min period of desiccation. This desiccation treatment was repeated for an hour. Thereafter scallops were intragonadally injected in both the male and female areas of the gonad with 0.2 ml serotonin (2 mM) hormone solution and placed back in the glass bowls. The number of scallops that released sperm and eggs following each spawning induction method was recorded. The number of eggs and D-veligers obtained per month were counted according to Helm *et al.* (2004):

Eggs / D-veligers counts in three sub-samples = A; B; C.

Mean = (A + B + C)/ 3 = D

Volume of sub-sample = E

Total Volume of cylinder = F

**Total volume of eggs = F/E x D**

### 2.3.6 Statistical analysis

Statistical analyses on data collected were carried out using Statistica version 6.1 (Statsoft Inc.) and Sigma Stat 3.1 (Systat Software Ic.). The Shapiro-Wilk test was used to test for normality and Levene's test was used to test for equal variance. A t-test or a Mann-Whitney U test was used to test for differences in the GSI between the two size groups (<70 mm; ≥70 mm) of scallops collected each month for data collected in 2004 - 05. The monthly mean GSI was established by pooling these two size groups.

The Kruskal-Wallis one way ANOVA by Ranks test was used to compare the mean GSI and mean oocyte diameters between months and the Dunn's Post hoc and Tukey HSD test was used establish which months were different respectively. The significance level (α) was set at 0.05.

Statistical analyses on data collected during 2010 - 11 were completed using Statistica version 6.1 (Statsoft Inc.). The Shapiro-Wilk test was used to test for normality in both the mean GSI and Mean oocyte diameters. The ANOVA test was used to test for differences in both the mean GSI and oocyte diameters between months and the Tukey HSD test was used to establish which months were different. The significance level (α) was set at 0.05.

## 2.4 RESULTS

### 2.4.1 GSI (2004 – 2005)

The numbers of scallops in 2004 - 05 dissected for GSI purposes and the numbers of gonads used for histological purposes differed from month to month (Table 2.2). In some months there were no histological analyses, as the fixed gonads were damaged. The size range of scallops collected in 2010 - 11 ranged from 72.1 - 104.8 mm in shell height (Table 2.2).

Table 2.2: Summary of sampling dates, shell height, number of scallops < 70 and ≥70 mm, sample size for histological analysis and monthly temperature collected in 2004 - 05.

| Date of Collection | Shell Height Range (mm) | Larger Group N (≥70 mm) | Smaller Group N (<70 mm) | Histology N | Monthly Temperature (°C) |
|---|---|---|---|---|---|
| 23 Aug. 2004 | 45 - 110 | 10 | 24 | 0 | 15 |
| 20 Sept. 2004 | 46 - 102 | 25 | 21 | 0 | 14.5 |
| 18 Oct. 2004 | 49 - 100 | 21 | 21 | 0 | 14 |
| 19 Nov. 2004 | 54 - 107 | 23 | 23 | 13 | 13 |
| 20 Dec. 2004 | 53 - 103 | 20 | 22 | 6 | 13 |
| 20 Jan. 2005 | 52 - 102 | 15 | 25 | 2 | 13 |
| 22 Feb. 2005 | 44 - 101 | 20 | 20 | 0 | 14 |
| 16 Mar. 2005 | 59 - 99 | 20 | 2 | 5 | 14 |
| 13 Apr. 2005 | 47 - 97 | 19 | 8 | 7 | 14 |
| 19 May 2005 | 39 - 101 | 20 | 7 | 8 | 14 |
| 23 Jun. 2005 | 52 - 79 | 14 | 12 | 4 | 15 |
| 18 Jul. 2005 | 43 - 102 | 10 | 18 | 6 | 15 |
| 15 Aug. 2005 | 46 - 95 | 20 | 4 | 7 | 14 |
| 27 Sept. 2005 | 45 - 84 | 17 | 8 | 3 | 14 |
| 18 Oct. 2005 | 81 - 98 | 13 | 0 | 7 | 14 |
| 05 Aug. 2010 | 82 - 98 | 15 | 0 | 15 | |
| 06 Sept. 2010 | 72 - 98 | 15 | 0 | 15 | |
| 18 Oct. 2010 | 75 - 104 | 15 | 0 | 15 | |
| 19 Nov. 2010 | 85 - 98 | 15 | 0 | 15 | |
| 13 Dec. 2010 | 81 - 98 | 15 | 0 | 15 | |
| 21 Jan. 2011 | 78 - 94 | 15 | 0 | 15 | |
| 21 Feb. 2011 | 78 - 100 | 15 | 0 | 15 | |
| 24 Mar. 2011 | 85 - 105 | 15 | 0 | 15 | |
| 11 Apr.2011 | 87 - 100 | 15 | 0 | 15 | |
| 19 May 2011 | 79 - 95 | 15 | 0 | 15 | |
| 28 Jun. 2011 | 72 - 93 | 15 | 0 | 15 | |
| 26 Jul. 2011 | 85 - 103 | 15 | 0 | 15 | |
| 26 Aug. 2011 | 76 - 93 | 15 | 0 | 15 | |
| 26 Sept. 2011 | 83 - 99 | 15 | 0 | 15 | |
| 26 Oct. 2011 | 83 - 95 | 15 | 0 | 15 | |
| 22 Nov. 2011 | 72 - 101 | 15 | 0 | 15 | |

*P. sulcicostatus* was found to be a functional hermaphrodite, with the gonads comprising both whitish testes and orange ovaries. The shell height of the scallops

collected during the study ranged from 39 - 110 mm. Only during December 2005 was there a significant difference ($p = 0.002$) in GSI between the two size groups (< 70 mm; ≥ 70 mm) of scallops. During this month the mean GSI for scallops < 70 mm was 7.2 % (± 1.8 sd, n = 22) and for scallops ≥ 70 mm was 5.2 % (± 1.6 sd, n = 20). Consequently the two size groups were pooled for further analysis.

A seasonal pattern was evident in GSI, which was lowest from November - February and highest from June - September (Fig. 2.2 A). The minimum mean GSI of 6.2 % (± 1.9 sd, n = 42) was recorded in December 2004 and the maximum of 14.4 % (± 4.0 sd, n = 24) in August 2005. As is evident from the standard deviation, there was significant variation in the GSI of individual scallops within each month, particularly from May - October 2005 (Fig. 2.2 A).

A clear seasonal cycle was evident in the GSI frequency, a higher percentage of individuals being in a vegetative stage from November - February (late spring - summer) and individuals occurring in the vitellogenesis stage present only in July - September (mid-winter - early spring) (Fig. 2.2 B). This pattern is similar to that obtained from the GSI.

### 2.4.2 Histological analysis (2004 - 2005)

Monthly mean oocyte diameter demonstrated a similar seasonal pattern to that of the GSI (Fig. 2.2 C), with a minimum mean diameter of 36.6 μm (± 3 sd, n = 13) in November 2004 and a maximum of 54.7 μm (± 2.7 sd, n = 7) in August 2005. There was a significant difference between these minimum and maximum values.

### 2.4.3 Environmental assessment (2004 - 2005)

Water temperature at the time of scallop collection during 2004 - 05 at 20 - 22 m depth ranged from 13.0 - 15.0 °C (Table 2.1). The temperature recorded from the diver's watch upon monthly collection of scallops indicated that the temperature were consistent.

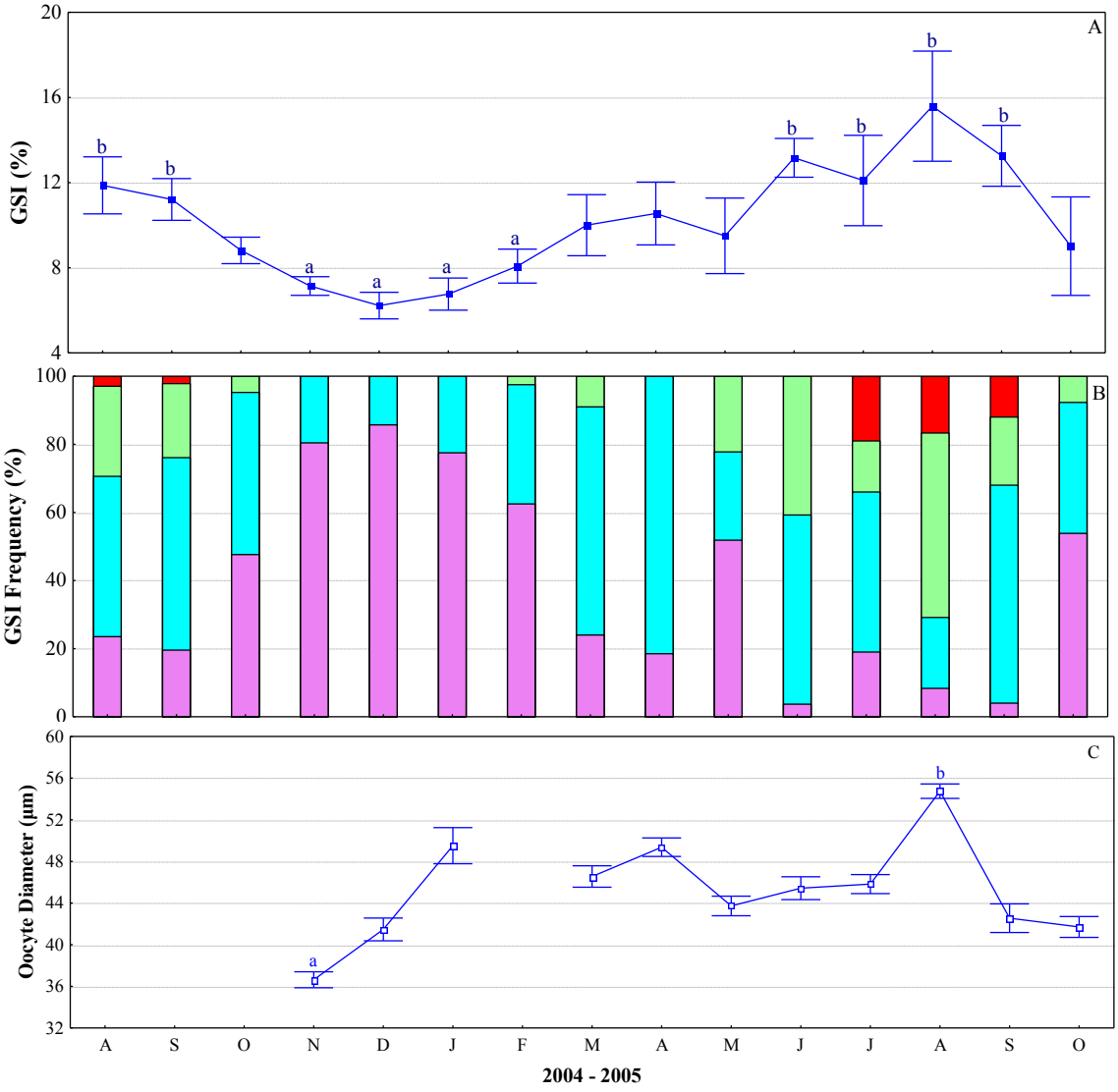


Figure 2.2: (A) Mean monthly GSI for *P. sulcicostatus*, indicating the mean ± the standard error. (B) Assessment of GSI representing four stages of gonad development, as indicated by different ranges of the GSI: 4-8% (▫ vegetative), 9-13% (▫ differentiation), 14-18% (▫ cytoplasmic growth), and 19 -22% (▫ vitellogenesis). (C) Mean monthly oocyte diameter for *P. sulcicostatus* ± standard error. Data missing from some months due to fixed gonads being damaged. Means not sharing the same superscript (a, b) are significantly different.

### 2.4.4 GSI (2010 - 2011)

A seasonal pattern was evident in the GSI, which was lowest from October - December 2010 and highest in June - September (Fig. 2.3 A). The minimum mean GSI value of 5.8% (± 1.7 sd, n = 16) was recorded in November 2011 and the maximum of 17.8% (± 4.8 sd, n = 16) in August 2010.

The GSI frequency displayed a similar seasonal cycle to that of the GSI. A higher percentage of individuals in a vegetative stage occurred from mid spring - summer (October - February) and individuals occurring in the vitellogenesis stage were present from late autumn to early spring (May - September) (Fig. 2.3 B).

### 2.4.5 Histological analysis (2010 - 2011)

Monthly mean oocyte diameter demonstrated a seasonal pattern (Fig. 2.3 C) with a minimum mean diameter of 34.5 μm (± 3.2 sd, n = 15) in July 2011 and a maximum of 53.4 μm (± 7.3 sd, n = 15) in August 2010 (Fig. 2.3 C). There was a significant difference between the minimum and maximum values.

The qualitative histological analysis was performed following the classification of Lasta & Calvo (1978). The female section of the gonads was assessed and grouped into the following stages:

A) Early maturation – follicles are defined and the interfollicular space is very visible. Up to 50% of the follicle may be occupied by lumen (Fig. 2.3 A).

B) Mid-maturation – follicles can occupy up to 75% of the gonad and the follicular lumen is much reduced. Interfollicular space is very limited (Fig. 2.3 B.

C) Ripe – gametes occupy the entire follicle with no interfollicular space (Fig. 2.3 C).

D) Spawned and recovering – ripe gametes are released and new germ cells are attached to the follicular wall (Fig. 2.3 D).

E) Partially spawned – follicles still maintain ripe gametes. Some gametes appear to be lax, due to the release of a few ripe gametes. A few phagocytes are present in the interfollicular space (Fig. 2.3 E).

F) Spent – follicles are completely empty (Fig. 2.3 F).

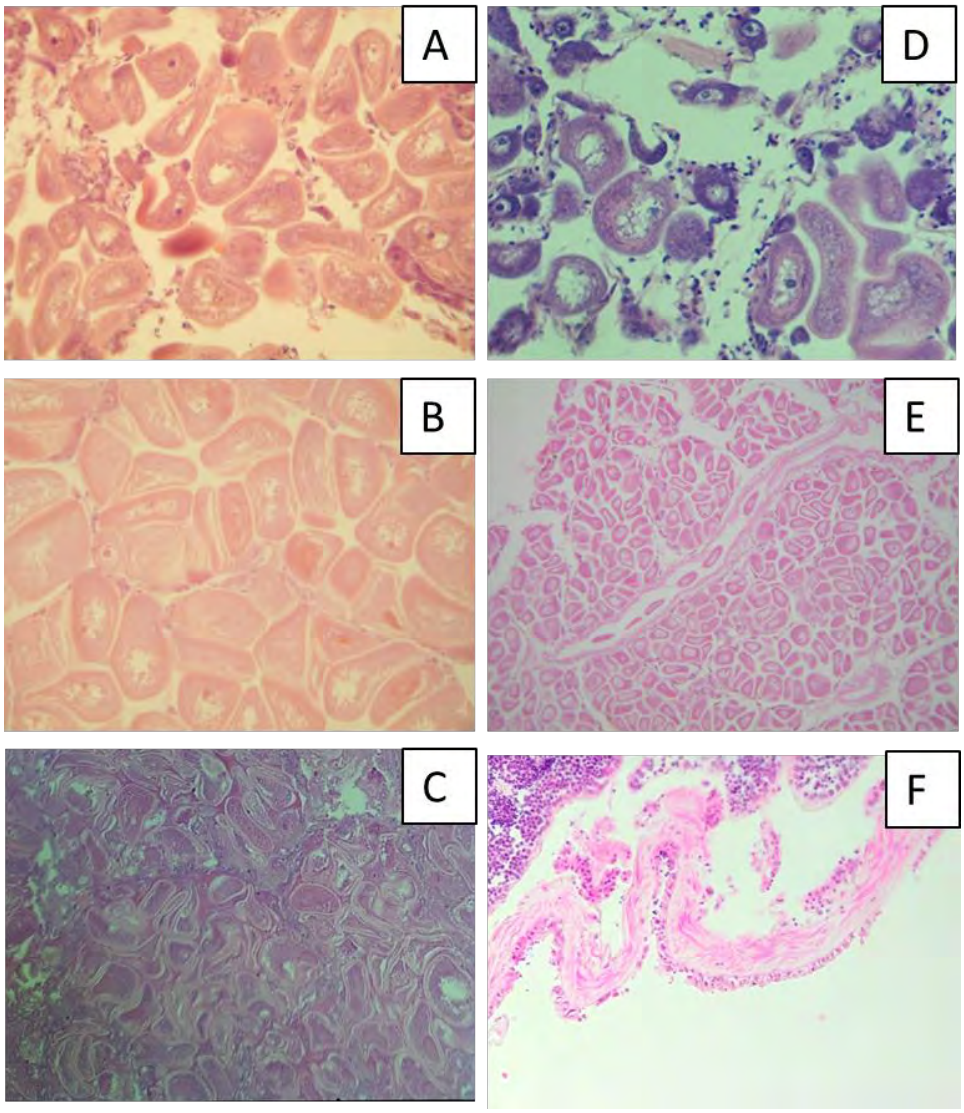


Figure 2.3: Photomicrographs of female gonadal stages of *P. sulcicostatus* from False Bay following the classification of stages after Lasta and Calvo (1978). (A) early-maturation, (B) mid-maturation, (C) ripe, (D) spawn and recovery, (E) partial spawn and (F) spent.

The qualitative histological analyses showed scallops collected monthly to be in different stages of gonad development, except in October 2011. Scallop gonads in the early maturation stage were present only in late spring through to mid - summer (November - January) (Fig. 2.4 D). The mid-maturation stage was present in most months, with the highest occurrence in September 2011 (Fig. 2.4 D). There is evidence of three spawning events in the months December, August and November

2011, as the spawn and recovery stages were present at 67, 67 and 100% respectively (Fig. 2.4 D). In most months partial spawning events occurred in 13 - 47% of the scallops (Fig. 2.4 D).

### 2.4.6 Environmental assessment (2010 - 2011)

Bottom temperatures at the study site in False Bay in 2010 - 11 showed a clear seasonal cycle, with higher temperatures in winter and spring, and lower temperatures in summer and autumn (Fig. 2.4 E). Temperatures during the period of study ranged from 9.7 - 17.6 ºC with a mean of 13.1 ºC (± 1.69 sd, n = 69198). Monthly mean temperatures ranged from a low of 11.4 ºC (± 1.2 sd, n = 4464) in January 2011 to a high of 15.6 ºC (± 1.3 sd, n = 4320) in November 2011.

Chlorophyll *a* concentrations also displayed a seasonal cycle, with highest values recorded in summer and early autumn (December – March) (Fig. 2.4 F). The highest mean chlorophyll *a* value of 14.1 mg $m^{-3}$ was recorded in February 2011 and the lowest mean value of 3.8 mg $m^{-3}$ in August 2010 (Fig. 2.4 F).

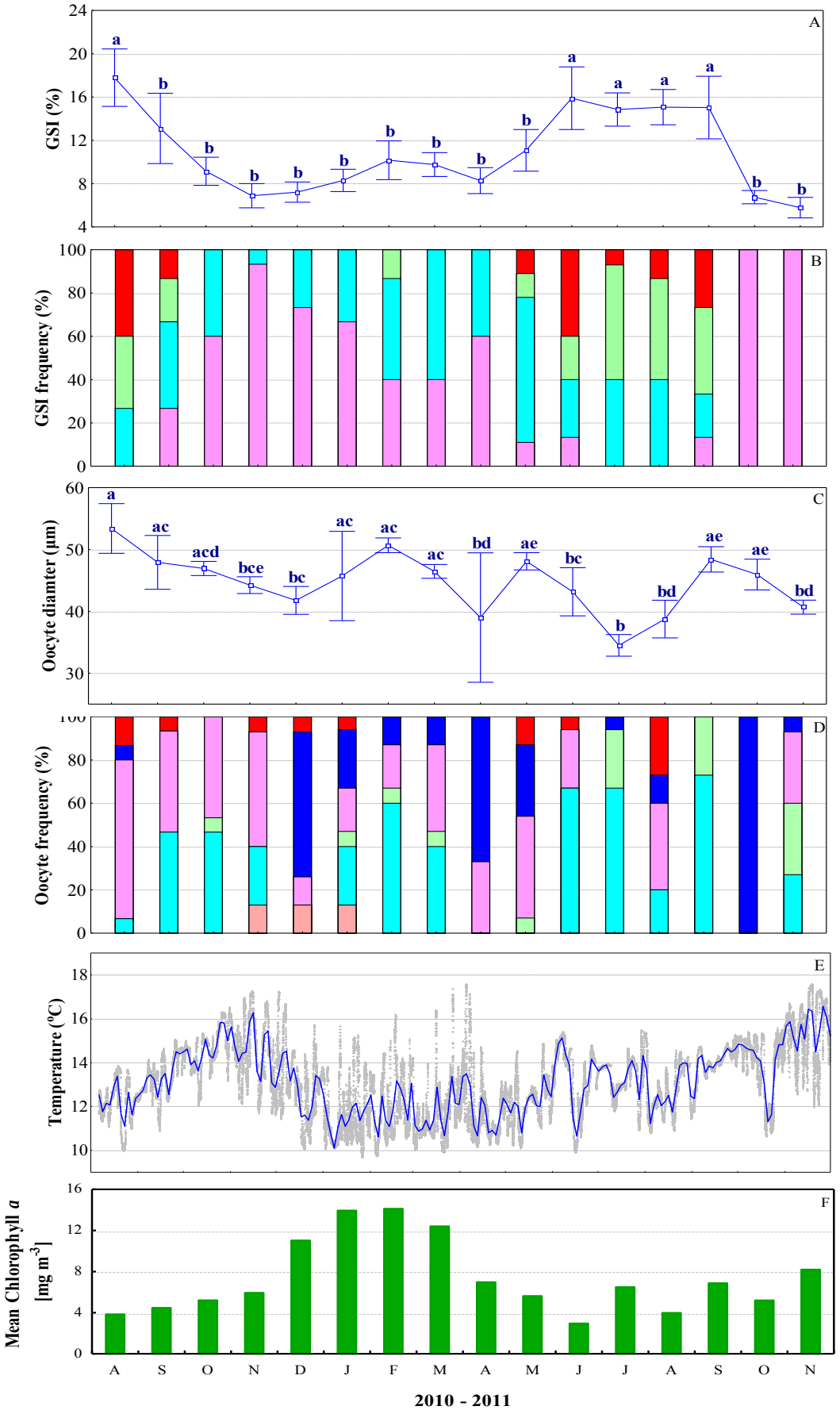
A
GSI (%)
B
GSI frequency (%)
C
Oocyte diamter (µm)
D
Oocyte frequency (%)
E
Temperature (°C)
F
Mean Chlorophyll *a* [mg m$^{-3}$]
A S O N D J F M A M J J A S O N
2010 - 2011

Figure 2.4 (A) Monthly mean GSI for *P. sulcicostatus*. (B) Qualitative assessment of GSI representing four stages of gonad development as indicated by different ranges of the GSI: 4-8% (vegetative), 9-13% (differentiation), 14-18% ( cytoplasmic growth), and 18 -22% ( vitellogenesis). (C) Monthly mean oocyte diameter for *P. sulcicostatus,* ± standard error. Means not sharing the same superscript (a, b) are significantly different. (D) Qualitative histological analyses indicated by six groupings of gonad histology: early maturation ( ), mid-maturation ( ), mature ( ), partial spawn ( ), spawn and recovery ( ) and spent ( ). (E) Temperature at 30 m depth measured every 10 minutes at the site of collection in False Bay. The blue line represents a negative exponentially weighted fit to 10 minute measurements. (F) Monthly mean cholorophyll *a* concentration at 10 m.

