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HIGH DENSITY REARING OF *Pteria penguin* LARVAE USING ALGAL PASTE

by

Jerome S. Taoi

A thesis submitted in fulfillment of the requirements for the degree of
Master of Science in Marine Science

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School of Marine Studies
Faculty of Science, Technology and Environment
University of the South Pacific

December, 2015
DECLARATION

Statement by Author

I, Jerome S. Taoi, declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with material submitted for the award of any other degree at any institution, except where due acknowledgement is made in the text.

Jerome S. Taoi

s11051766

16th December, 2015

Statement by Supervisors

The research in this thesis was performed under my supervision and to my knowledge is the sole work of Mr. Jerome S. Taoi.

Prof. Ciro Rico
Principal Supervisor

16th December, 2015

Prof. Paul Southgate
External Supervisor

16th December, 2015
DEDICATION

I would like to dedicate this thesis to the Lord Almighty for the obstacles overcome during this research and to three most influential people in my life, Mom, ‘little’ brother Jake and to my mentor, the late Mr. Johnson Seeto.
ACKNOWLEDGEMENTS

I would like to thank the Australian Centre for International Agricultural Research (ACIAR) who through their USP-ACIAR scholarship scheme enabled this Thesis project to be carried out and successfully completed.

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ABSTRACT

There is a rich history of pearl oysters being cultured that goes back some 2000 years in China. Over the course of history, many new techniques were attempted to perfect the culture of round pearls and it was not until 1916 when Kokichi Mikimoto, a Japanese aquaculturalist, successfully developed the technique. Since the advent of cultured pearls by Mr. Mikimoto, much progression has been made in pearl oyster farming which has included the rearing of larvae on site. *Pteria penguin*, the Mabe pearl oyster, is the main species used, as the name suggests, in the culture of the Mabe pearl and it is an important oyster species in the cultured pearl industry. The rearing of this species in the high-density flow through system is the main focus of this larval rearing study.

Larval rearing techniques include the static system, which has been the traditional system of rearing in many oyster farms; and the newly introduced and adapted, flow-through system. The traditional static system involves labor intensive, time-consuming procedures in the rearing of the *P. penguin* larvae. This system involves rearing in large tanks (up to 50 000 liters in volume) at low densities (< 10 larvae / ml), where the water is completely exchanged every 1 to 2 days. The flow-through systems, on the other hand, involve continuous water exchange (enabled by the fitting of banjo sieves), allows larval rearing densities to be increased (up to 100 larvae / mL) and tank sizes to be reduced (from 10 000 L to 250 L). Thus, it is better suited for regions that have problems with space requirements (tankage capacity for larval rearing) and high labor costs. However a major issue of concern for the flow-through system is the sieves could be clogged by the algal feed.

In this study, tests were carried out at the at the Tongan mariculture facility in Sopu, Nuku’alofa, to compare larval growth (as measured by shell length) between the larvae reared in the static systems and flow-through. Average shell length (± SE) on Day 13 for the Static System Tanks (ST) larvae was 113 (± 1.85) µm compared to 90.3 (±0.73) µm for Flow-through Tank (FT) larvae, t = 3.0591, df = 38, p value = 0.004056. The larval populations in both treatments saw a steady decline, with the ST showing the highest mortality on Day 2. Larval survival at day 12 was higher for the ST with a count of 131, 250 larvae / 150 L, while FT count 2 was 97,500 larvae / 150 L.
Furthermore, in this study, comparison tests on the performance of the sieve designs were carried for the Flow-through system tank at the School of Marine Studies (SMS) at University of the South