### 2.4.7 Spawning induction

The frequency of gonad stages assessed visually according to Williams & Babcock (2004) on *P. sulcicostatus* displayed variations of gonad stages within 15 out of 16 monthly collections of scallops (Fig. 2.5). May 2011 was the only month where only one stage of gonad development occurred. The highest percentage of ripe gonads (stage 8) was found in the scallops collected in June 2011. The highest percentage of spent individuals was found in scallops collected in January 2011.

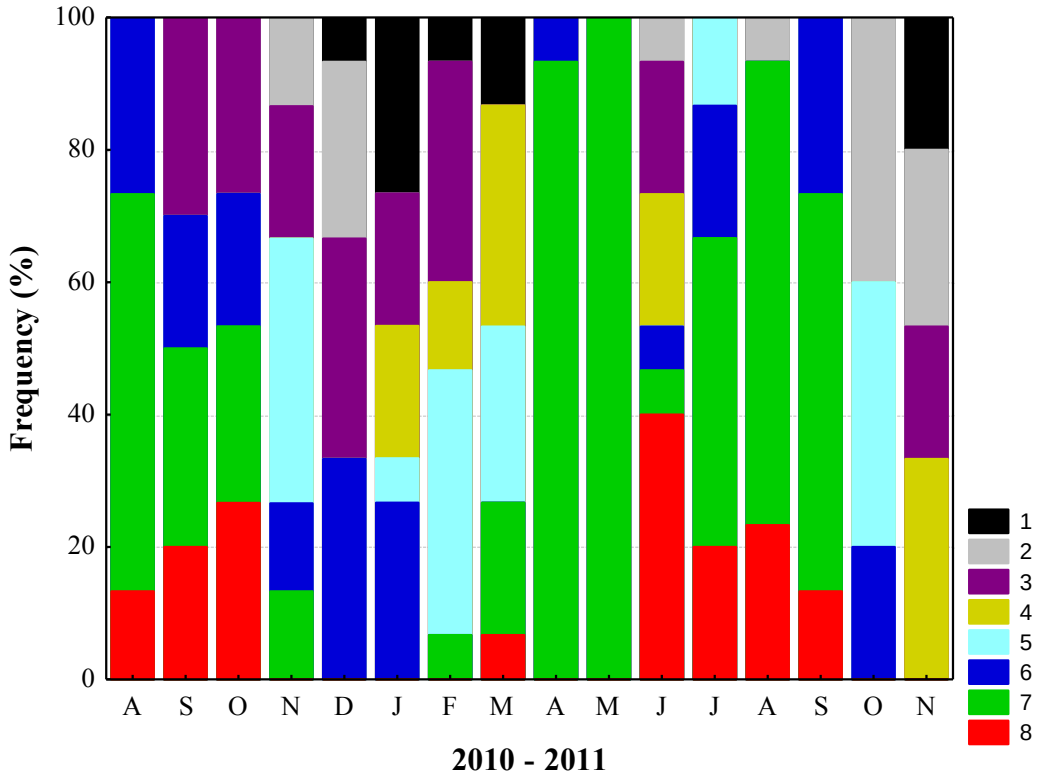


Fig. 2.5 Percentage frequency of each visual grade in scallops collected monthly from False Bay from August 2010 – November 2011.Visual grades 1 – 8 is described according to Williams & Babcock in the materials section.

The hormonal injection of serotonin was the first method that possibly triggered spawning, however, the combination of the other inductiob methods could also have triggered spawning. The number of scallops releasing sperm was notably higher than those producing eggs. On average 17.2% of scallops released eggs and 79.6% sperm throughout the experimental period. Whereas spermazoa were released in all months, oocytes were not released in either November or December (Fig. 2.6). In the months where eggs were obtained, successful fertilisation took place and D-veligers were developed (Fig. 2.7).

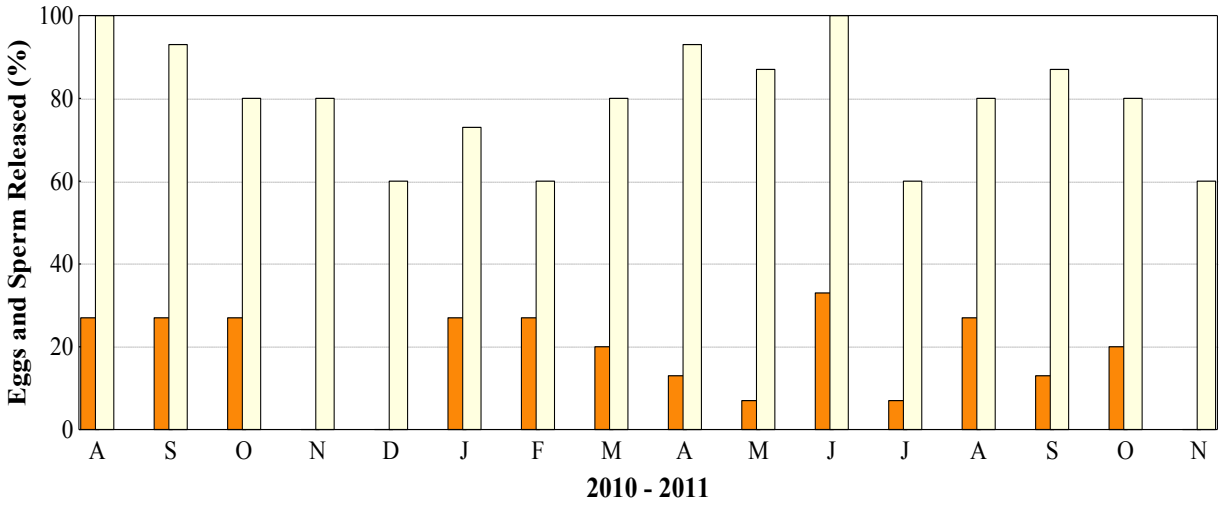


Figure 2.6: The percentages of animals releasing sperm (□) and eggs (■) in each month following the injection of the hormone seretonin.

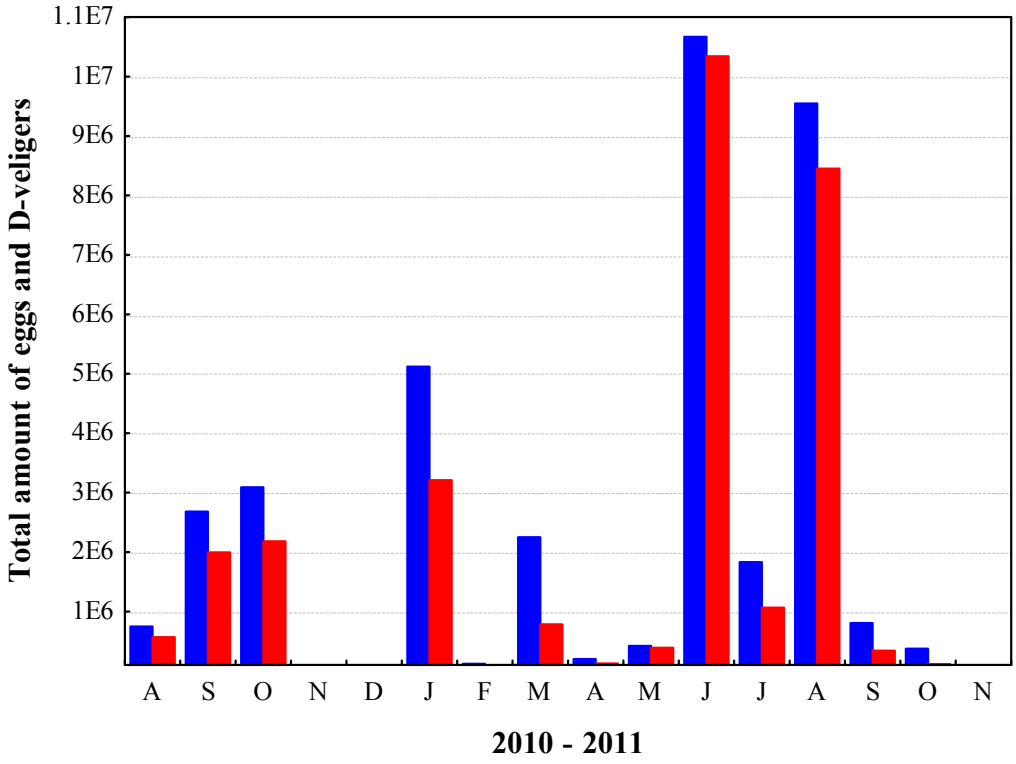


Figure 2.7: The number of eggs (■) and D-veligers (■) obtained following the injection of the hormone seretonin.

## 2.5 DISCUSSION

### 2.5.1 GSI

The reproductive cycle of *P. sulcicostatus* in False Bay demonstrated a similar seasonal trend for both periods of study from August 2004 - October 2005 and August 2010 - November 2011. The GSI, supported by histological analysis, showed spawning to occur predominantly in autumn and winter, followed by decrease in the mean GSI and oocyte diameter. However, minor spawning events throughout the year cannot be excluded, as oocytes appeared to develop throughout the reproductive cycle. This trend in spawning period is similar to that of the Australian scallops *P. fumatus* and *P. alba* and the New Zealand *Chlamys zelandiae,* which also exhibit peak spawning activity in winter through to mid - spring (Booth 1983, Sause *et al*. 1987, Young *et al*. 1999). These trends in spawning differ from those of most other scallop species that spawn primarily in summer, following an increase in water temperature. Such species include the Peruvian scallop *Argopecten purpuratus* (Wolff 1988), *Chlamys tehuelcha* from Argentina (Lasta & Calvo 1978), *Pecten maximus* from Norway (Strand & Nylund 1991) and several other species (Shafee 1980, Beninger 1987, Roman *et al*. 2002, Williams & Babcock 2005).

The maximum GSI for *P. sulcicostatus* of 14.4% in 2004 - 05 and 17.8% in 2010 - 11 tended to be lower than those reported for many other species, but similar to that reported for *Argopecten irradians* from Florida (14.5%; Barber & Blake 1983). The higher GSI reported for *Aequipecten tehuelchus* from Argentina (25%; Narvarte & Kroeck 2002), *Pecten maximus* from Norway (38.8%; Strand & Nylund 1991) and *Patinopecten caurinus* from British Columbia (21%; MacDonald & Bourne 1987) are

likely to be a function of a higher degree of synchronicity in spawning within these species.

### 2.5.2 Histology

Recognisable stages in the reproductive cycle of a population typically include activation, gamete growth, ripening of gametes, spawning, and an inactive or "resting" period (Sastry 1979). In this study the low frequency (0 - 26.7%) of totally spent gonads demonstrated the absence of a post-spawning resting period in the False Bay population. Instead, gametes began developing immediately after spawning and newly developing oocytes were present all year. In August 2010 oocyte diameters were at their highest and in July 2011 the oocytes diameters were at their lowest. From the clear seaonal cycle demonstrated over the study periods, one would expect a much higher oocyte diameter in July 2011. However, a sharp dip in temperature occurred in June 2011, which demonstrates the possible impact exogenous factors could have on the reproductive cycle. Unfortunately this study does not have environmental data prior to August 2010 in order to explain the maximum oocyte diameter in August 2010. It is, however, possible that variation in the timing of gametogenesis could play a role. Since the reproductive cycles for the two study periods are similar, it appears that exogenous factors played a role in having maximum and minimum oocytes diameters during winter in 2010 and 2011 respectively (Rodman & Capuzzo 1983, Magnesen & Christophersen 2008, Parsons *et al*. 1992).

Considerable variability in the GSI and oocyte diameter between individuals within each month also implies a low level of synchronicity in *P. sulcicostatus*. In the scallop *Placopecten magellanicus* from North Carolina, individuals occurring at greater depth

had a reduced rate of gamete development and were less synchronous, with greater reabsorption of oocytes (MacDonald & Thompson 1986a, Barber *et al*. 1988, Schmitzer *et al*. 1991). *P. sulcicostatus* has been found in False Bay between 22 and 70 m, with the densest populations occurring at approximately 40 m (De Villiers 1976). It is, therefore, possible that the occurrence of this species in relatively deep waters contributes to the lack of synchronicity, but this needs further investigation.

### 2.5.3 Environmental assessment

Water temperature and food availability are the parameters most often cited as controlling the reproductive cycle of scallops (Sastry 1979, Barber & Blake 1981, Cruz & Villalobos 1993, Wada *et al*. 1995). For *A. irradians, P. fumatus* and *P. maximus* the parameter most important in triggering spawning is temperature. These species are typically subjected to large temperature differences between winter and summer, ranging from 6-13 °C (Barber & Blake 1983, Strand & Nylund 1991, Young *et al*. 1999).

Monthly temperatures taken by divers during 2004 - 05 study showed no trend and temperatures were mostly consistent. However, temperatures collected continuously during the 2010 - 11 study showed a sharp decline of 7º C from spring to summer. False Bay forms an integral part of the southern Benguela upwelling regime and demonstrates strong seasonality in water column stratification (Shannon 1985). Little or no stratification is evident in winter, but the system becomes strongly stratified in summer, owing to solar heating of the surface waters and the inflow of cold bottom water into the Bay following upwelling on the adjacent shelf (Boyd *et al*. 1985, Eagle

& Orren 1985, Swart & Largier 1987). Consequently, surface temperatures have been shown to decrease by approximately 5 ºC in winter and bottom temperatures by 1 - 3 ºC in summer (Atkins 1970). These observations are consistent with the temperatures recorded during this study. Winter spawning and a decline in the GSI appear therefore to coincide with the transition to spring and an increase in bottom temperatures. However, it remains questionable as to whether this increase in temperature alone is able to control the reproductive cycle of *P. sulcicostatus* in False Bay, as reproduction is an energetically expensive process and therefore requires adequate food supply (Barber & Blake 1983).

Coincident with seasonal stratification are variations in phytoplankton biomass and the consequent availability of food for scallops. False Bay is an area of high phytoplankton biomass and this tends to peak in late summer, when the Bay is strongly stratified (Hutchings *et al*. 2006, Pitcher & Weeks 2006). These results coincide with the chl *a* data recorded during the 2010 - 11 study period. Food availability to scallops is likely to be greatest following the decay of these late summer blooms and their sedimentation to the benthos. It is at this time that the GSI is highest. Larger oocyte diameters were also present with higher chlorophyll *a* levels. However, the quantitative analysis indicated that partial spawning occured mostly when chlrophyll *a* levels were higher. The intrusion of cold, food-depleted bottom waters into the Bay during summer conditions coincide with a decline in the GSI. However, GSI and oocyte diameters build up fast after the decline in early summer and late winter, initiated by a higher level of chlrophyll *a* followed by spawning events in late autumn and winter. It thus seems likely that temperature, together with

food availability, control the reproductive cycle of *P. sulcicostatus* in False Bay. The relatively high phytoplankton biomass throughout the year may nevertheless explain the low frequency of spent individuals, and the presence of newly developing oocytes year-round, contributing to the low percentage of synchronicity.

### 2.5.4 Spawning Induction

Due to the low synchronicity mentioned above, spawning induction of *P. sulcicostatus* is difficult, as animals are not all in the same reproductive phase, as is the case where animals undergo a resting period. Various different spawning induction techniques, such as thermal shock, food deprivation, desiccation and injection with the hormones dopamine and serotonin were tested on *P. sulcicostatus* prior to this study. These are the methods most often used in scallop spawning induction (Culliney 1974, Matsutani & Nomura 1982, Gibbons & Castangna 1984, Velasco *et al*. 2007). Previous spawning induction trials in *P. sulcicostatus* showed that intragonadal injection with serotonin consistently resulted in the release of spermatozoa and oocytes, whereas spawning success following induction by the other techniques was at best sporadic. Spawning success was only achieved once using food deprivation and twice using the dessication and thermal shock method out of ± 10 spawning induction attempts per method while gonads were ripe in the previous studies. During the above few spawning induction sucesses, sperm and eggs were obtained within an hour. It is for this reason that all four methods were used for an hour during this experiment. Since the previous methods were used when gonads were individually assessed as being mature, this study also wanted to see if more

success could be achieved during other stages of gonad development, hence monthly collection from the wild would ensure this.

The frequency of the gonads collected throughout 16 months in this study showed that most scallops were in developing stages, and that the presence of mature gametes was present in most months. This indicates that it would be possible to induce scallops to spawn in most months throughout the year. During this study seretonin appeared to be the only method that triggered the release of both spermatozoa and eggs, although a combination of spawning techniques could have also contributed to succesfull spawning. Similar success has been achieved in species such as *P. yessoensis* and *P. albicans* (Matsutani & Nomura 1982, 1984, Tanaka & Murakoshi 1985), but in other species, such as *P. ziczac* and *A. ventricosus,* serotonin has been shown to induce only the release of spermatozoa (Vélez *et al*. 1990, Monsalvo-Spencer *et al*. 1997). However, serotonin has been described to release immature gametes, which could lead to reduced fertilisation and high moratalities in larvae (Braley, 1992). Nontheless, in this study the amount of larvae obtained versus the amount of eggs released shows that development of larvae were indeed successful in all the months that eggs were obtained. However, whether or not spat will develop throughout the year needs further investigation. Success with a combination of spawning induction techniques has been achieved in other scallop species (Parsons & Robinson 2006). It is therefore possible that a combination of all four spawning techniques could have contributed to spawning induction, although this is considered unlikely, as demonstrated by previously mentioned spawning induction trials. The release of no

eggs in the summer months November and December is possibly a consequence of spawning activity occurring in winter.

### 2.5.5 Conclusion

The results from this study shows that the reproductive cycle of *P. sulcicostatus* has two distinct spawning period in late summer – early autumn and winter - early spring, which in turn indicates the best time to collect, condition and spawn this species. The environmental data collected, suggest that both temperature and food availability trigger spawning and could be used in further spawning induction experiments. However, temperature and food availability may not be the only contributing factors controlling the reproductive cycle. Other exogenous factors and endogenous factors could also contribute, but have not been investigated in this assessment of the reproductive cycle. The information obtained in this study will also aid management with recruitment and population dynamics if a possible fishery for this species should ever exist. Furthermore, the results from this study indicate that it may be possible to spawn *P. sulcicostatus* throughout the year, due to the absence of a resting period and a low percentage of synchronicity. However, it is still advised that prior conditioning be considered before spawning induction, as although no resting period occurs, some primary oocytes may need some conditioning for further development before spawning can take place. Broodstock conditioning also increases the nutritional content of ova, which is fundamental for larval growth and survival (Caers *et al*. 1999).

# CHAPTER 3:

## Effect of artificial conditioning on gonad development in the scallop *Pecten sulcicostatus*

## Contents

## 3.1 Abstract

This study investigated whether *Pecten sulcicostatus* can be successfully conditioned in a controlled environment to produce ripe gametes. Two experiments are reported on, the first comparing experimental conditioning of broodstock of *P. sulcicostatus* under three different feed concentrations (approximating 4, 16 and 33 million cells liter$^{-1}$) and the second comparing rates of gonad conditioning over different time periods (1, 2 and 4 weeks) using the recommended concentration from the first experiment.

Gonad status was assessed in all experimental animals by the gonadosomatic index (GSI), GSI frequency, and by qualitative (oocyte stage) and quantitative (oocyte diameter) histological studies. For comparative purposes gonad status in the wild population was also assessed at the beginning and end of the study for the first experiment and throughout the entire experimental period for the second experiment. Measures of the inflow and outflow concentrations of algae into and from the broodstock tanks also allowed clearance and ingestion rates of the scallops to be calculated.

Clearance rates in medium and high food concentrations remained high for two weeks, before gradually declining. Ingestion rates followed a similar pattern to that of inflow of algal concentration. Gonad maturation was clearly influenced by feed concentration, with both GSI and oocytes diameters increasing with increased feed concentration.

For the second experiment the mean GSI values increased gradually from week 1 to 4. Week 4 was significantly different to week 1 and 2 (Tukey, $P< 0.05$). Mean oocyte diameters of experimental scallops increased gradually up to week 2, followed by a decrease in week 4.

This was supported by the oocyte frequency, which demonstrated that after two weeks of conditioning the gonads of some animals were spent and partially spawned. There was no difference between the conditioned animals and those collected from the wild.

This study indicated that scallops can be conditioned successfully in a laboratory. A high food concentration produced the fastest rate of gonad maturation. However, depending on the gonad status at the start of conditioning, not more than two weeks of conditioning is required at a high food concentration to avoid uncontrolled spawning activity.

Key words: broodstock conditioning, spawning, feed concentration, gonadosomatic index, histology

## 3.2 Introduction

Broodstock conditioning is required to ensure the continuous provision of spat throughout the year for operation of a bivalve hatchery. Several studies have shown that scallops can undergo gametogenesis outside their natural reproduction cycles if artificially conditioned under adequate rearing conditions (Sastry 1979, Barber & Blake 1991, Heasman *et al*. 1995, Andersen & Ringvold 2000). The primary environmental cue regulating bivalve reproduction is temperature (Orton 1920, Yamamoto 1951, Sastry 1979). However, egg production is also dependent on food supply in certain bivalve species (Barber & Blake 1991).

The natural reproductive cycle of *P. sulcicostatus* comprises a peak spawning period during the austral southern winter (June - August) and early spring (September) (Arendse *et al*. 2008). Given the relatively limited spawning window for this species, it is important for farming purposes to determine whether *P. sulcicostatus* can be conditioned in a controlled environment to produce ripe gametes throughout the year. The aim of this study was to investigate the effect of food concentration and conditioning period on gonad development of laboratory maintained *P. sulcicostatus*. It was hypothesized that an increase in food concentration from 5 - 40 million cells $L^{-1}$ would increase rate of gonad development in *P. sulcicostatus* and that scallops conditioned at the recommended food concentration determined from the first study, would show a reduced conditioning period as compared to natural stocks**.**

## 3.3 Materials and methods

Two separate experiments were conducted. The first experiment compared rates of gonad conditioning at three different food concentrations. The second experiment compared rate of gonad conditioning over different time periods using the recommended food concentration determined from the first experiment, to that of scallops collected from the wild over the same time period.

### 3.3.1 Conditioning food concentrations

Scallops were collected from False Bay, on the south-west coast of South Africa, at a depth of 20 - 22 m on 19 March 2008 (autumn). A total of 137 scallops of mean shell height 85.92 ± 6 mm SD were collected by divers and transported for one hour in a Styrofoam box covered with moist (soaked in sea water) paper towels back to the laboratory. In the laboratory, the scallop shells were cleaned in seawater, in order to remove the majority of fouling organisms, before being placed in conditioning tanks. The broodstock conditioning tanks were 1.2 by 0.8 m with a water depth of 0.3 m. Each tank contained a false net bottom. A total of nine tanks, each containing 13 scallops, were used to provide three replicate tanks per food concentration. The remaining 20 scallops collected were used for immediate assessment of gonad condition. Scallops from the wild were also collected at the end of the experiment for similar comparative analyses.

The broodstock diet was a mixture of the microalgae *Chaetocerous muellerii*, *Isochrysis galbana* (Tahitian strain) and *Pavlova lutherii*, using a 70:15:15 ratio respectively, based on cell number. The three food concentrations compared were a low concentration of 5 million cells $L^{-1}$, a medium of 20 million cells $L^{-1}$ and a high concentration of 40 million cells $L^{-1}$. The algal diet was continuously added in a flow-to-waste system. Temperature (±14 $^{\circ}$C) (refer

to Chapter 2) and day length (L: D= 10:14) were set according to winter (June - August) conditions for the duration of the experiment.

In order to assess if a link occurs between food concentration and gamete production, clearance and ingestion rates were calculated by measurements of inflow and outflow of algae into and out of the broodstock tanks. The inflow of algae was determined by taking triplicate samples of algae from the feeding pipe going into the broodstock tanks. The algal concentration of the inflowing algae was counted on a Z2 coulter counter. For the outflow of algae, triplicate samples were collected at the outflow area of the broodstock tanks. Algal counts from the outflow area were counted on a Z2 coulter counter. The flowrate was measured daily, by measuring the amount of water at the outflow area per minute. Clearance rates (F) were calculated as follows: (Riisgård 2001)

$$F = Fl\ (C_1 - C_2)/\ C_2$$

where Fl is the flow rate through the tanks, and C1 and C2 are the concentration of algal cells flowing into and out of the tank respectively. Ingestion rates (IR) were calculated as follows (Lu & Blake 1997).

$$IR = CR \times C/n$$

Where CR is the clearance rate, C is the algal concentration and n is the number of individuals.

### 3.3.2 Gonadosomatic index (GSI) and histology

The state of gonad development in scallops collected from the wild at the beginning and at the end of experiment, as well as of the conditioned scallops, was assessed by means of gonado-somatic index (GSI) and histological examinations. The method used to calculate

GSI and asses quantitative and qualitative histological investigations are the same as outlined in Chapter 2 of this thesis.

### 3.3.3 Conditioning period

A total of 155 scallops were collected from False Bay on 9 March 2010. The broodstock diet was a mixture of the microalgae *Chaetocerous muellerii*, *Isochrysis galbana* (Tahitian strain) and *Pavlova lutherii*, using 70:15:15 ratios respectively, based on cell number. The mean feed concentration was 40 million cells $L^{-1}$, which had been shown to be the food concentration which increased the rate of gonad maturation faster than the other two experimental food concentrations (based on the results of experiment 1). Three groups, containing 45 scallops each, were conditioned over different time periods of 1, 2 and 4 weeks. The rate of conditioning was assessed by means of gonadosomatic index (GSI) and histological examination. Scallops were also collected from the wild at the start of the experiment and after each time period (week 1, 2 and 4), to compare artificial gonad conditioning to that of scallops remaining in the natural environment.

### 3.3.4 Statistical analysis

Statistical analyses for the broodstock conditioning study were conducted using Statistica version 6.1. The Shapiro-Wilk test was used to test for normality. The GSI data were transformed logarithmically before the ANOVA test was used to test for differences in the GSI between concentrations. The Tukey HSD was used to determine which concentrations were significantly different. Kruskal Wallis ANOVA was used to test for differences in mean oocyte diameters between algal concentrations, and a Multiple comparison of mean ranks for all groups test was used to establish which concentrations were significantly different.

For the conditioning period investigation, the ANOVA test was used to test for differences in the GSI between the different weeks and oocyte diameters. The Tukey *post hoc* Test was used to determine which conditioning period was significantly different for both the GSI and oocyte diameters. The significant level (α) was set at 0.05.

## 3.4 Results

### 3.4.1 Conditioning food concentrations

Actual measured food concentrations in the low food tanks averaged 3.93 million cells $L^{-1}$ (range1.7 - 5.5 million cells $L^{-1}$), 15.9 million cells $L^{-1}$ (range 6.8 - 23 million cells $L^{-1}$) in the medium food tanks and 32.8 million cells $L^{-1}$ (range13 - 46 million cells $L^{-1}$) in the high food tanks (Fig. 3.1 A). The clearance rate of animals in the high food concentration decreased gradually after two weeks of conditioning, while those of animals in the low feed concentration increased gradually throughout the conditioning period (Fig. 3.1 B). The clearance rates of animals in the medium and high food concentration were the highest of all treatments initially, decreasing towards the end of the experiment (Fig. 3.1 B). The mean clearance rates of animals in the low, medium and high food concentration were 8 (± 4.8 sd), 15 (± 5.3 sd) and 12 (± 5.9 sd) L $h^{-1}$ $scallop^{-1}$, $P < 0.05$ respectively. The ingestion rates of animals in the low, medium and high food concentration (Fig. 3.1 C) followed a similar pattern to that of the inflow of algal cells (Fig. 3.1 A). The mean ingestion rates of animals increased progressively from the low, medium to high food concentration and averaged 33 (± 2.3 sd), 238 (± 11.1 sd) and 379 (± 22 sd) million cells $h^{-1}scallop^{-1}$, $P < 0.05$ (Fig. 3.1 C).

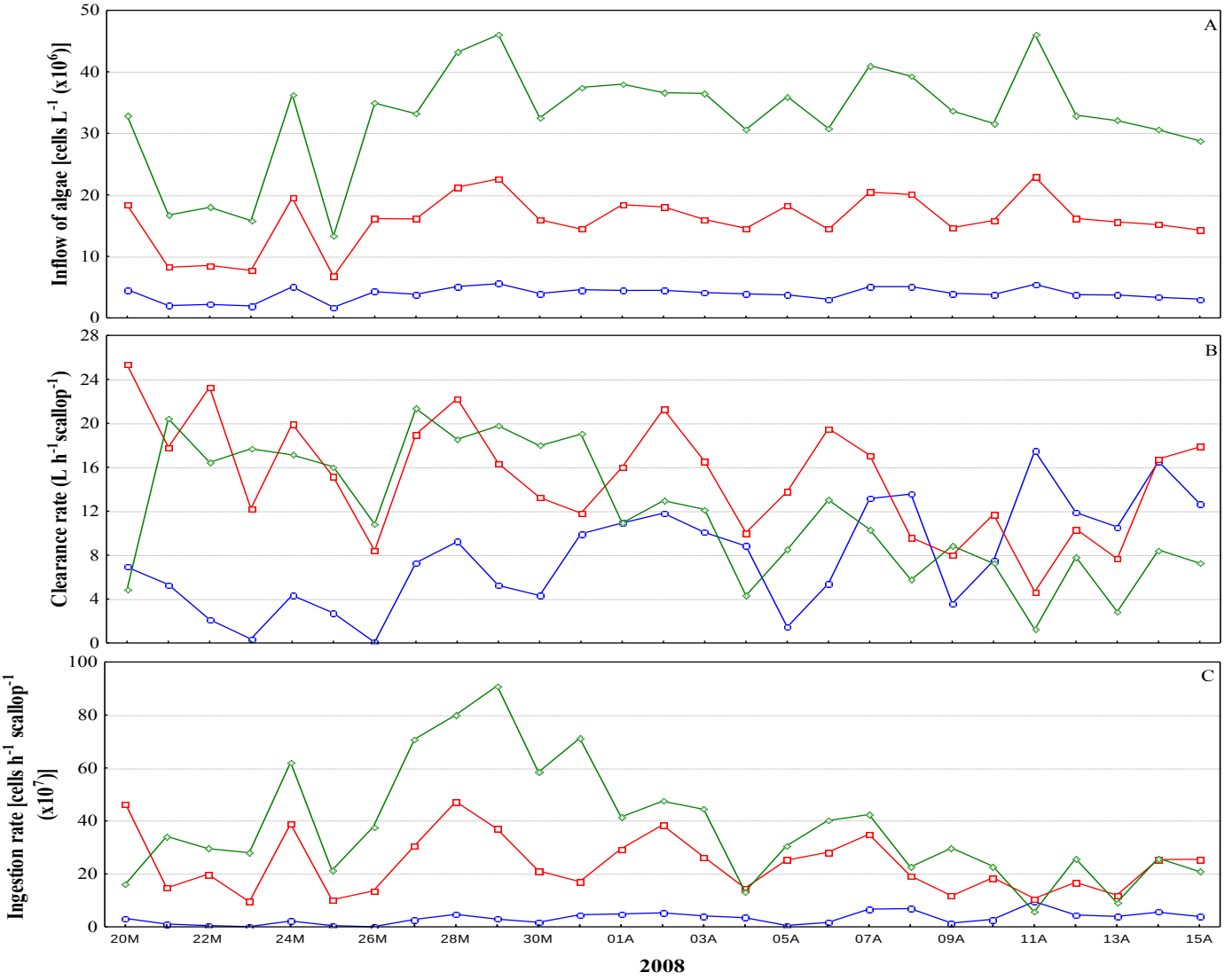


Figure 3.1: (A) inflow of algal cells and (B) Clearance rates (C) Ingestion rates at low (—), medium (—) and high (—) food concentration.

### 3.4.1.1 GSI

The mean GSI values increased with an increase in food concentration. After 26 days the mean GSI at the three food concentrations were 9.4 % ± 2.5 SD; 10.4 % ± 2.95 SD and 14.2% ± 3.4 SD at low, medium and high feed concentration respectively (n = 39) (Fig 3.2 A). The mean GSI of the scallops collected from the wild increased from 9.4% ± 2.6 SD -

15.4 % ± 3.4 SD (n = 20) at the end of the experiment. The mean GSI obtained in the initial, low and medium food concentrations were not significantly different. The mean GSI obtained of the high feed concentration and scallops collected from the wild at the end of the experiment were not significantly different (Tukey, $P < 0.05$).

A clear pattern was evident in the GSI frequency, with a higher percentage of individuals being in a vegetative stage in the low feed concentration and the lowest percentage of individuals in a vegetative stage in the high feed concentration (Fig. 3.2 B). In turn, the highest percentage of scallops undergoing cytoplasmic growth stage occurred in the high food concentration and lowest percentage in the low food concentration. Gonads undergoing vitellogenesis stage were only present in the high food concentrations and in the scallops collected from the wild at the end of the experiment.

#### 3.4.1.2 Histology

Mean oocyte diameter (Fig. 3.2 C) demonstrated a similar pattern to that of feeding rate (Fig. 3.1 B) and GSI (Fig. 3.2 A), with a minimum mean diameter of 58.48 µm ± 2.23 SD (n = 39) in the low food concentration and a maximum of 74.99 µm ± 3.07 SD (n = 39) in the high food concentration. The mean oocyte diameters of the scallops collected from the wild at the beginning and end of the experiment increased from 68.95 ± 9.06 SD - 74 µm ± 2.05 SD (n = 20). There was a significant difference between oocyte diameters (Multiple comparison of Mean Ranks, $P < 0.05$) conditioned at the various food concentrations (Fig. 3.2 C).

The qualitative histological analyses, indicated by four groupings of gonad histology, showed scallops from each food concentration and those collected from the wild to contain different

stages of gonad development (Fig. 3.2 D). The mid - maturation (MM) stage was the dominant stage in all food concentrations (58.2 - 64%) and in scallops collected from the wild (60 - 75%) (Fig. 3.2 D). A small percentage of scallops in the spawn and recovery (SR) stage were present in most groups (indicating spawning in some individuals), except the high feed concentration and in scallops collected at the end of the experiment. Scallops in the partial spawn (PS) were present in all groups with the highest percentage occurring in scallops conditioned in the medium food concentration (Fig. 3.2 D).

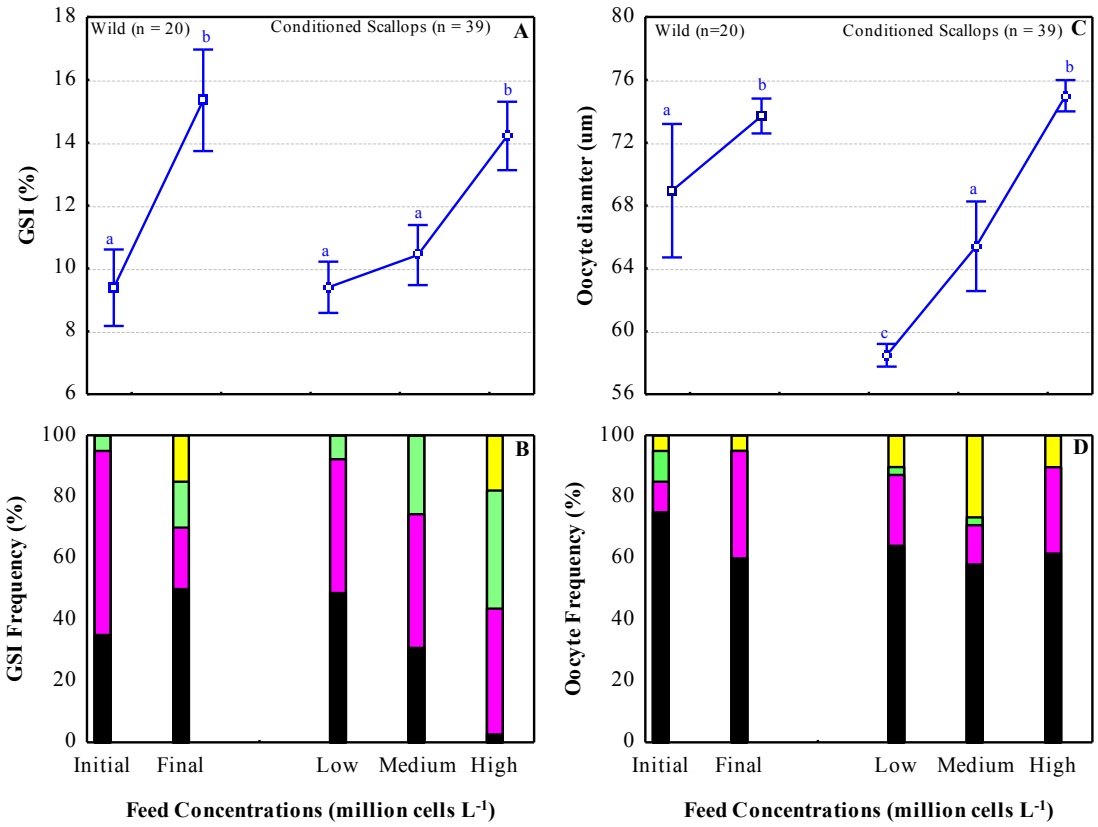


Figure 3.2: (A) GSI for *P. sulcicostatus* at different food concentrations, low, medium and high. Means not sharing the same superscript (a, b,) are significantly different. Bars correspond to error bars B) Qualitative assessment of GSI representing four stages of gonad development as indicated by different ranges of the GSI: 4-8% (■ vegetative), 9-13% (■ differentiation), 14-18% (■ cytoplasmic growth ) and 19-22% (■ vitellogenesis). (C) Oocyte diameters for *P. sulcicostatus* at different food concentrations, low, medium and high. Bars correspond to error bars. Means not sharing the same superscript (a, b, c) are significantly different. D) Percentage frequency of occurrence of 4 oocyte development stages. Qualitative histological analysis indicated by four groupings of gonad histology: mid - maturation ( ■, mature ( ■ spawn and recovery ( ), ■ artial spawn ( ). ■

### 3.4.2 Conditioning period

The mean food concentration during one, two and four weeks of conditioning was ± 39 million cells $L^{-1}$ (range 38 - 53 million cells$L^{-1}$), 40 million cells $L^{-1}$ (range 35 - 58 million cells $L^{-1}$) and 39 million cells $L^{-1}$ (range 31.5 - 64 million cells $L^{-1}$) respectively (Fig. 3.3 A). The clearance rates of the scallops were high during the first week of conditioning and decreased thereafter (Fig. 3.3 B). The mean clearance rates of the scallops during one, two and four weeks of conditioning were 30 (± 7.2 sd), 19 (± 8.9 sd) and 16 (± 7.4 sd) L $h^{-1}$scallop$^{-1}$ $P < 0.05$ (Fig. 3.1B). The ingestion rates of the scallops during conditioning (Fig. 3.3 C) followed a similar pattern to that of the inflow of algal cells (Fig. 3.3 A). The mean ingestion rates of scallops during one, two and four weeks of conditioning were 1130 (± 2.2 sd), 774 (± 4.1 sd) and 600 (± 4 sd) million cells indiv$^{-1}$ $h^{-1}$,$P < 0.05$ (Fig. 3.3C).

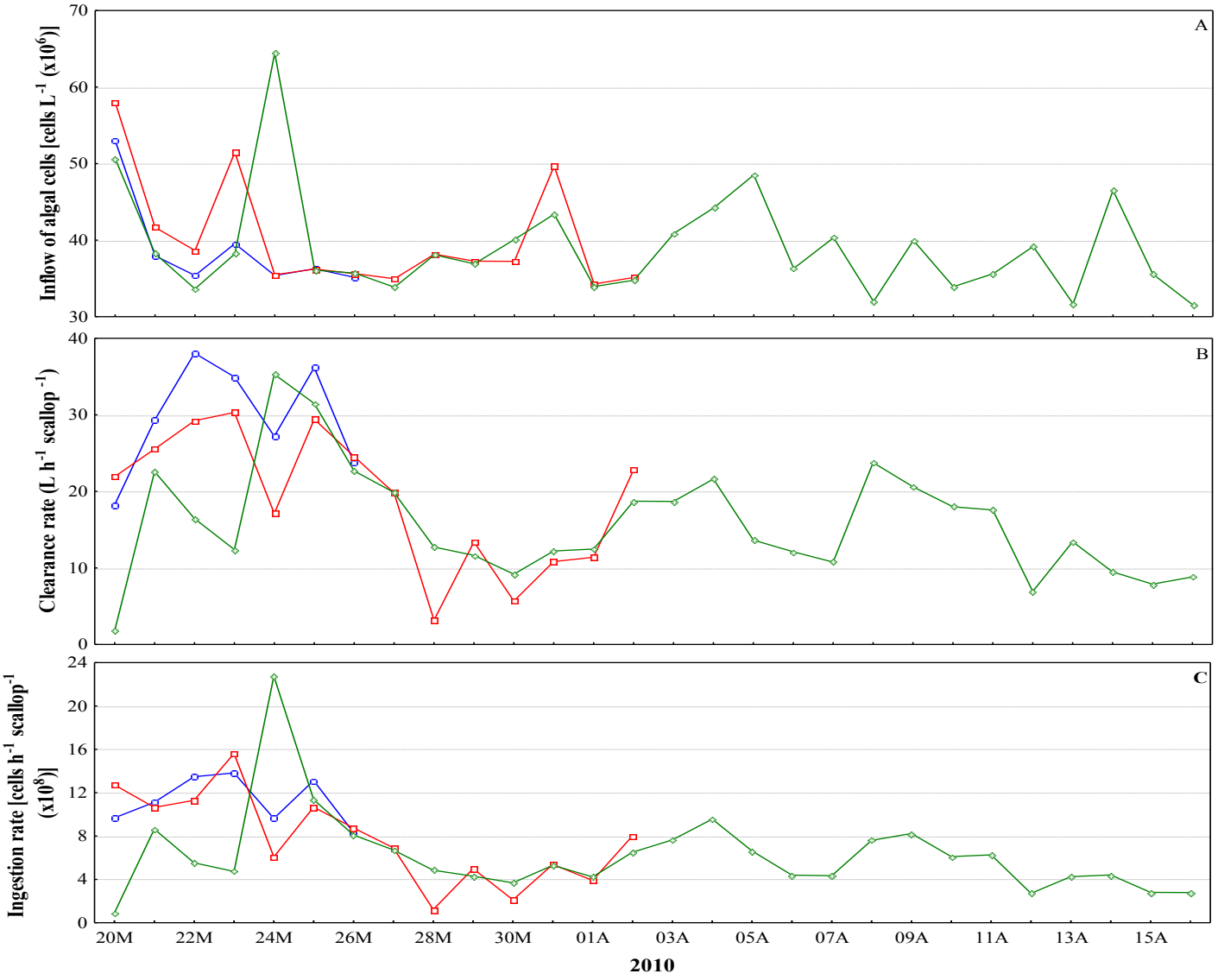


Figure 3.3: (A) Inflow of algal cells and (B) Clearance rates (C) Ingestion rates of scallops conditioned over a period of 1 ( ), 2 ( ) and 4 ( ) weeks.

### 3.4.2.1 GSI

The mean GSI of the scallops collected from the wild at the beginning and end of the experiment decreased from an initial 7.7 ±1.9 SD (n = 20) to 7 ± 1.7 SD (n = 20) in week one and thereafter increasing steadily to 10.4 ± 2.1 SD (n = 20) in week four (Fig. 3.4 A). A mean

GSI value of 7.1± 1.9 SD (n = 45) was obtained after the first week of conditioning, increasing steadily over the next few weeks to 11.6 ± 2.1 SD (n = 45) after four weeks of conditioning (Fig. 3.4 A). The mean GSI value of scallops after four weeks of conditioning was significantly different to the mean GSI values of scallops conditioned for one and two weeks (Tukey, $P < 0.05$).

In terms of GSI frequency, scallops from the wild were mostly in the vegetative stage (GSI values of 4 - 8%) throughout the experimental period (Fig. 3.4 B). This occurrence is similar to scallops after the first week of conditioning (Fig. 3.4 B). A small percentage of scallops undergoing the vitellogenesis stage (GSI values of 19 - 22%) were present only after four weeks of conditioning (Fig. 3.4 B).

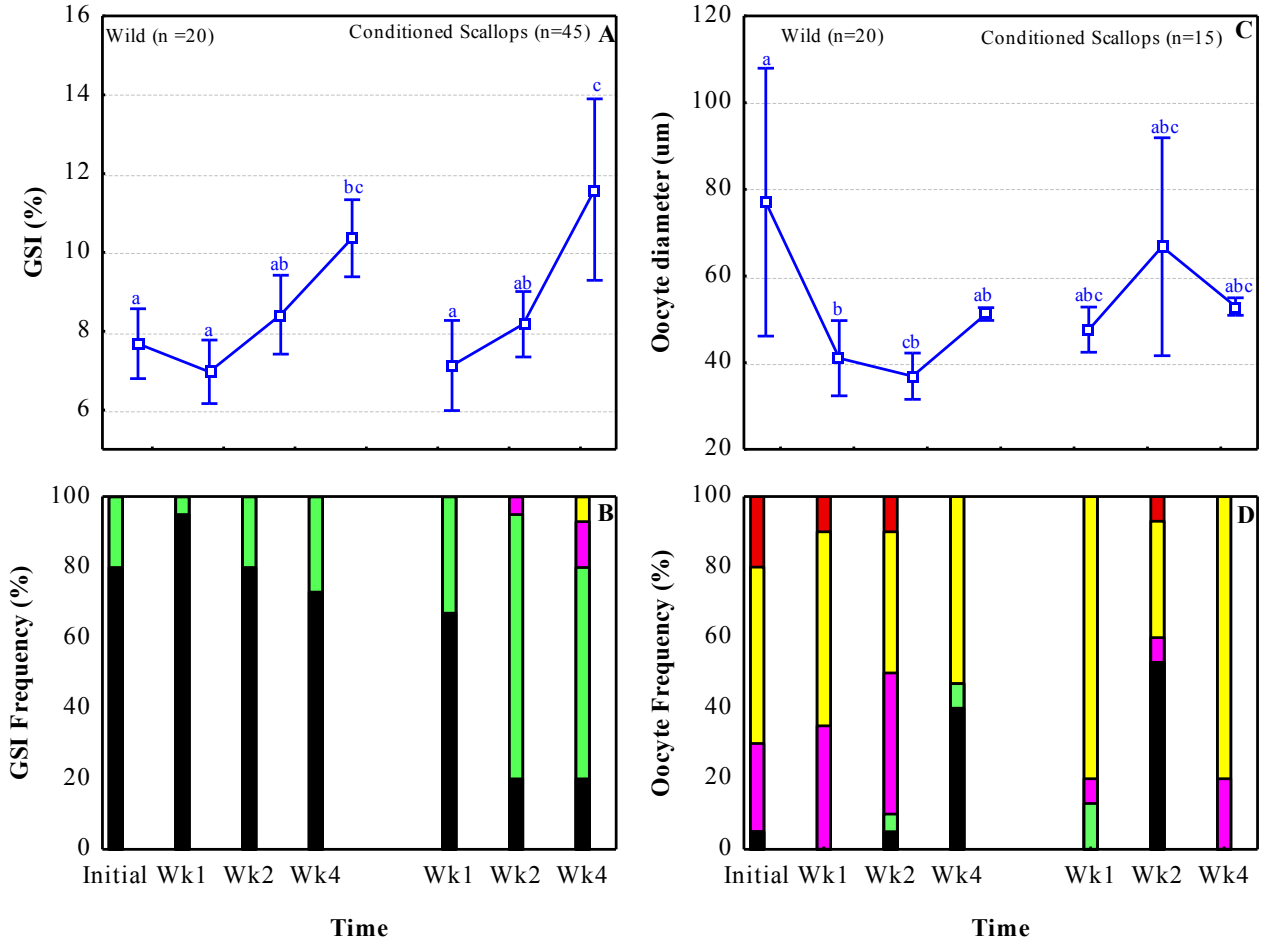


Figure 3.4: A) GSI of *P. sulcicostatus* over a conditioning period of 1, 2 and 4 weeks. Means not sharing the same superscript (a, b, c) are significantly different. Bars correspond to error bars B) Qualitative assessment of GSI representing four stages of gonad development as indicated by different ranges of the GSI: 4-8% ( vegetative), 9-13% ( differentiation), 14-18% ( cytoplasmic growth ) and 19-22% ( vitellogenesis). C) Oocyte diameters at ± 40 million cells $L^{-1}$ food concentration over a conditioning period of 1, 2 and 4 weeks. Means not sharing the same superscript (a, b, c) are significantly different. Bars correspond to error bars D) Percentage frequency of occurrence of 4 oocyte development stages. Qualitative histological analysis indicated by five groupings of gonad histology: mid - maturation ( ), mature / ripe ( ), spawn and recovery ( ), partial spawn ( ) and spent ( ).

### 3.4.2.2 Histology

The mean oocyte diameters of scallops collected from the wild at the beginning and end of the experiment decreased from 76 to 37µm and then increased in the last two weeks to 51µm. Mean oocyte diameters of experimental scallops (Fig. 3.4 C) was minimal at 48µm after

week 1, then increased in week 2 to 67µm, followed by a decrease in week 4 to 53µm. There was no significant difference between the three groups (week 1, 2 and 4) (Tukey, $P < 0.05$) (Fig 3.4 C).

The oocyte frequency indicated a high percentage of partial spawning (PS) stage throughout the experiment, with peaks in week 1 and 4 (Fig. 3.4 D). Individuals that were described as spent (S) were found in week 2 and in the first three groups of scallops collected from the wild. The mid-maturation (MM) stage was present only in week 2 in the conditioned scallops and in scallops collected from the wild at the beginning, week 2 and at the end of the experiment (Fig. 3.4 D).

## 3.5 Discussion

### 3.5.1 Conditioning food concentrations

The clearance rates of animals exposed to medium and high food concentrations were elevated above that for the low food concentration for the first two weeks of conditioning. Thereafter, there was a tendency for high and medium clearance rates to decrease over the experimental period. The clearance rate of animals conditioned in the low food concentration showed a different pattern, increasing gradually throughout the period of conditioning. The overall effect was that after two weeks clearance rates at all feeding levels, though highly variable, were of similar magnitude. It appeared as if the scallops in the medium and high food concentration took a week or more to acclimatise to the algal concentration. This trend is different to that of the adult species *Argopecten irradians concentricus*, where the clearance rates of the animals displayed a strong inverse, linear relationship to that of the

algal concentrations (Palmer 1980). Also, in juvenile *Argopecten irradians irradians* and *Argopecten irradians concentricus,* the clearance rates of animals declined rapidly with an increase in algal concentration (Kuenstner 1988, Lu & Blake 1997). The clearance rate of adult *Placopecten magellanicus* and *Mya arenaria* also decreased with an increase in algal concentration (Bacon *et al*. 1998). The clearance rate indicates that an even higher food concentration can be used for conditioning purposes. However, a too high food concentration could result in energy being transferred for scallop growth instead of gonad conditioning. Also a too high food concentration could result in the production of pseudofeces.

The ingestion rate of *P. sulcicostatus* was elevated at increased food availability and decreased in concert with falling clearance rates over the study period. Ingestion rates for high and medium rations remained elevated over those for the low ration, although the difference was not as marked as at the start of the study. It is impossible to speculate on what these findings mean for potential somatic growth and reproductive development without knowledge of absorption efficiencies at the different rations. However, as absorption efficiency generally decreases with consumption (Peirson 1983), it is possible that the absorbed ration and scope for growth are similar, or at least not as divergent as ingestion rates suggest, at all three rations. This is similar to the situation in juvenile *Argopecten irradians concentricus,* where ingestion rate also increased with an increase in algal concentration (Lu & Blake 1997).

The GSI followed the same trend as that of the food concentration, as it continued to increase with higher food concentrations across the entire range tested. A significant difference in gonad development in the three food concentrations was only found when broodstock were fed algal concentrations above ± 15 million cells $L^{-1}$. The highest mean GSI of 14.2% in

April 2008 was comparable to the highest mean GSI of 14.4% found in August 2005 in a study describing the reproductive cycle of *P. sulcicostatus* in natural stocks (Arendse *et al*. 2008). These data were supported by the GSI frequency, which showed that scallops undergoing vitellogenesis were only present in the high food concentration group. This is similar to the findings of Villalaz (1994), who described the gonadal dry weight of *Argopecten ventricosys* as increasing significantly after being fed a high ration for 40 days.

Although the GSI, GSI frequency and oocyte diameters indicate that a higher food concentration should be considered, the oocyte frequency analyses indicated that a natural spawning event was triggered during the four week conditioning period, suggesting that a shorter period for conditioning should be assessed in the second experiment, using the high food concentration. However, the variation in the food concentrations throughout the experiment could have induced a spawning event, as the gonads were mostly in a mid-maturation stage from the start of the experiment and therefore scallops in the vitologenesis stage should be used for the second experiment.

The mean GSI of conditioned scallops followed a similar trend to scallops collected from the wild. There was no significant difference in GSI between scallops collected from the wild at the beginning of the experiment and that of scallops conditioned at the low and medium food concentrations. There was also no significant difference in GSI between scallops collected from the wild at the end of the experiment and those conditioned at a high food concentration. This indicates that the artificial environment created for the broodstock in the laboratory is adequate for successful artificial conditioning of *P. sulcicostatus*. This differs from the findings of Martinez *et al*. (1992) who found that during artificial conditioning of *Argopecten purpuratus* for 48 days, the gonads did not become fully ripe, but that individuals

placed in the ocean during the same period reached maturity. This study therefore demonstrates that food concentration had a significant impact on gonad development in laboratory-conditioned *P. sulcicostatus*. In addition, the rate of gonad development in scallops held in the laboratory and exposed to the high food concentration was similar to that of natural stocks, indicating that feeding conditions in the field are not limiting.

### 3.5.2 Conditioning period

The ingestion rates were elevated for the first few days, followed by a decrease in ingestion rates. Again, without knowledge of the absorption efficiency, it can only be assumed that as the absorption efficiency decreased with consumption, so did the growth over a specific time period.

The mean GSI of 11.6% in April 2010, obtained after four weeks of conditioning, was similar to that recorded for natural stocks (10.6%) by Arendse *et al*. (2008) on 13 April 2005. The mean GSI values from both the wild stock and those conditioned after four weeks in April 2010 were lower than the GSI values obtained in the first experiment in April 2008. This could be attributed to a higher GSI value obtained at the beginning of the experiment in 2008 (9.4%) to that in 2010 (7.68%). Also the environmental conditions are likely to vary for each year. Arendse *et al*. (2008) also observed *P. sulcicostatus* to have a low spawning synchronicity, meaning that individual scallops are all in different stages of development.

The GSI frequency showed mature eggs were present only after four weeks of conditioning. This is similar to in *Argopecten ventricosus***,** where gonad and reproductive maturity were reached after 27 days when fed a diet of a 6:3:1 ratio of *Isochrysis* spp, *Chaetoceros* spp. and

*Tetraselmis* spp. (Monsalvo-Spencer *et al*. 1997). Contrary to the GSI results, oocyte diameters increased from week 1 to 2, followed by a decrease. The decrease in oocyte diameters suggests that spawning had taken place. This was further supported by the oocyte frequency, which indicated that some scallops were spent and partially spawned. Arendse *et al*. (2008) also showed that minor spawning events occur throughout the year, as the oocytes develop almost instantly after spawning activities, in the absence of a post-spawning resting period. Although the mean GSI was highest after four weeks and some scallops were in the cytoplasmic and vitellogenesis stage, the histological results indicates that two weeks of conditioning at 40 million cells $L^{-1}$ is adequate to be able to spawn broodstock in an controlled environment. Spontaneous spawning was indicated after two weeks of conditioning. Although, once again it can be speculated that variation in food concentration throughout the experiment could have triggered an induced spawning event, the qualitative histological results do show that the majority of scallops in week 2 were in the mid-maturation stage and some scallops were even ripe.

False Bay, the site where animals were collected, is situated within the southern region of the Benguela, where wind-induced upwelling occurs from September to March and peaks during spring and summer (Shanon 1966, Andrews & Hutchings 1980). An inflow of cold, bottom, nutrient-rich water occurs in False Bay owing to upwelling conditions occurring on the adjacent shelf (Boyd *et al*. 1985, Eagle & Orren 1985, Swart & Largier 1987). These nutrient-rich waters provide the scallops with an abundance of phytoplankton during upwelling conditions. False Bay has been previously described to have high phytoplankton biomass throughout the year (Hutchings *et al*. 2006, Pitcher & Weeks 2006). After upwelling season, food availability to scallops may still remain high in the form of detritus after the decay of summer blooms. Therefore, there is an abundance of food available for scallops

throughout the year in False Bay, in the form of phytoplankton or detritus. It is known that scallops rely on either detrital material or phytoplankton as a food source (MacDonald *et al*. 2006). This is possibly why gonad development in wild scallops could follow the same trend as the scallops artificially conditioned in this study.

### 3.5.3 Conclusion

In conclusion, this study showed that an increase in food concentration increased the rate of gonadal development of *P. sulcicostatus,* but this rate was not higher than that in scallops collected from the wild during the same period. As the population of *P. sulcicostatus* has been shown to be insufficient to support a fishery and are not easily found in their natural distributional area due to depth (De Villiers 1976), it is imperative that this species can be successfully conditioned in a hatchery environment, as continuous collection of broodstock from the wild may be limited. Further testing of higher food concentrations is required to ascertain if higher food densities would increase the rate of gonad development compared to wild populations. However, although the clearance rates suggest that an even higher concentration can be used for conditioning, at higher food concentrations could more energy might be converted to shell growth, or might result in increased pseudofeces production, instead of increasing gonad development. Further studies should therefore not just include higher food concentration, but also focus on different phytoplankton species at different food concentrations to assess the proper nutritional requirements for gonad development. Alternatively, it would appear that food availability is not limiting in the wild, due to the comparable development with laboratory-conditioned scallops. The food concentration and

period of conditioning should be managed carefully during broodstock conditioning to prevent uncontrolled spawning activities.

# CHAPTER 4

## Effect of temperatures on larval rearing and settlement on different substrates

## Content

## 4.1 ABSTRACT

The effect of temperature on the growth and survival of larvae of *P. sulcicostatus* (D-larvae to pediveliger) was investigated. Larvae were cultured at 12.5, 15.5, 18 and 22 ºC. The growth rates of larvae compared favourably to those of other commercial scallop species. Larvae cultured at 22 ºC had the fastest growth and development rates, with the majority reaching pediveliger stage 14 days post fertilization. However, survival was low at all four temperatures. As there was no correlation between temperature and survival, other factors, such as high larval density, sub-optimal food concentration, or bacterial infection, could have contributed to low survival. Following development of pediveligers, settlement on different substrata in a light and dark environment was investigated. The three substrata used were 150 µm poly-amide mesh, polyethylene string and crushed oysters. Successful settlement occurred on poly-amide mesh in both light and dark environment. Settlement on crushed oysters only occurred in the light environment. No settlement occurred in the control (no substrate), or on the polyethylene string. The results thus showed that the larvae of *P. sulcicostatus* were best cultured at 22 ºC and settled on poly-amide mesh. However, further studies to improve larval survival should be undertaken. Other settlement substratata and factors to increase settlement success also require further investigation.

Key words: D-larvae, pediveliger, growth rate, survival, settlement

## 4.2 INTRODUCTION

The collection of scallop spat from the wild is often insufficient and too unreliable for the needs of aquaculture operations. Therefore scallop hatcheries have become necessary to provide a regular supply of scallop spat. Initial attempts to culture scallops in hatcheries were problematic due to high mortality rates of larvae (Dabinett 1989).

Light intensity, temperature and salinity all have an effect on larval development and growth. However, the effect of temperature on larval rearing is considered the most important parameter and is most frequently tested in investigations, as it can be easily manipulated. Temperature plays a major role in the success and rate of development of larvae (Lough & Gonor 1973, Falmagne 1984). Larval growth can stagnate, or be reduced, when temperatures during larval rearing are too low. On the other hand, high temperatures can permit rapid growth, but at the same time increase the growth of bacteria and ciliates in the water supply and thereby increase mortality (Gruffydd & Beaumont 1972, Beaumont & Budd 1982, Krassoi *et al*. 1997). Temperature optima in the embryonic and larval stage may also differ between species (Heasman *et al*. 1996).

Larval settlement of benthic invertebrates has been described to be influenced by water flow, light, chemical cues, types of substratum, community strucures, molecular and bacterial films (Roberts *et al*. 1991). Previous studies has shown that most marine bottom invertebrates produce pelagic larvae and that in some species the larvae becomes photonegative during metamorphosis and thereby prefer shaded or dark areas to settle (Thorson 1964). Very little is known about the chemical, biotic and physical features that attract pectinid species to settle

on certain substrata, also in many cases substrate settlement appear species specific (Nicolas 1999). For example, *A. purpuratus* settle on red algae (Cantillanez 2000), *P. magellanicus* on chitin (Harvey *et al*. 1997), and *P. maximus* on biogenic amines (Cochard *et al*. 1989). Physical and chemical stimulation can also induce or increase larvae settlement, e.g. the use of potassium chloride, sodium chloride, water turbulence and temperature (Le Pennec *et al*. 2003). However, most studies on pectinid settlement have focussed on substrate preferences (Hodgson *et al*. 1989, Pearce & Bourget 1996, Lyton & Riquelme 2008).This study therefore investigated the effect of temperature on growth and survival of larvae of *P. sulcicostatus* from D-larvae to pediveligers. The study further investigated the suitability and relative performance of several substrata for settlement of pediveligers and whether *P. sulcicostatus* are photosensitive or photonegative during settlement.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Broodstock conditioning

Thirty scallop broodstock ranging from 70 – 90 mm in shell height were collected from False Bay and conditioned for two weeks prior to spawning. Water temperature was set at ±14 $^{\circ}$C and day length at L: D= 10:14; conditions shown to be optimal for *P. sulcicostatus* (Arendse *et al*. 2008). Scallops were fed a mixture of the microalgae *Chaetocerous muellerii*, *Isochrysis galbana* and *Pavlova lutherii*, using a 70:15:15 ratio respectively, based on cell number. The food was continuously added at a concentration of 2 x$10^{7}$ million cells $L^{-1}$ in a flow-to-waste system.

### 4.3.2 Spawning induction

After two weeks of conditioning, the gonads of the broodstock were visually investigated to see if they were potentially ripe. A total of 12 scallops were used for spawning induction. The gonads of the scallops were gently wiped with an ear bud dipped into 70% ethanol, in order to prevent possible infection. The scallops were intragonadally injected in both the male and female areas of the gonad with 0.2 mL serotonin (2 mM) hormone solution and placed into individual glass bowls containing filtered seawater. The number of scallops that released sperm and eggs were recorded.

### 4.3.3 Fertilisation

Cross fertilisation was maximized by adding sperm from one scallop to eggs from another scallop. Two ml of sperm were added to every L of egg suspension. Following the addition of sperm, the eggs were allowed to stand for 60 min for fertilisation to take place. Thereafter, the eggs were checked under a dissecting microscope to confirm successful fertilization. The total number of fertilized eggs was counted according to Helm *et al.* (2004):

Fertilized egg counts in three sub-samples = A; B; C.

Mean = (A + B + C)/ 3 = D

Sub-samples were divided into several droplets in a petri-dish. Following, each fertilised egg was counted in each droplet under a disecting microscope.

Volume of sub-sample = E

Total Volume of cylinder = F

**Total volume of eggs = F/E x D**

### 4.3.4 Effects of temperature on larval culture

Larval growth and survival were determined at four temperatures, 12.5, 15.5, 18 and 22 º C. Four 5 L beakers containing 4000 mL of filtered seawater (0.2 µm) were held at each temperature. Results from previous pilot larval rearing experiments using densities of 3000, 5000, 10 000 and 15 000 larvae per liter indicated that a higher density of larvae may improve the survival rate, as survival (although low) was higher than that found at the other experimental densities used. Therefore, approximately 70 000 D-larvae were placed into each beaker. Every Monday, Wednesday and Friday water changes took place, at which time larvae were counted (using same method as described for counting fertilized eggs) to determine survival rate during development. Five larvae from each beaker were also sampled for growth measurement. During sampling, each beaker was agitated using a perforated plunger to ensure even distribution of larvae. The larvae were fed a mixture of the microalgae *Chaetocerous muellerii*, *Isochrysis galbana* and *Pavlova lutherii*, using a 1:1:1 ratio respectively, based on cell number. Initial food concentrations of 1.5 $x10^7$ million cells $L^{-1}$ were increased to 2 $x10^7$ million cells $L^{-1}$.

### 4.3.5 Growth measurement of larvae

Larvae samples were placed into a 25 ml settling chamber and measured under an Olympus IX 50 microscope with X10 objective. An eye-piece micrometer was used to measure valve length of each larva. The measurements of the valve length were then used to determine the mean valve length, growth rate and specific growth rate of larvae cultured at each of the four temperatures.

### 4.3.6 Settlement of pediveligers

Pediveligers ready for metamorphoses were identified according to size and morphology. The settlement experiment took place on three substrata and a control, each placed in both dark and light environments. The three substrata were polyethylene string, 150 μm poly-amide mesh and fine pieces of oyster shell and the control had no substratum. Four replicates of each of the four series were set up. In each case the substratum was placed in 90 x 90 mm sterilised petri dishes containing 100 ml of 0.2 μm filtered seawater. A total of 10 pediveligers were then placed in each petri dish. After 24, 48 and 72 h the number of attached spat was counted using a dissecting microscope.

### 4.3.7 Statistics

Statistical analyses for the larvae rearing study were conducted using Statistica version 6.1. The Shapiro-Wilk test was used to test for normality. The ANOVA test was used to test for differences in growth and survival of larvae between the four temperatures. The Tukey HSD post hoc test was used to establish significant difference in growth and survival of larvae at the four temperatures. The significant level ($\alpha$) was set at 0.05.

## 4.4 RESULTS

### 4.4.1 Spawning and fertilization

All 12 scallops induced to spawn released sperm and two scallops released eggs. A total of ± 1.4 million eggs were counted, of which 912 500 were fertilised. Of 350 000 fertilised eggs maintained at each of the four temperatures 285 000 D-larvae developed at 12.5 ºC, 290 000 at 15.5 ºC, 300 000 at 18 ºC and 330 000 at 22 ºC.

### 4.4.2 Stages of larval development and pelagic life cycle

D-larvae developed after six days post fertilisation at 12.5 ºC, four days post fertilisation at 15.5 ºC and two days post fertilisation at both 18 and 22 ºC. The development of pediveligers was fastest at 22 ºC with the first few pediveligers appearing 11 days post fertilisation (Fig. 4.1 D). Larval development did not proceed beyond the eyed veliger stage at 12.5 ºC (Fig. 4.1 A). The valve length of the larval stages was similar in size at the four temperatures, with minimum mean D-larvae length of 107 µm and maximum 230 µm (Table 4.1).

The pelagic life cycle of *P. sulcicostatus* from oocyte to pediveliger were obtained fastest at 22 ºC. Once post-fertilisation of the oocyte (Fig. 4.2 A) took place the first polar body (Fig. 4.2 B) appeared within 15 – 30 min, indicating successful fertilisation. Following fertilisation, cell division took place and the first cell cleavage (Fig. 4.2 C) developed after 60 min post fertilisation. The second (Fig. 4.2 D) and third (Fig. 4.2 E) cleavages developed 4 hours post fertilisation, followed by the morula stage (Fig. 4.2 F), blastula (Fig. 4.2 G) and gastrula (Fig. 4.2 H) stage, which developed within 12 hours post fertilisation. The

trocophore (Fig. 4.2 I) stage was reached 21 hours post fertilisation. The majority of D-larvae (Fig. 4.2 J), veligers (Fig. 4.2 K), umboned veligers (Fig. 4.2 L), eyed veligers (Fig. 4.2 M) and pediveligers (Fig. 4.2 N) were reached within 2, 4, 9, 11 and 14 days post fertilisation respectively.

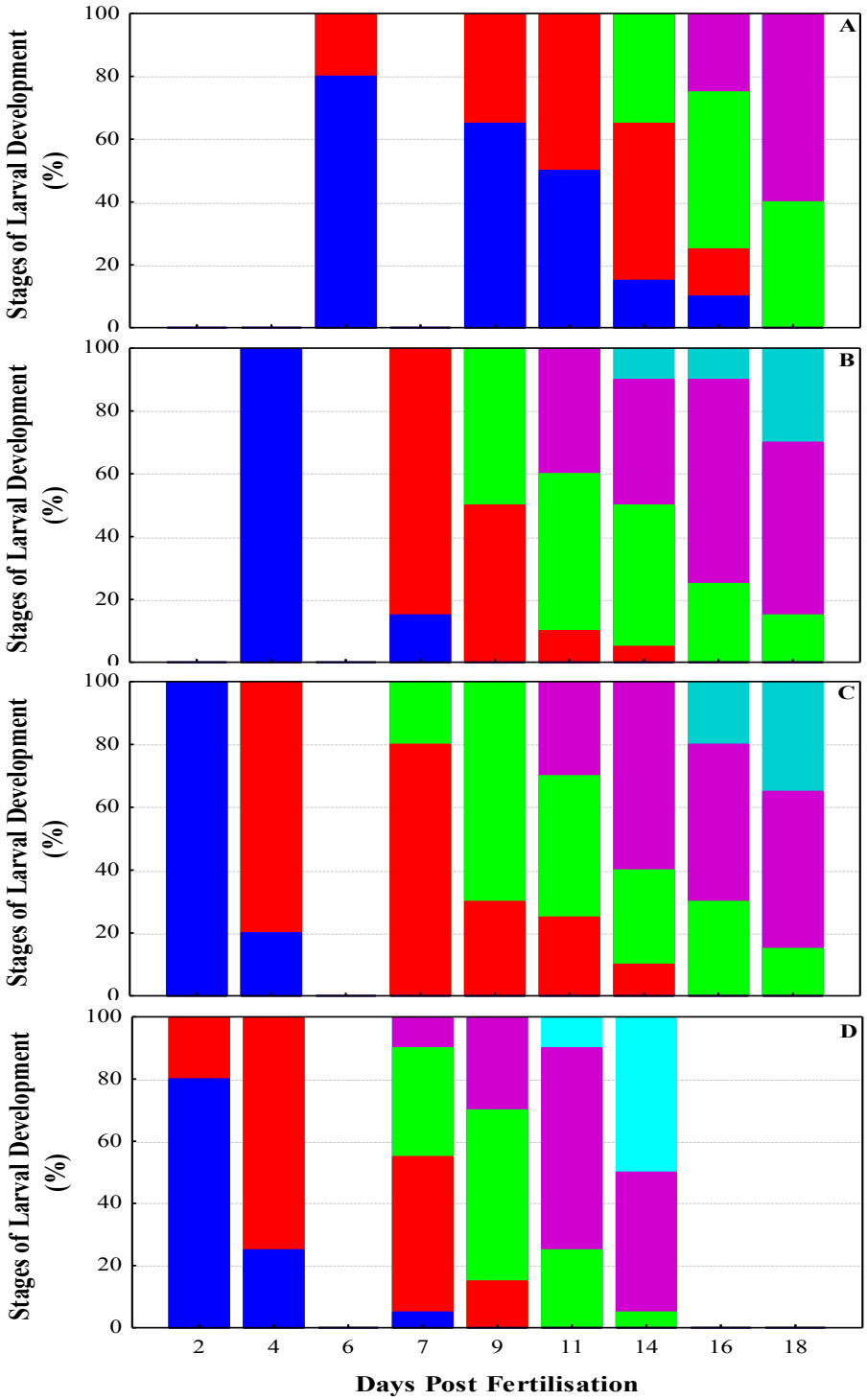


Figure 4.1: Development of D-larvae ( ), veligers ( ), umboned veligers ( ), eyed veligers ( ) and pediveligers ( ) post fertilisation at four temperatures, (A) 12.5 ºC, (B) 15.5 ºC, (C) 18 ºC and (D) 22 ºC

Table 4.1: Mean valve lengths of larval development stages cultured at four temperatures

| **Larval development stage** | **Mean valve length (µm) at 12.5 °C** | **Mean valve length (µm) at 15.5 °C** | **Mean valve length (µm) at 18 °C** | **Mean valve length (µm) at 22 °C** |
|---|---|---|---|---|
| D-larvae | 107 (range 92 - 120; ±5.9 sd, n = 24) | 109 (range 101 - 110; ±3.3 sd, n = 8) | 108 (range 101 - 110; ±4.6 sd, n = 17) | 108 (range 101 - 110; ±4.2 sd, n = 20) |
| veliger | 124 (range 120 - 138; ±5.9 sd, n = 25) | 137 (range 120 - 147; ±10.1 sd, n =30) | 134 (range 120 - 147; ±10.7 sd, n = 56) | 134 (range 120 - 166; ±12.4 sd, n = 56) |
| umboned veliger | 161 (range 156 - 166; ±4.7 sd, n = 19) | 161 (range 156 - 166; ±4.7 sd, n = 27) | 161 (range 156 - 166; ±4.6 sd, n = 65 | 162 (range 156 - 166; ±4.7 sd, n = 55) |
| eyed veliger | 176 (range 175 - 184; ±3.3 sd, n = 8) | 184 (range 174 - 203; ±10.4 sd, n = 40) | 180 (range 174 - 202; ±7.5 sd, n = 51) | 183 (range 174 - 203; ±7.7 sd, n = 50) |
| pediveliger | | 225 (range 211 - 276; ±21.4 sd, n = 8) | 225 (range 212 - 276; ±23.3 sd, n = 21) | 230 (range 212 - 276; ±24.6 sd, n = 20) |

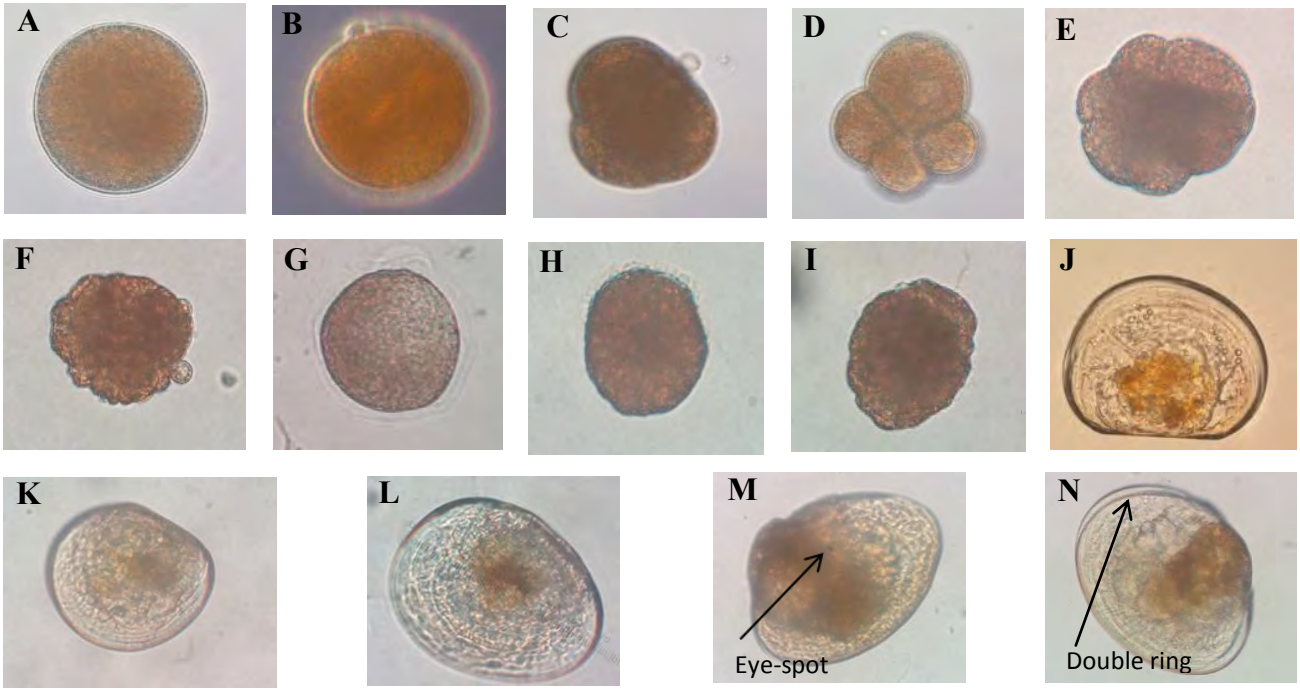


Fig. 4.2 Pelagic life cycle of *P. sulcicostatus* from oocyte to pediveliger stage at 22 ºC: (A) oocyte, (B) polar body, (C), first cleavage, (D) second cleavage, (E) third cleavage, (F) morula, (G) blastula(H) ciliated gastrula, (I) trocophore larva, (J) D-larva, (K) veliger, (L) umboned veliger, (M) eyed veliger, (N) pediveliger.

### 4.4.3 Growth of larvae

Larvae size, as represented by valve length, was determined at approximately two day intervals (Monday, Wednesday and Friday) (Fig. 4.3 A). Growth statistics of the larvae cultured at four temperatures are presented in Table 4.2. There was no significant difference between growth of the larvae at the four temperatures (Tukey, $P < 0.05$) (Fig. 4.3).

Growth rates and specific growth rates followed an almost identical pattern (Fig. 4.3 B and C). After an initial increase, both measures of growth at 15.5 and 22 ºC tended to decline throughout the larvae development period (Fig. 4.3 B and C). At 18 ºC both measures of growth first increased followed by decrease, then increased again followed by another decrease. At 12.5 ºC both measures of growth increased steadily over the larvae development period and decreased just before complete mortality occurred. The minimum growth rate at 15.5, 18 and 22ºC (3.22, 2.68 and 4.83 μm day $^{-1}$ respectively) were higher than the minimum at 12.5 ºC (0.46 μm day$^{-1}$).

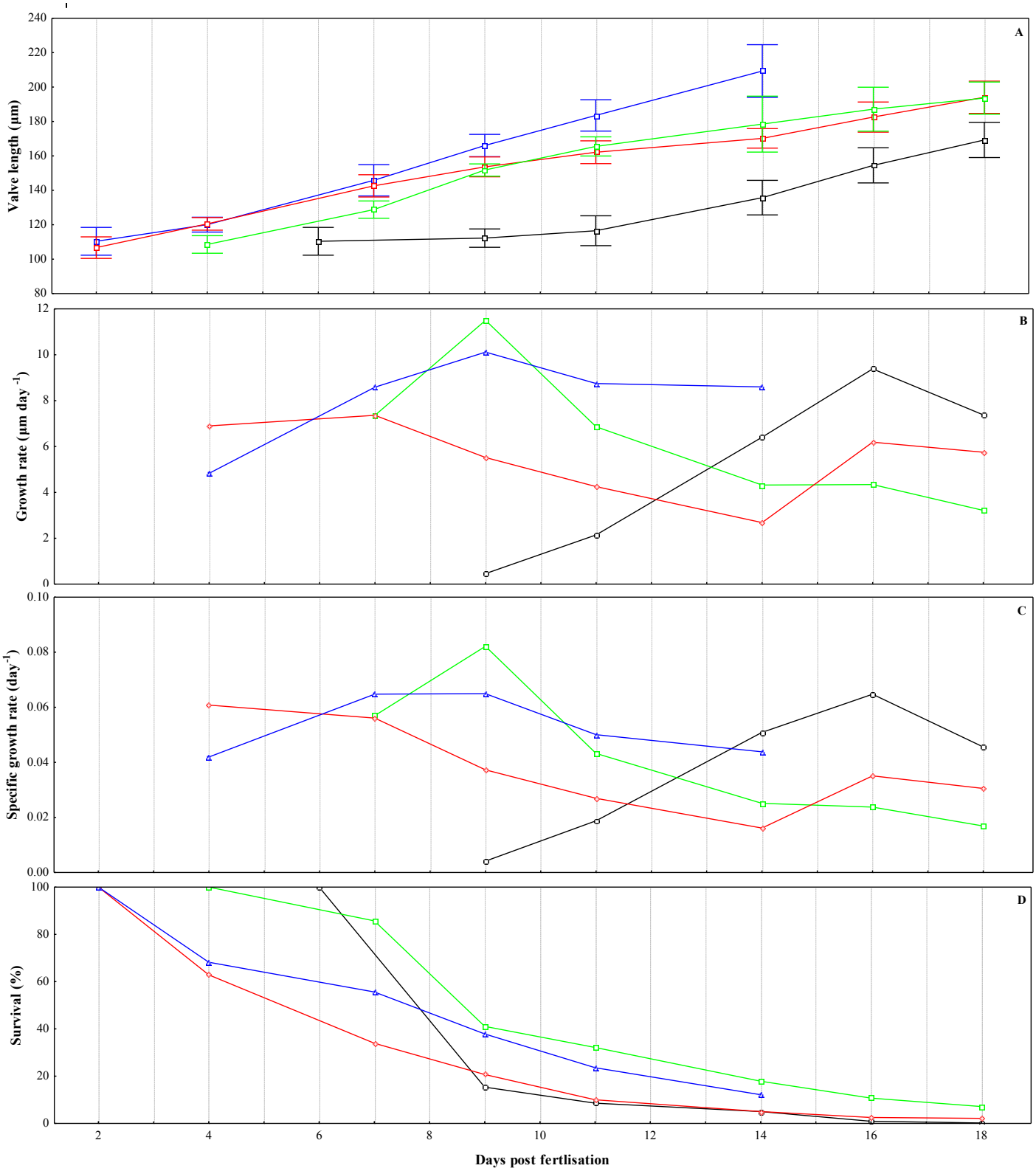


Figure 4.3: Larval development of *P. sulcicostatus* post-fertilisation at 12.5 (----) 15.5 (----), 18 (----) and 22 °C (----) (A) mean valve length (error bars denote ± SE), (B) growth rate, (C) specific growth rate and (D) survival

Table 4.2: Summary of growth statistics of larvae (D-larvae to pediveligers) cultured at four temperatures

| Temp (ºC) | Period of larval rearing (fertilised to pediveligers) | Length of grow-out (days) | Initial valve length (µm) | Maximum valve length (µm) | Mean growth rate (µm day$^{-1}$) | Mean specific growth rate (day$^{-1}$) |
|---|---|---|---|---|---|---|
| 12.5 | 24 Nov - 12 Dec 2014 | 18 (to eyed veliger stage) | 92 (range 101 - ,120; ± 6.5 sd, *n* = 5) | 169 (range 156.4 - 184; ± 8.2 sd, *n* = 5) | 5.16 (range 0.46 - 9.39) | 0.05 (range 0.041 - 0.065) |
| 15.5 | 24 Nov - 12 Dec 2014 | 18 | 109 (range 101 - 110; ± 4.1 sd, *n* = 5) | 193 (range 166 - 276; ± 20 sd, *n* = 20) | 6.27 (range 3.22 - 11.50) | 0.03 (range 0.016 - 0.061) |
| 18 | 24 Nov -12 Dec 2014 | 18 | 107 (range 101 - 110; ± 5 sd, *n* = 5) | 194 (range 166 - 230;± 20 sd, *n* = 20) | 5.52 (range 2.68 - 7.36) | 0.04 (range 0.016 - 0.082) |
| 22 | 24 Nov - 08 Dec 2014 | 14 | 110 (range 110 - 120; ± 6.5 sd, *n* = 5) | 209 (range 174 - 248; ± 33 sd, *n* = 20) | 8.17 (range 4.83 - 10.12) | 0.03 (range 0.045 - 0.064) |

### 4.4.4 Larval survival

Larval survival was counted from the D-larvae stage, therefore starting at different days post fertilisation for larvae exposed to the experimental temperatures (Fig. 4.3 D). Survival rate declined rapidly at 12.5 ºC. When the pediveliger stages were reached at 15.5, 18 and 22 ºC the percentage survival was 7.1, 2.1 and 12.4 % respectively. Larvae reared at 12 ºC were all dead 14 days post fertilisation. There was no significant difference in survival at the different temperatures (Tukey, $P < 0.05$).

### 4.4.5 Settlement of pediveligers

No settlement occurred in the control, nor on the polyethylene string in either light or dark environments (Fig. 4.4 A and B). Settlement occurred on crushed oysters only in the light environment (Fig. 4.4 A). The highest settlement occurred on poly-amide mesh in the light environment. It took approximately 72 hours for settlement to be completed in both environments. A settlement success of 43% was obtained.

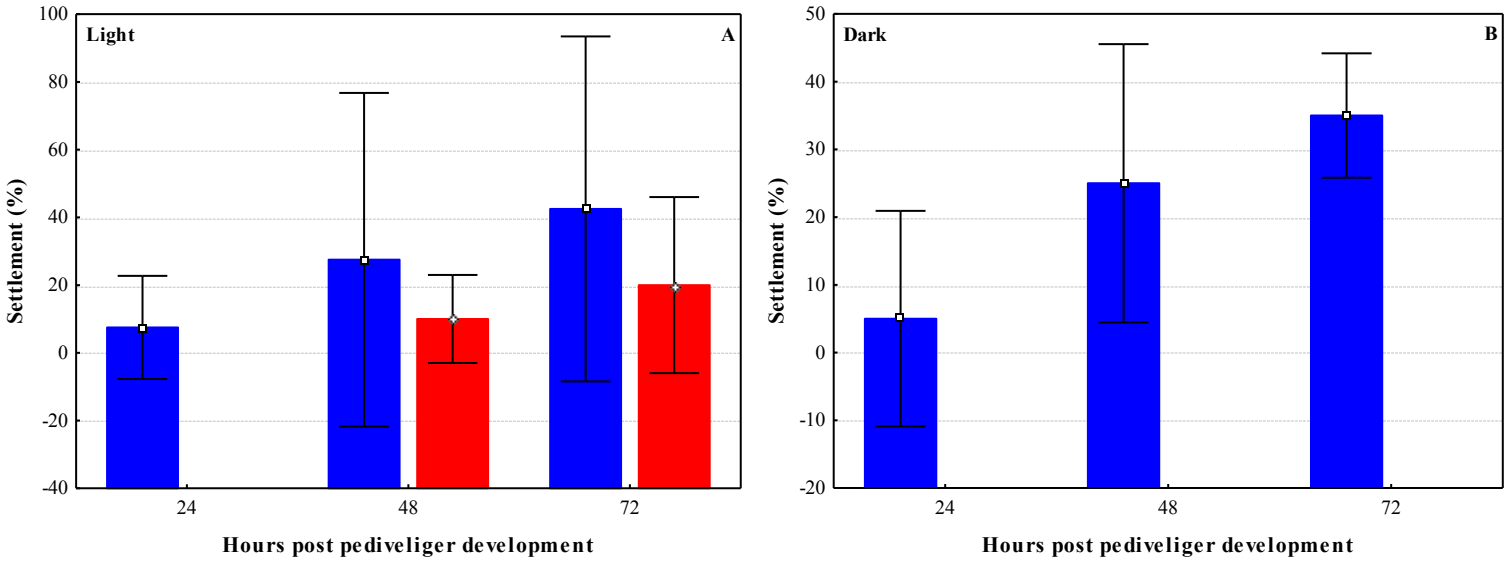


Figure 4.4: Larval settlement in the light (A) and dark (B) on 150 µm poly-amide mesh ( ) and crushed oyster shells ( ). Note no settlement occurred in control (no substrate) and on polyethylene string

## 4.5 DISCUSSION

### 4.5.1 Stages of larval development

The development rate reported here, of 48 h from fertilised eggs to D-larvae of *P. sulcicostatus* cultured at 18 and 22 ºC, was similar to those of other commercial species. For example, *Chlamys asperrimus* (17.5 ºC), *Chlamys apercularis* (17 ºC), *P. fumatus* (18 ºC),

*Argopecten gibbus* (23 ºC), *A. irradians* (24 ºC), *P. maximus* (20 ºC) and *P. fumatus* (21ºC) all reached the D-larvae stage 48 h post-fertilisation (Sastry 1965, Costello *et al*. 1973, LePennec 1974, 1982, Rose & Dix 1984, Heasman *et al*. 1996). D-larvae of *P. sulcicostatus* obtained 96 h post-fertilisation when cultured at 15.5 ºC was also similar in duration to that of larvae of *Placopecten magellanicus* maintained at 15 ºC (Culliney 1974). At 12.5 ºC the D-larvae stage was reached after 144 h and was much slower than in most commercial scallop species.

The rate of development of *P. sulcicostatus* larvae from fertilisation to pediveligers cultured at 15.5 and 18 ºC were similar to those of *P. maximus* (18 ºC) and *P. fumatus* (18 ºC), which reached the pediveliger stage after 16 d (Paulet *et al*. 1988, Heasman *et al*. 1996). The rate of larval development of *P. sulcicostatus* cultured at 15.5 and 18.5 ºC was, however, faster than in *P. albicans* (15 and 19 ºC) and *Placopecten magellanicus* (15 ºC), which reached the pediveliger stage after 28, 21, 35 d respectively (Culliney 1974, Hotta 1977, Tanaka 1985). The rate of larval development of *P. sulcicostatus* of 14 d to pediveliger stage at 22 ºC was the same as that of *Argopecten irradians* at 21.5 ºC, but slower than the 11 d required by *P. fumatus* to reach the pediveliger stage at 21 ºC (Loosanoff & Davis 1963, Heasman *et al.* 1996).

The valve lengths were similar at all temperatures for the embryogenic stages. The valve lengths of the pediveligers (211 - 276 µm) during this experiment were similar to those of other commercial species. For example, *P. maximus* (200 – 250 µm; Gerhard *et al*. 1989), *Placopecten magellanicus* (230 - 260 µm; Naidu *et al*. 1989), *Nodipecten nodosus* (207 – 214 µm; Rupp 1994) and *Argopecten purpuratus* (230 µm; Von Brand *et al*. 2006). The pelagic life cycle of *P. sulcicostatus* is thus similar to those reported for other Pectinid species (Ibarra

*et al*. 1997, Minchin 2002, Le Pennec *et al*. 2003, Velasco *et al*. 2007, Loor *et al*. 2015). It has been described that the rate of embryonic development of bivalve species depends mainly on water temperature (Wright *et al*. 1983, Le Pennec *et al*. 2003). Embryonic development of *P. sulcicostatus* was fastest at 22 ºC.

### 4.5.2 Growth of larvae

The valve length of *P. sulcicostatus* larvae cultured at 22 ºC increased faster than at the other three experimental temperatures. The period of larval development in scallops is temperature dependent and it is often found that larval growth increases with temperature (Bayne 1983). However, a temperature higher than the optimum temperature can cause growth to decline and mortality to increase (Hodges & Bourne 1988). The mean temperature in the natural environment at 4 - 13 m depth in False Bay was 14.5 ºC (range 9.8 – 21.6 ºC) from 5 August 2010 – 4 February 2012 (unpublished data). Taking the temperature range in the natural environment into consideration and the growth results of this experiment, it appears that 22 ºC may be the optimal temperature for *P. sulcicostatus* larvae.

The mean growth rates of *P. sulcicostatus* larvae at the experimental temperatures was similar to that of other commercial species, such as *Nodipecten nodosus, Chlamys Hastata* (16 ºC) and *Placopecten magellanicus,* which have growth rates of 6 – 8, 5.8 and 6.8 µm day$^{-1}$ respectively (Hodgson & Burke 1988, Rupp & Parsons 2006). The growth rates of scallop larvae have been classified as low (3 – 3.5 µm d$^{-1}$), moderate (3.5 – 5.5 µm day $^{-1}$) and high (5.5 – 7.5 µm d$^{-1}$) by Cochard & Gérard (1987). Following this classification the growth rate of the larvae of *P. sulcicostatus* would be regarded as high.

### 4.5.3 Larval survival

High larvae mortality is a worldwide hindrance in the development of viable scallop hatcheries (Bourne & Hodgson 1991, Robert & Gérard 1999). Larvae survival for pectinids is generally between 15 – 30 % (Helm *et al*. 2004). Low rates of larval survival throughout this study were experienced at all the experimental temperatures. As no correlation was found between temperature and survival, the low survival rate could have been attributed to other factors, such as larva density and/or sub-optimal food concentration or bacterial infection.

Larval density is an important factor to consider in larval rearing, as it has previously been shown that low larvae densities in certain scallop species produce high survival rates (Loosanoff & Davis 1963, Gruffydd & Beaumont 1972). Therefore the density of 17 500 per L used in this study could possibly be responsible for the low survival rate. However, Ibarra *et al*. (1997) found that a density of 20 larvae per mL gave satisfactory growth and survival. Food nutrition has also been described to play an important role in larval rearing (Whyte *et. al.* 1990, Baker & Mann 1994, Pernet & Tremblay 2004, Gagné *et al*. 2010). Quality gametes, obtained through sufficient nutrition during broodstock conditioning, increases the survival rate in scallop larvae (Racotta *et al*. 1998, Navarro *et al*. 2000). Also, larvae require sufficient lipid reserves, essential fatty acids, sterols and carbohydrates during growth and metamorphosis (Whyte *et. al.* 1990, Baker & Mann 1994, Pernet & Tremblay 2004, Gagné *et al*. 2010). Therefore, the low larval survival rate during this experiment could be attributed to inadequate nutrition during broodstock conditioning and larval rearing. Thus the feed concentration of 1.5 x$10^7$ million cells $L^{-1}$ followed by 2 x$10^7$ million cells $L^{-1}$ used in this experiment may not be adequate for such a high density of larvae cultured. However, this requires further investigation by conducting a particle clearance experiment on larvae, not

only to determine optimal filtration rate, but also to prevent over-feeding, as high algal densities could increase bacterial numbers, which could also decrease larval survival (Helm *et al*. 2004). Future studies will therefore investigate the effect of different densities on larval survival and development.

### 4.5.4 Settlement of pediveligers

As this was the first assessment of settlement of *P. sulcicostatus* larvae, only substrate preferences were tested. From the three substrata investigated in this study *P. sulcicostatus* larvae only settled on the poly-amide mesh and crushed oyster shells. Settlement in light and dark environments was also investigated to assess whether this species is negatively phototrophic, as in the case for both *P. magellanicus* and *M. yessoensis* (Culliney 1974, Golikov & Scarlato 1970). Since *P. sulcicostatus* larvae settled on poly-amide mesh in both light and dark conditions, this suggests that its larvae is not phototrophic. Poly-amide mesh and crushed oyster shells substrata are suitable for larval settlement of *P. sulcicostatus*. The low settlement on these substrata could perhaps be attributed to some pediveligers dying. Mortalities of pediveligers during settlement are usually caused by behavioural changes, or because larvae are induced to settle too early or late (Culliney 1974). Future work will include various settlement times after pediveliger development, other substrata and chemicals to act as settlement cues.

## 4.6 Conclusion

This study showed that the larvae of *P. sulcicostatus* should be cultured at 22 ºC, as the fastest growth and development occurred at this temperature. The growth rate of larvae of *P.*

*sulcicostatus* was similar to that of other commercial species. As there was no correlation between temperature and survival, it is suggested that other factors, such as food concentration and larval densities be investigated in order to improve survival rate. It is also suggested that other substrata be investigated for settlement. In order to improve settlement, the effect of the size of pediveligers on settlement should be investigated.

# CHAPTER 5:

## Growth and survival of the South African scallop *Pecten sulcicostatus* in suspended culture

## Contents

## 5.1 Abstract

This study presents the results of grow-out trials for the endemic South African scallop *Pecten sulcicostatus*, and contributes to ongoing investigations as to the suitability of this species for commercial culture. Hatchery-reared juvenile *P. sulcicostatus* were grown-out in suspended culture during three experimental periods, 9 February 2010 - 15 February 2011 (Experiment 1), 02 March 2011 - 15 February 2012 (Experiment 2) and 20 December 2011 - 19 December 2012 (Experiment 3). During Experiment 1 juvenile scallops were placed in a suspended culture system at 5 m depth in Saldanha Bay, on the west coast of South Africa. Subsequent growth was assessed monthly through increments in shell height in relation to changing environmental conditions, as determined through continuous measures of temperature and chlorophyll *a* concentration. Upon termination of the experiment, the mean shell height of 45.1 mm represented an increment of 38.2 mm over a year. The mean growth rate of 0.10 mm $day^{-1}$ (specific growth rate of 0.0046 mm $day^{-1}$) compared favorably with other commercially-cultured scallop species and exceeded previous estimates of growth of naturally occurring populations of *P. sulcicostatus*. Scallop growth was poorly correlated with both temperature and chlorophyll *a* concentration, but survival was closely aligned to temperature regime, exhibiting low survival during midsummer.

Experiments 2 and 3 compared growth and survival of hatchery produced juveniles suspended above (5 m) and below (10 m) the thermocline line in Saldanha Bay. Although favourable growth rates (0.10 mm $day^{-1}$) were again achieved, survival remained low, and despite lower mean temperatures at 10 m depth survival was significantly lower here (2.3%) than at 5 m (32.2%). Possible causes for the lower survival with increasing depth are extreme

temperature fluctuations associated with vertical adjustments in the thermocline depth and a higher incidence of infection by parasites, owing to closer proximity to the sediments.

Keywords: grow-out, Saldanha Bay, juvenile scallops, suspended culture, growth rate

## 5.2 Introduction

A major determinant of the economic feasibility of scallop culture is the growth rate of the species of interest, as cost of production is largely determined by length of grow-out period. Japan was the first country to successfully cultivate scallops in the late 1960's and today several species of scallops are grown successfully grown to commercial size in suspended culture (Widman & Rhodes 1991, Parsons & Dadswell 1992, Côté *et al*. 1993, Román *et al*. 1999). Several aquaculture methods are used, including pearl nets, ranching, bottom plastic trays and lantern nets. The success of any particular method depends largely on the species being farmed and the local environment.

This study reports on the first direct measures of growth and survival of hatchery-produced juveniles of *Pecten sulcicostatus* in suspended culture in Saldanha Bay, a semi-enclosed system on the west coast of South Africa. Saldanha Bay is located outside the natural range of distribution of *P. sulcicostatus,* but is considered suitable for the culture of shellfish, as it offers the protected waters needed for long-line and raft cultivation. It is also a highly productive system, owing to its strong link to the adjacent Benguela upwelling system, resulting in subsurface nitrate input and high chlorophyll levels for most of the year (Grant *et al*. 1998, Pitcher & Calder 1998). Saldanha Bay currently supports the bulk of shellfish production in South Africa, as there are few other protected sites on the South African coast suitable for culture of shellfish. The influence of environmental conditions on growth and survival of *P. sulcicostatus* was specifically examined in relation to high resolution measures of temperature, phytoplankton biomass and bay currents.

## 5.3 Materials and methods

### 5.3.1 Scallop grow-out

Juvenile *P. sulcicostatus* were grown-out in suspended culture during three experimental periods, 9 February 2010 - 15 February 2011 (Experiment 1), 02 March 2011 - 15 February 2012 (Experiment 2) and 20 December 2011 - 19 December 2012 (Experiment 3). Juvenile scallops were reared from wild broodstock collected by scuba divers from 30 m depth in False Bay (Fig. 5.1 A). Broodstock were maintained at approximately 16 °C and conditioned on a feed mixture of *Chaetoceros muelerii*, *Isochrysis tahitian* and *Pavlova lutherii* (ratio of 70:15:15). Spawning of each cohort was induced by intragonadal injection of the hormone serotonin on 16 November 2009, 19 October 2010 and 23 August 2011 respectively. Fertilized eggs were placed into 150 L conical tanks under static conditions pending development of D-veligers. Once formed, veligers were maintained in a flow-through upwelling system at 18 °C. Following development of eye spots, veligers were settled onto 150 µm mesh trays and placed in raceways. Both veligers and spat were maintained on a feed mixture of *Chaetoceros muelerii*, *Isochrysis tahitian* and *Pavlova lutherii* (ratio of 1:1:1). Hatchery-produced juvenile scallops (Fig. 5.1 B) were placed in suspended culture at an experimental grow-out site located on an oyster farm in Saldanha Bay ($33^{o}$ 01.6416 S; $18^{o}$ 00.9621 E). The first cohort of juvenile scallops ranged from 4.5 - 11.0 mm shell height (mean 6.9 mm, ± 1.26 sd, n = 254) and were placed at 5 m depth. Both the second and third cohorts were split and deployed at 5 and 10 m depth. The second cohort ranged from 13.1 - 29.8 mm shell height (mean of 20 mm at 5 m, ± 1.39 sd, n = 86; mean of 18.94 mm at 10 m, ±1.28 sd, n = 87) and the third cohort from 11.2 - 18.8 mm shell height (mean of 15.3 mm at 5 m, ± 1.7 sd, n = 46; mean of 14.8 mm at 10 m, ± 1.8 sd, n = 47).

Transportation from the hatchery to the site of grow-out was undertaken in-water and water temperature was maintained at <20$^{o}$ C. At the grow-out site, juvenile scallops were suspended in five stacked circular cages (diameter 60 cm, height 11 cm, mesh size 18 mm; Fig. 5.1 C), each of which was divided into four compartments.

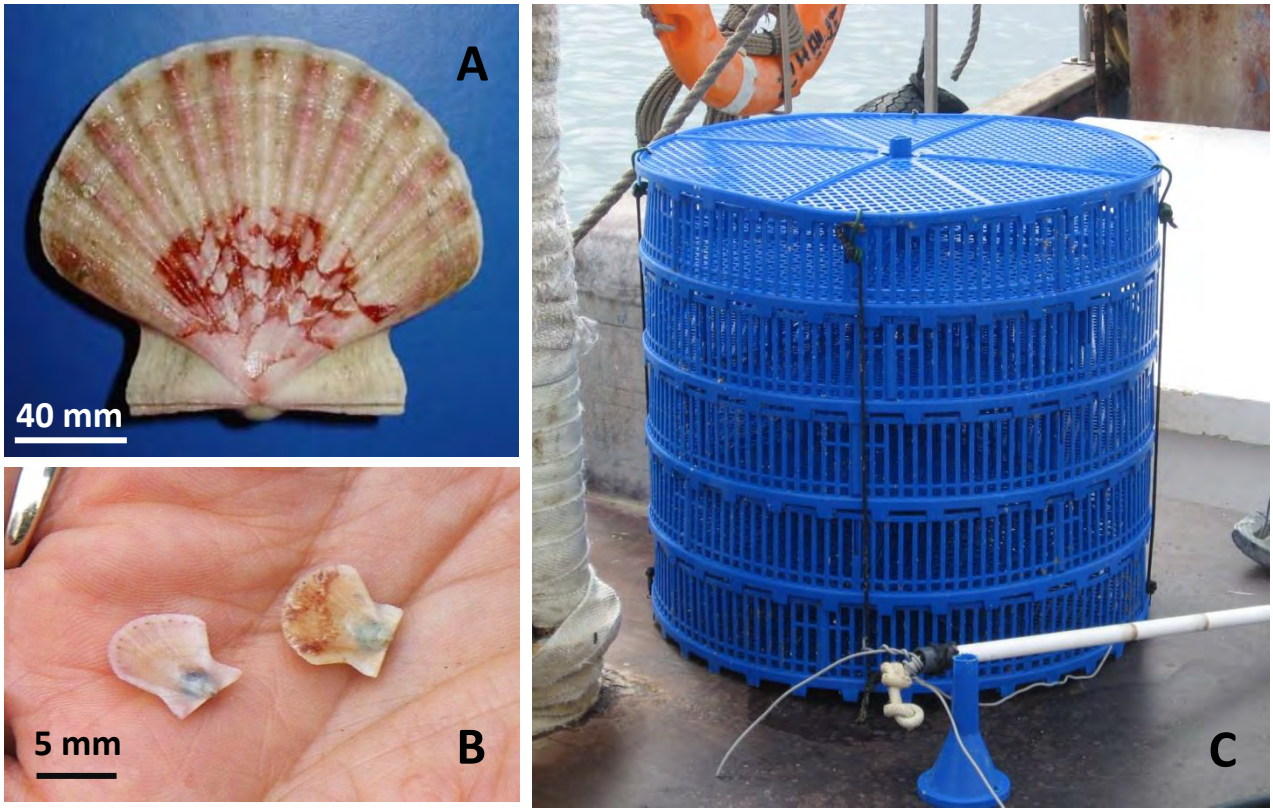


Figure 5.1: (A) *P. sulcicostatus* broodstock collected from False Bay at a depth of 30 m, (B) hatchery-reared juvenile scallops, and (C) plastic circular rigid cages used for the grow-out of scallops.

Owing to their small size, juvenile scallops were placed in smaller mesh liners (length 20 cm, width 9 cm, height 9 cm, mesh size 3 mm), each of which were placed into one of the compartments of the circular plastic cages. Approximately 21 juvenile scallops from Cohort 1 and 11 juvenile scallops from Cohorts 2 and 3 were placed into each of the liners, which were retrieved at approximately monthly intervals and cleaned through removal of all fouling organisms. At this time scallop growth was determined through measures of shell height

(maximum distance between dorsal [hinge] and ventral margins) using Vernier calipers. Emersion times for cleaning and measuring scallops were kept to < 1 hr through placement of cages in holding tanks. Growth rate (GR) was determined as: $GR = (SH_{t2} - SH_{t1}) / (t_2 - t_1)$, and specific growth rate (SGR) as: $SGR = ln\,(SH_{t2}/\,SH_{t1}) / (t_2 - t_1)$, where *SH* is shell height on consecutive sampling dates $t_1$ and $t_2$. Scallops were redistributed among the liners following mortalities in order to maintain similar stocking densities. Following retrieval of the remaining scallops after termination of Experiment 1, the somatic tissue was fixed in Davidson's fixative for histopathology examination (Austin & Austin 1989). Following retrieval of the remaining scallops after termination of Experiment 3, the shells were brought back to the laboratory for assessment of infection by parasites.

### 5.3.2 Environmental parameters

A Starmon temperature recorder and a Turner Designs SCUFA (Self-Contained Underwater Fluorescence Apparatus) were attached to the scallop cages and set to record every 10 minutes. The SCUFA allowed phytoplankton biomass to be tracked through *in vivo* estimates of chlorophyll *a* (Chl *a*). Default calibration settings, as determined by the turner Designs facility using primary standards of Chl *a,* were employed. An Acoustic Doppler Current Profiler (ADCP) was bottom-mounted at the grow-out site to measure currents throughout the water column. The monthly retrieval of scallop cages allowed for these instruments to be cleaned and for the data to be uploaded. Surface water samples were also collected for phytoplankton analysis at these monthly intervals during the grow-out period of Cohort 1. The water samples were fixed in buffered formalin and phytoplankton enumerated following the method of Utermöhl (Hasle 1978).

### 5.3.3 Statistical analysis

Statistical analyses were carried out using STATISTICA®, version 6. The Pearson correlation coefficient test was used to measure the extent to which growth rates were related to either temperature or Chl *a*. The Shapiro-Wilk test was used to test for normality.

## 5.4 Results

### 5.4.1 Scallop grow-out

Scallop size, as represented by shell height, was determined at approximately monthly intervals following retrieval and measurement of scallops (Fig. 5.2 A). Changes in shell height for the three cohorts are presented in Table 5.1. There was no significant difference between growth of scallops at the two different depths (Turkey, $P < 0.05$), nor was there any significant difference between growth of scallops between the three cohorts (Turkey, $P < 0.05$).

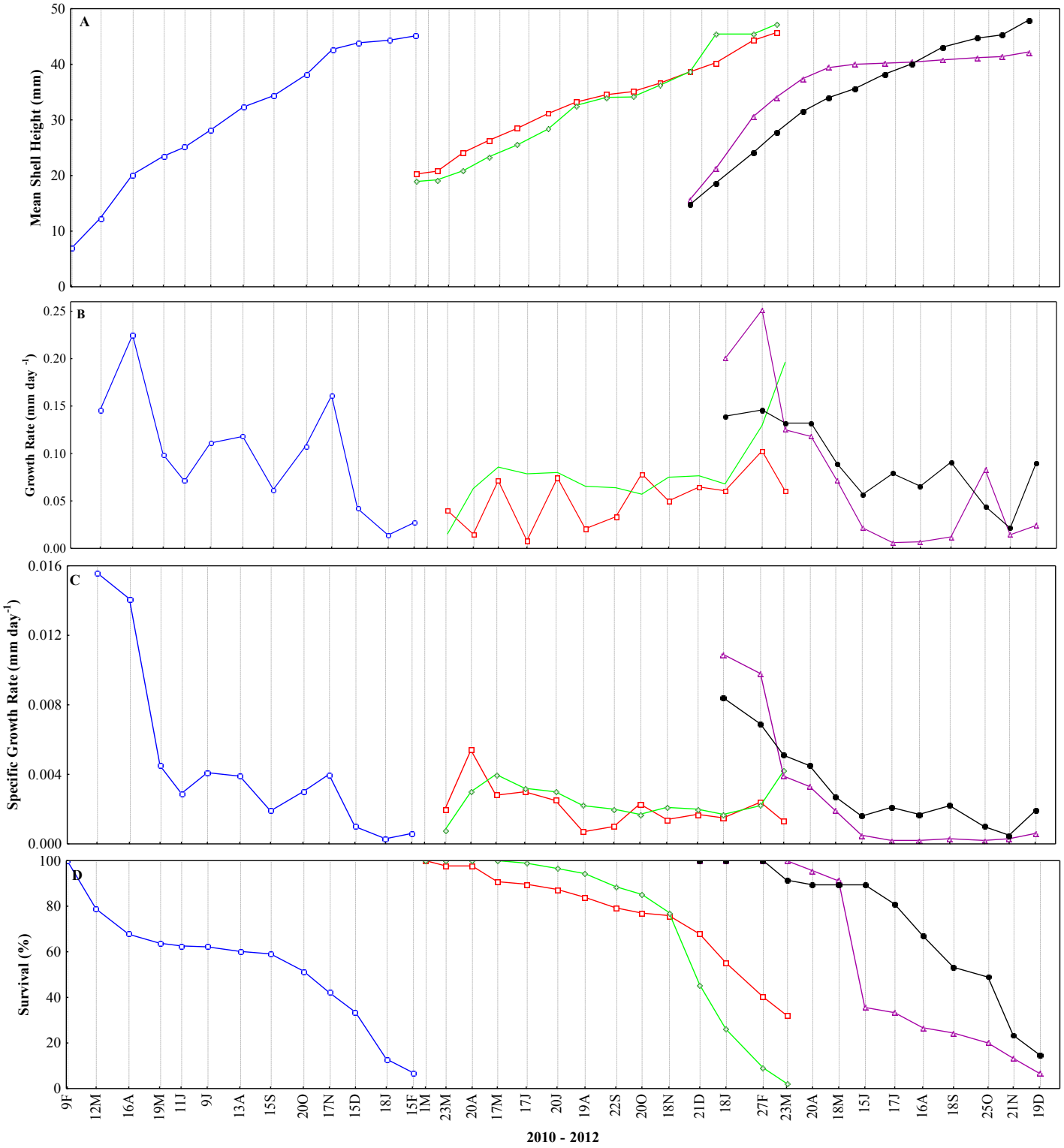


Figure 5.2: (A) Mean size, (B) growth rate and specific growth rate (C), and (D) survival of *P.sulcicostatus* cultured at 5 ( —— , —— , —— ) and 10 m ( —— , —— ) depth from 9 February 2010 - 19 December 2012 in Saldanha Bay.

Table 5.1: Increase in scallop size represented by shell height, growth rate and specific growth rate for the three cohorts over grow-out period

| Cohort | Period of grow-out | Depth (m) | Length of grow-out | Initial shell height (mm) | Maximum shell height (mm) | Shell Height increment (mm) | Mean growth rate (mm $day^{-1}$) | Mean specific growth rate ($mmday^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 Feb 2010 – 15 Feb 2011 | 5 | 378 | 6.9 (range 4.5 – 11; *n* = 254) | 45.1(range 42.1 - 48.7; *n* = 18) | 38.2 | 0.1 (range 0.01 - 0.23) | 0.0046 (range 0.0003 - 0.0156) |
| 2 | 2 Mar 2011 – 23 Feb 2012 | 5 | 358 | 20 (range 12.2 - 29.8; *n* = 87) | 44.2 (range 32.7 - 56.8; *n* = 31) | 24.2 | 0.07 (range 0.008 - 0.1) | 0.006 (range 0.0007 -0.056) |
| 2 | 2 Mar 2011 – 23 Feb 2012 | 10 | 358 | 18.9 (range 14 - 27.8; *n* = 88) | 45.2 (*n* = 1) | 26.3 | 0.08 (range 0.01 - 0.2) | 0.007 (range 0.0008- 0.039) |
| 3 | 20 Dec 2011 – 19 Dec 2012 | 5 | 364 | 14.4(range 12.8 - 19.2; *n* = 46) | 40 (range 27.6 - 47.3; *n* = 5 | 25.6 | 0.07 (range 0.06 - 0.25) | 0.002675 (range 0.0002 - 0.0109) |
| 3 | 20 Dec 2011 – 19 Dec 2012 | 10 | 364 | 14.1(range 11.6 - 18.4; *n* = 47) | 45.9 (range 39.2 - 53.6; *n* =5 | 31.8 | 0.09 (range 0.02 - 0.15) | 0.003217 (range 0.0005 - 0.0084) |

Growth rates and specific growth rates determined from monthly measurements showed considerable variations of up a 40 fold (growth rates) and 80 fold (specific growth rates) during grow-out periods (Table 5.1). Both measures of growth tended to decline during the course of grow-out, particularly specific growth rate, which was notably higher for smaller scallops (Fig. 5.2 B and C). The high growth rates characteristic of the initial two months of grow-out were notably reduced in all three cohorts of scallop juveniles deployed during 2010 – 2012, as the last three months of measurement showed a decrease in shell height increment. The histological results of the remaining scallops after 12 months from the first cohort indicated that the gonads were all in advanced gonad development stages (mid-maturation and mature / ripe gonads) and some scallops appeared to have spawned.

### 5.4.2 Environmental parameters

At 5 m the temperature ranged between 10.5 and 21.7 °C and at 10 m depth between 9.4 and 20.4 ºC over the experimental period (Fig 5.3 A). Water temperatures at both 5 and 10 m depth exhibited clear seasonal patterns, with lower temperatures in autumn and winter and higher temperatures in spring and summer. The temperature ranges during autumn - winter and spring - summer were 11.2 – 19.8 ºC and 10.5 – 21.7 ºC respectively at 5 m depth. At 10 m depth the temperature ranges during autumn - winter and spring - summer were 10.1 – 19.6 ºC and 9.4 – 20.4 ºC respectively.

Winter deployments at both depths were also characterized by low variability, with temperatures varying by < 3 °C during autumn and winter months, while spring and summer

deployments were characterized by high variability, with temperatures varying between 3 - 11.2 °C.

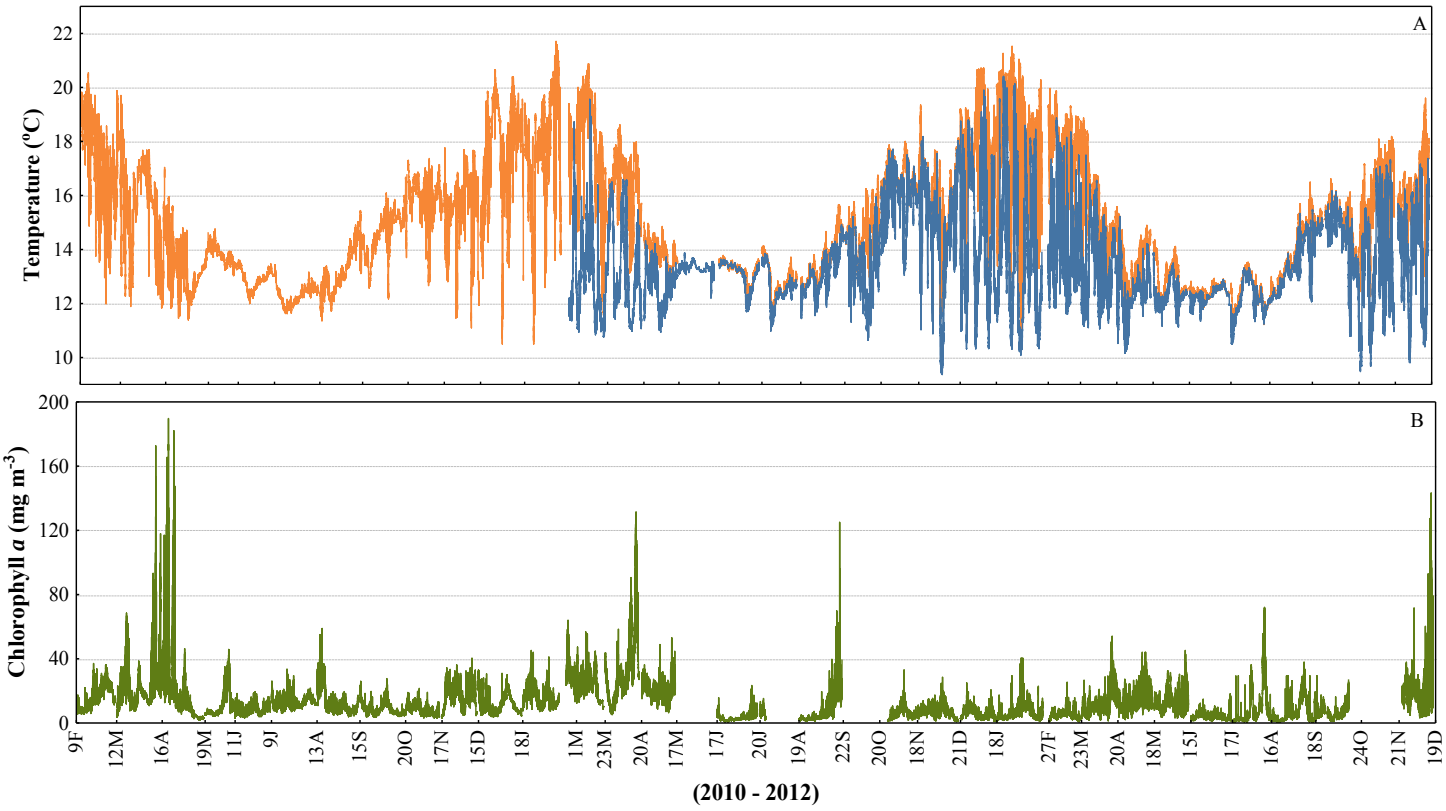


Figure 5.3: (A) Temperature at 5 (---) and 10(---) m depth and (B) Chlorophyll *a* concentration at 5 m depth as measured at 10 minute intervals for the period 09 February 2010 - 19 December 2012.

Chl *a* concentrations at 5 m depth ranged between 0.5 and 189.7 mg $m^{-3}$ during 9 February 2010 - 19 December 2012 (Fig. 5.3 B). Mean concentrations of Chl *a* for the approximate monthly intervals of instrument deployment ranged from 8.4 mg $m^{-3}$ to 23.2 mg $m^{-3}$ throughout the experimental period. Seasonality was evident in Chl *a* concentrations, in that winter and summer concentrations tended to be low, while concentrations in spring and autumn were highest.

Diatoms were a prominent component of the phytoplankton community from 9 February 2010 - 15 February 2011, dominated by species of *Chaetocerous*, *Ditylum*, *Pseudo-nitzschia*, *Thalassionema*, *Thalassiosira* and *Skeletonema*. Observations of exceptional high Chl *a* concentrations (> 60 mg $m^{-3}$) could be attributed to dinoflagellate blooms, eg. during March and April 2010 high biomass dinoflagellate blooms were dominated by *Ceratium* and *Prorocentrum* species.

Vertical profiles of current velocities measured from 17 May 2011 - 22 March 2012 showed consistent shear at 4 m depth, with current velocities consistently > 90 mm $s^{-1}$ at < 4 m depth and typically < 50 mm $s^{-1}$ at > 4 m depth. Scallop deployments at 5 and 10 m depths therefore appeared to be subjected to similar current velocities (Fig. 5.4).

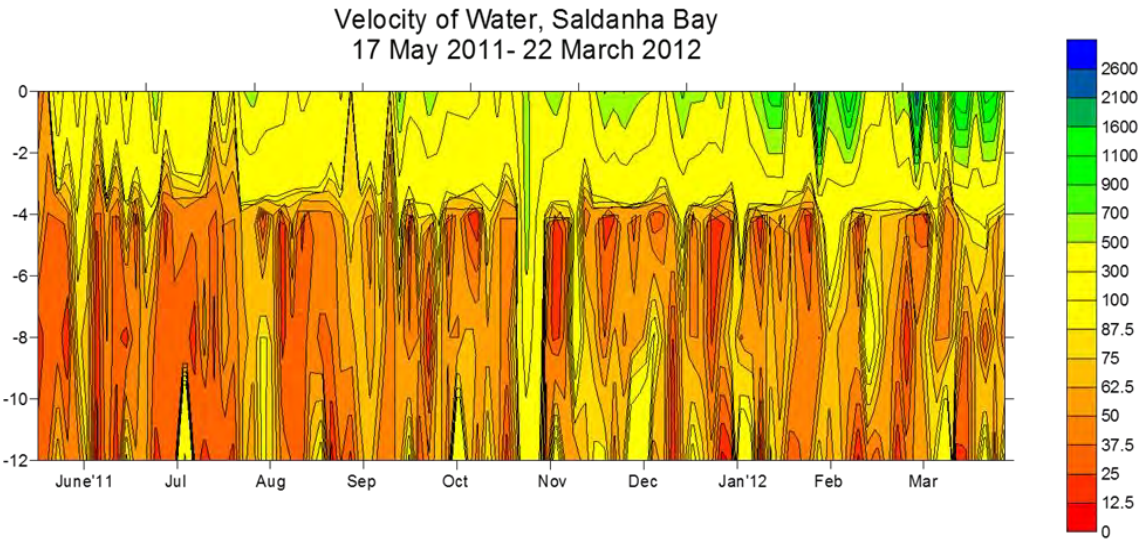


Figure 5.4: Profile of water velocity obtained from a bottom mounted Acoustic Doppler Current Profiler (ADCP) at the site of grow-out (mm $s^{-1}$).

### 5.4.3 Scallop survival

Monthly rates of survival for the first cohort of juvenile scallops grown at 5 m depth during Experiment 1 ranged from 78.8 – 7.1 % (Fig. 5.2 D). Initial survival remained high, with only a few mortalities occurring with the approach of winter, at which time survival ranged between 63.8 – 60.2 % per month (16 April 2010 – 15 September 2010). Survival decreased with the onset of spring and severe mortalities were recorded from mid-spring – mid-summer (20 October 2010 - 17 January 2011, range 18.3 – 61.2%), with an overall survival of only 7.1 % of the initial stock by the end of the experiment.

Monthly rates of survival for the second cohort of juveniles grown at 5 and 10 m depth during Experiment 2 ranged from 97.7 – 32.2% and 100 – 2.3% respectively (Fig. 5.2 D). Low survival at 5 m depth occurred during summer (21 December 2011 – 27 February 2012, ranging from 55.2 – 40.2%). At 10 m depth low survival occurred from late autumn throughout summer (18 November 2011 – 27 February 2012, ranging from 45.5 – 2.3%). The overall survival at 5 and 10 m depth were 32.2 and 2.3% respectively.

Monthly rates of survival for the third cohort at 5 and 10 m depth during Experiment 3 ranged from 100 – 6.7% and 100 – 14.9% respectively (Fig. 5.2 D). Low survival rates at 5 m depth were found in mid-winter (15 June 2012, 33.3%) and again in mid-autumn to early summer (25 October 2012 – 19 December 2012, range 20 – 6.7%). At 10 m depth, low survival occurred in late autumn – early summer (21 November 2012 – 19 December 2012, range 48.9 – 14.9%). The overall survival rates at 5 and 10 m depth were of 6.7 and 14.9 % respectively.

Scallop survival appeared to be closely aligned to temperature regime during the grow-out period in Experiment 1 (Fig. 5.5 A and B), with high survival when temperatures were low and temperature fluctuations were small (< 2 ºC) and low survival when temperatures increased and fluctuated markedly (> 10 ºC). In Experiment 2 a correlation between temperature and survival appeared at 5 and 10 m depth (Fig. 5.5 C). Scallop survival appeared to be closely aligned to temperature fluctuation (5 - 11º) at both depths during the grow-out period (5.5 D). During Experiment 3 scallop survival appeared not to be aligned to either temperature or temperature fluctuation (Fig. 5.5 E and F).

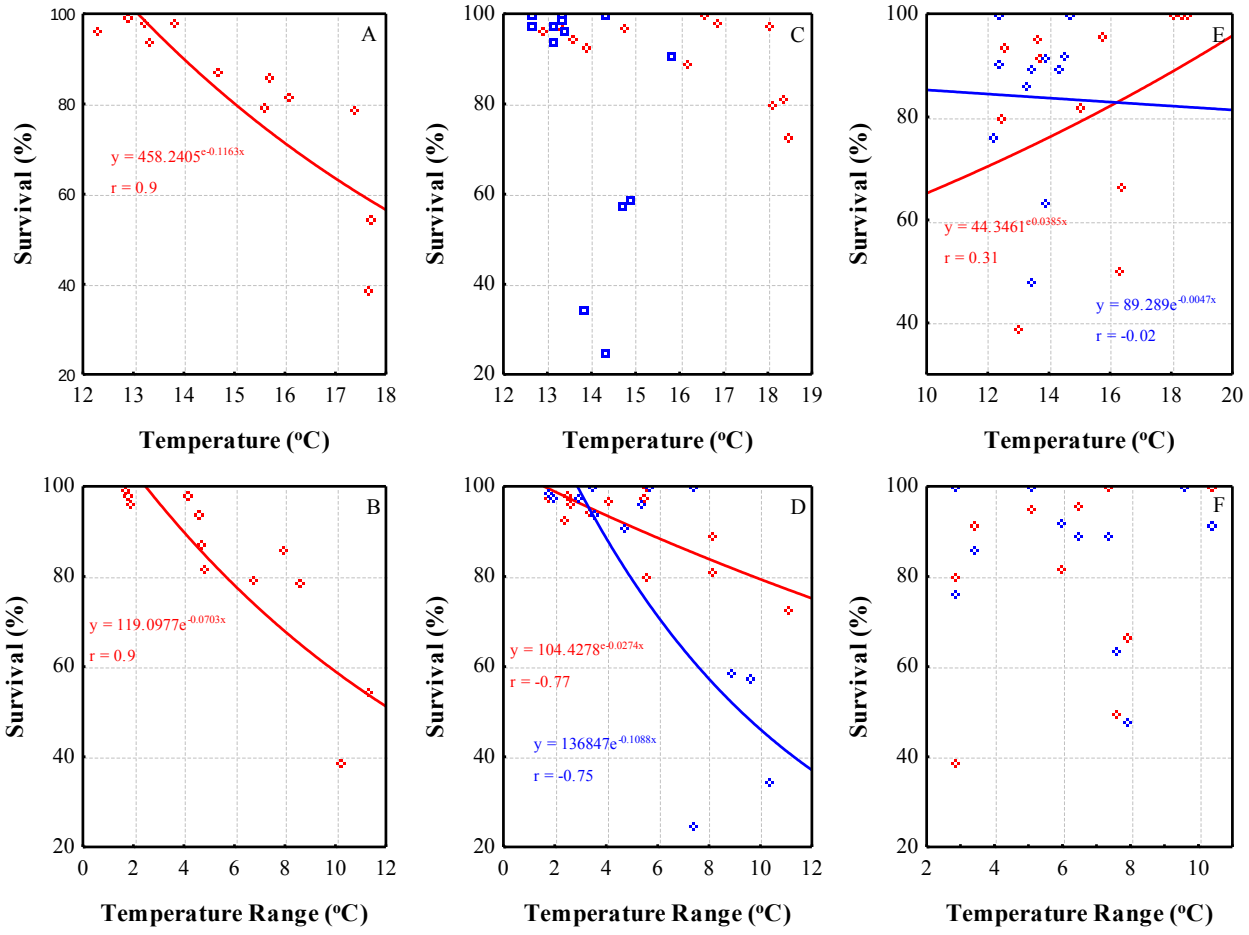


Figure 5.5: Relationship between scallop survival and (A, C, E) mean temperature and (B, D, F) mean temperature range for each of the monthly intervals during experiment 1, 2 and 3 respectively at 5 (——) and 10 m ( ——) depth.

The health examination of the remaining scallops following termination of Experiment 1 showed no significant abnormalities and no internal parasites. Gonad development was advanced in all animals, two of which appeared to have recently spawned. Following termination of Experiment 3 spionid polychaetes were found in the remaining scallops.

## 5.5 Discussion

### 5.5.1 Scallop growth

The mean growth rates determined for *P. sulcicostatus* were similar to those reported for several other commercially cultured pectinids (Table 5.2), such as *Chlamys farreri* (Zhikong scallop), which accounts for over 80% of the cultured production of scallops in China (Zhang *et al.* 1956, as cited by Guo & Luo 2006). They were also similar to those of the Japanese scallop *Patinopecten yessoensis*, the main culture species in Japan (Ventilla 1982); *Pecten maximus* (Great scallop), the main species of interest in European aquaculture (Louro *et al*. 2005, Christophersen & Magnesen 2001) ; *Argopecten purpuratus* (Northern scallop), the only scallop cultivated in Chile (Von Brand *et al.* 2006) and *Placopecten magellanicus* (sea or giant scallop), which dominates scallop culture in the Northwest Atlantic (Kleinman *et al.* 1996, Côté *et al*. 1993).

Table 5.2: Comparative growth rates of various commercial cultured pectinids

| Species | Country | Size | Mean Growth (mm $day^{-1}$) | Temperature (ºC) | Reference |
|---|---|---|---|---|---|
| *Pecten sulcicostatus* | South Africa | 46 mm after 14 months | 0.07 – 0.10 | 10.1 – 21.7 | |
| *Chlamys farreri* (Zhikong scallop) | China | 50 mm after 18 months | 0.09 | > 28 | Zhang *et al*. 1956, as cited by Guo & Luo 2006 |
| *Patinopecten yessoensis* (Japanese scallop) | Japan (Saroma Lake) | 30 mm after 8 months | 0.10 | > 23 | Ventilla 1982 |
| *Pecten maximus* (Great scallop) | Norway | | 0.05 – 0.13 | 5 – 15 | Christophersen & Magnesen 2001 |
| *Pecten maximus* (Great scallop) | Spain (Galician coast) | 17 mm after 57 -85 days | 0.16 – 0.23 | 12 – 20 | Louren *et al*. 2005 |
| *Argopecten purpuratus* (Nothern scallop) | Chile | 75 – 85 mm after 14 – 18 months | 0.16 | | Von Brand *et al*. 2006 |
| *Placopecten magellanicus* (Sea or Giant scallop) | Canada (Nova Scotia, Lunenberg Bay) | 41 mm after 9 months | 0.07 – 0.12 | 2 – 16 | Kleinman *et al*. 1996 |
| *Placopecten magellanicus* (Sea or Giant scallop) | Canada (Gulf of St Laurence | 42.5 – 48 mm after 12 months | 0.04 – 0.12 | | Côte *et al*. 1993 |

The growth rate of *P. sulcicostatus* in suspended culture in Saldanha Bay was notably higher than that estimated by De Villiers (1976) through the analysis of size-frequency distributions and annular growth rings of natural populations of *P. sulcicostatus* in False Bay. Consequently the size of scallops for the three cohorts at different depths, following grow-out in Saldanha Bay, is notably larger than scallops of an estimated equivalent age in False Bay (shell height of 25 mm at the age of 12 months; shell height of 45 mm at the age of 24 months (De Villiers 1976). It is possible that these differences may be a consequence of error in the estimates of De Villiers (1976), owing to the uncertainty of the age of scallops at the time of formation of the first conspicuous “annular ring”. Alternatively, the growth of

scallops may be considerably slower in the bottom water environment of False Bay, owing to lower temperatures and food levels, compared to those in the shallow water environment of Saldanha Bay. Conversely, the scallops *Aequipecten opercularis* and *Crassodoma gigantean* has been described to have slower growth near the surface (MacDonald & Bourne 1989, Román *et al*. 1999). However, it has has been described that scallop growth is slower when grown at below a critical depth, where environmental conditions such as temperature, food availibity and turbity reaches suboptimal levels (MacDonald & Thompson 1985a, Côté *et al*. 1993, Lodeiros & Himmelman 2000, Fréchette & Daigle 2002).

The growth of *P. sulcicostatus* in Saldanha Bay needs also to be considered in context of the reproductive state, as the energy requirement for production of gametes in marine bivalves is high, resulting in a coupling between reproductive cycle and energy available for growth, with the cost of reproduction likely to increase with size and age (Thompson & MacDonald 2006). Wildish & Saulnier (1992) and Kleinman *et al*. (1996) described variation in scallop juvenile growth rates to be seasonal, due to the substantial allocation of energy for gonad production. In *Argopecten ventricosus*, growth rate decreased during the spawning season and mortality increased significantly, due to an increase in temperature (Maeda-Martínez *et al*. 1997). Although reproductive development was not followed during grow-out in Saldanha Bay, histological examination of the remaining scallops at termination of Experiment 1, showed gonad development to be advanced. Therefore, transfer of energy to gonad development, created by metabolic demand of gametogenesis, may have been a contributing factor to declining growth rates, as indicated by decreasing increments in shell height when scallops were ± 10 months old. The environmental conditions at the grow-out depths in Saldanha Bay are condusive for reproductive outputs. MacDonald & Thompson (1985a, b, 1986a) demonstrated that *Placopecten magellanicus* from deep water had less reproductive

output, reduced rates of gamete development and less energy allocated to reproduction due to sub-optimal environmental conditions. However, this needs further investigation.

### 5.5.2 Environmental parameters

Scallop growth rate has often been correlated with environmental conditions, particularly temperature and food availability (Thompson & MacDonald 2006). Such associations are common in systems that demonstrate strong seasonality, particularly in environments that experience very cold and unproductive winters, resulting in much reduced growth (Thompson & MacDonald 2006). Here the combined effects of ration and temperature on energy balance are considerable, with ration often being the most influential single factor (e.g., MacDonald & Thompson 1985b, 1986a). The poor correlation of scallop growth rate with either temperature or Chl *a* concentration in Saldanha Bay is a likely consequence of absence of a particularly cold and unproductive winter. Saldanha Bay is recognized as a highly productive system, and abundance of phytoplankton in the upper water column, as reflected by the high Chl *a* concentrations recorded during this study, is likely to provide an environment of surplus food. The Chl *a* / carbon concentrations are higher in the upper water column of Saldanha Bay due to its link to the Benguella upwelling system (Pitcher & Calder 1998) and is also expected to provide significantly higher nutritional value, compared to that encountered by *P. sulcicostatus* in the bottom waters of False Bay where organic detritus is likely to be the predominant food source. Thus, given the surplus high quality food supply of Saldanha Bay, it is unlikely that scallop growth is limited by food ration at any time of the year.

Saldanha Bay is stratified during summer, as a result of surface warming and the penetration of cold bottom water forced by coastal upwelling on the shelf (Monteiro & Largier 1999).

However, there was only a 1.6 ºC temperature difference between the mean temperatures at 5 (mean 15.0 ºC) and 10 m (13.4 ºC) depth throughout the entire experimental period. Although there was a slight difference between the mean temperatures at the two depths, the temperature ranges were very similar (range 10.5 - 21.7 $^{\circ}$C, difference 11.2 $^{o}$ C at 5 m and range 9.4 - 20.4 ºC, difference 11 ºC at 10 m depth). Although scallop growth was poorly correlated with temperature, it was evident that low survival rates coincided with maximum temperatures and the highly variable temperature regime characteristic of mid-summer during grow-out period during the first and second experiments.

Current speed may also affect scallop growth. Previous studies on other pectinids demonstrated that current speed was not a contributing factor for scallop growth (Claereboudt *et al*. 1994, Eckman *et al*. 1989). In *Placopecten magellanicus*, where current speeds of < 0.9 and > 0.16 m.s$^{-1}$ were recorded, no influence on scallop growth in suspended culture was described (Claereboudt *et al*. 1994). Eckman *et al*. (1989) also described growth in *Argopecten irradians concentricus*, to be unaffected when exposed to a current speed of 1.7 - 3.9 cm s$^{-1}$. However, growth rate of *Pecten maximus* grown in suspended culture increased at a current speed of 0.3 m s$^{-1}$ (Wilson 1987). In contrast, Laing (2002) described strong tidal currents as causing excessive buffeting of scallops in nets or cages, which can lead to reduced growth and mortality. In this experiment, the current speed recorded during the grow-out period 17 May 2011 - 22 March 2012 demonstrated consistent shear at the two depths of scallop grow-out and therefore did not influence scallop growth.

### 5.5.3 Scallop Survival

With the absence of any evidence of disease or abnormality during Experiment 1 it seemed as if low survival rates was possibly attributable to the high summer temperatures and/or extreme temperature fluctuations. There was also a strong correlation between survival and temperature and temperature fluctuations during Experiment 2 which could also have been responsible for the low survival rates. However, during the grow-out period of Experiment 3 the correlation between survival and temperature and/or extreme temperature fluctuations was not strong. Therefore temperature and/or high temperature fluctuations were possibly not the only contributing factors to low survival rates. Attempts to raise the Japanese scallop (*Patinopecten yessoensis*) outside its natural distribution resulted in low survival rates of 40% in summer, attributed to water temperatures > 22 $^{o}$C (Deguchi *et al.* 1975 as cited by Ventilla 1982). Also in China, the Zhikong scallop (*Chlamys farreri*), which forms the bulk of cultured scallop production, experienced low survival rates of 40 - 20% in 1997 - 98 in areas that experienced abnormally high temperatures exceeding 28 $^{o}$C (Guo & Luo 2006).

During the grow-out period of Experiment 3 low survival rates occurred from late autumn (18 May 2012) untill the end of the grow-out period at both depths. Heavy fouling occurred during this period, indicating that fouling could also be a contributing factor to high mortalities. However, in some months it was observed that fouling was more dense than in other months, irrespective of cages being cleaned on a monthly basis. In *Euvola ziczac* fouling only slightly affected increase in tissue mass, and could have been a contributing factor causing increase in scallop mortality (Lodeiros & Himmelman 1996). In the Bay scallop, *Argopecten irradians concentricus* (Say) very low survival rates were encountered

during summer, partially as a result of heavy fouling during grow-out in suspended culture in Tampa Bay, Florida (Lu & Blake 1997).

Additionally, spionid polychaetes could have been a contributing factor to low survival rates, as the remaining scallops at termination of the grow-out experiment were found to have a few spionid polychaetes. Although the effect of parasites was not followed during the grow-out period, a few scallops were found to have broken hinges, causing growth to cease and ultimately death to occur thereafter. Polychaete worms have been described as burrowing into the inner shell surface close to the adductor muscle attachment site of bivalves, causing the adductor muscle to weaken (Mc Gladdery *et al*. 2006). The weakened abductor muscle in effect impairs shell closure, affects swimming and feeding behavior and causes breakage in the hinge (Mc Gladdery *et al*. 2006).

## 5.6 Conclusion

This study is a first assessment of growth and survival of the South African scallop *P. sulcicostatus* during intermediate grow-out. The study specifically examined the growth and survival of scallops in Saldanha Bay as influenced by food availability, ambient temperature and water currents, thereby providing an assessment of the suitability of Saldanha Bay for commercial culture of *P. sulcicostatus.* Although acceptable growth rates were achieved, in that they compared favourably with other commercially cultured species, the low survival rates of scallops observed during grow-out indicated suboptimal conditions.

Initial size is known to have a significant influence on survival of scallops transferred to a sea -based system and the decrease in survival rate during the first month of grow-out in Saldanha Bay may have been a function of the stress of transfer and the fragility of small animals. Repeated handling during monthly assessments of growth may also have contributed to the low survival rate. However, this study indicates that the high and/or variable surface temperature during mid-summer and presence of parasites could all be contributing factors causing low scallop survival rate. It is, therefore, recommended that further studies of scallop grow-out be conducted, investigating the effect of parasite infection and fouling on growth and survival. It is also recommended that other grow-out sites be explored.

# CHAPTER 6

## FINAL DISCUSSION

One of the aims of the marine aquaculture policy of South Africa is to expand the resource base from the few species currently being farmed to a more diverse array of species. Since scallops have a high market value and have been farmed for decades globally, the local scallop species *Pecten sulcicostatus* has been identified as a possible species to be farmed in South Africa. Indeed, this is the only scallop species in South Africa large enough to be of interest to the aquaculture industry. However, *P. sulcicostatus* has been poorly studied and its biology and life history were largely unknown prior to the current study. This thesis therefore investigated the life cycle and culture potential of this species in order to assess its viability for farming purposes.

Understanding the reproductive cycle was imperative to provide information on natural spawning cycles, which in turn indicate the best time to collect broodstock for conditioning and spawning purposes. Documentation of the reproductive cycle was thus a primary aim of the thesis. The results showed that *P. sulcicostatus* has a peak spawning period during winter to early spring, with minor spawning events occurring in autumn. The study indicated that corresponding changes in phytoplankton biomass could control the reproductive cycle of *P. sulcicostatus*. Although a similar reproductive cycle was demonstrated five years later, it is recommended that a follow-up study be conducted in future to ensure that the reproductive cycle has not changed as a result of possible environmental changes. Furthermore, the histological investigations showed lack of a resting period and low percentage of

synchronicity occurred in this species, as the individual scallops started developing gametes immediately after a spawning event or reabsorption of oocytes. The lack of a resting period and low synchronicity indicates that artificial conditioning of *P. sulcicostatus* for spawning purposes could ensure a supply of ripe gametes throughout the year. However, spawning induction could be problematic, as the individual scallops would be in different oocyte maturation phases.

As predicted from the natural reproductive cycle of *P. sulcicostatus*, the species was successfully conditioned in a controlled environment, irrespective of the food concentration used. However, a higher food concentration increased rate of gonad development. Furthermore, depending on the development stage of gametes, *P. sulcicostatus* can be conditioned within two weeks, using a high food concentration. It is recommended that further studies be conducted on the rate of gonad conditioning, using a high food concentration on broodstock undergoing different stages of gamete development. The gonad stages of the broodstock must be visually assessed and documented in order to assist a farmer to identify the condition of broodstock. The knowledge of the time period for conditioning will also prevent over-feeding of the broodstock in order to cut down on the financial impact of live food production on a farm or hatchery.

Given the low synchronicity of *P. sulcicostatus,* spawning induction was problematic. Various spawning induction methods were tested, such as thermal shock, food deprivation, desiccation and using the hormone serotonin. Spawning induction by serotonin was the only method that was consistently successful for *P. sulcicostatus*. Future studies should use gonads that are all visually in the same stage of gonod development for conditioning and spawning

induction purposes, whereupon the different spawning induction techniques should be retested for *P. sulcicostatus*. After successful spawning induction, larval rearing was investigated. Larvae were cultured at 12.5, 15.5, 18 and 22 ºC from fertilised eggs up to pediveligers. Although the pediveliger stage was reached in 15.5, 18 and 22 ºC, growth and development were fastest at 22 ºC. The growth rate obtained also compared favourably to those of other commercial species. However, survival of larvae was very low. As there was no correlation between temperature and survival, it is suggested that other factors, such as larval density and food concentration, be investigated in order to increase survival. Settlement of pediveligers was successful on oyster shells and poly-amide mesh. It is recommended that poly-amide mesh be used for settlement purposes, as a higher survival was obtained on this substratum relative to that on oyster shells. Future studies should investigate pediveligers settlement on other substrata and chemical inducers.

Hatchery-reared spat were grown out in suspended culture in Saldanha Bay at two depths. Growth rates compared favourably to those of other commercial species. However, survival was low. The low survival could be attributed to the temperature regime, fouling, parasite infection and/or handling stress. Further grow-out studies are recommended to investigate the effect of parasite infection on growth and survival. As the environment in Saldanha Bay may not be conducive for scallop grow-out, it is recommended that other grow-out sites also be explored. However, in South Africa there are few sheltered bays and Saldanha Bay and Algoa Bay are the two sites that have been zoned for aquaculture. It is recommende that grow-out trials be conducted in Algoa Bay, as it forms part of the species' natural distribution. However, Algoa Bay has higher water temperatures than found in Saldanha Bay. An attempt to grow-out juveniles in effluent water of abalone farms is also recommended as a possible by product.

In conclusion, the thesis indicates that *P. sulcicostatus* can be successfully conditioned and spawned throughout the year. Larvae can be cultured from fertilised egg to pediveligers within 14 days and successfully settled on poly-amide mesh or oyster shells. The growth rates of larvae are favourable compared to those of other commercial species. Spat grown-out in suspended culture also have growth rates favourable relative to those of other commercial species and the local species is estimated to reach market size within 2 - 2.5 years in an environment such as Saldanha Bay. Research to date has demonstrated that *P. sulcicostatus* has similar traits to other commercial species (Table 6.1). Therefore, the research demonstrates the potential of *P. sulcicostatus* as a viable species for aquaculture in South Africa; however, increasing survival of both larvae and spat is a prerequisite to commercial culture. Therefore, future work should focus on increasing survival of both larvae and spat grown-out in the natural environment.

Table 6.1: Aspects of *P. sulcicostatus* compared to those of other commercial species

| **Experimental investigations** | ***Pecten sulcicostatus*** | **Commercial species with similar aspects to *P. sulcicostatus*** | **References** |
|---|---|---|---|
| Reproductive cycle | Highest mean GSI (%) value obtained: 14.4 % in 2004 - 05 and 17.8% in 2010 - 11 | *Argopecten irradians* (Florida) 14.5%, | Barber & Blake 1983 |
| | Major spawning period: winter – early spring | *P. fumatus* (Australia), *P. alba* (Australia), *Chlamys zelandiae* (New Zealand) | Booth 1983, Sause *et al*. 1987, Young *et al*. 1999 |
| Spawning Induction | Seretonin releases both male and female gametes | *P. yessoensis*, *P. albicans* | Matsutani & Nomura 1982, 1984, Tanaka & Murakoshi 1985 |
| Broodstock conditioning | High food concentration increased rate of gamete development | *Argopecten ventricosys* | Villalaz 1994 |
| Larval rearing | Time frame from fertilized egg to pediveligers at 22 ºC: 14 days | *Argopecten irradians* at 21.5 ºC | Heasman *et al.* 1996 |
| | Valve length of pediveligers: 211 - 276 µm | *P. maximus* (200 – 250 µm), *Placopecten magellanicus* (230 - 260 µm), *Nodipecten nodosus* (207 – 214 µm) and *Argopecten purpuratus* (230 µm) | Gerhard *et al*. 1989, Naidu *et al*. 1989, Rupp 1994, Von Brand *et al*. 2006 |
| | Growth rate of larvae 5.16 – 8.17 µm $day^{-1}$ | *Nodipecten nodosus* (6 – 8 µm $day^{-1}$), *Chlamys Hastata* (5.8 µm $day^{-1}$) , *Placopecten magellanicus* (6.8 µm $day^{-1}$) | Hodgson & Burke 1988, Rupp & Parsons 2006 |
| Grow-out of spat | Growth rate of juveniles: 0.07 – 0.1 mm $day^{-1}$ | *Chlamys farreri* (0.09 mm $day^{-1}$), *Patinopecten yessoensis* (0.1 mm $day^{-1}$), *Placopecten magellanicus* (0.07 – 0.12 mm $day^{-1}$), *Pecten maximus* (0.05 – 0.13 mm $day^{-1}$) | Zhang *et al*. 1956, as cited by Guo & Luo 2006, Ventilla 1982, Kleinman *et al*. 1996, Christophersen & Magnesen 2001 |

# CHAPTER 7

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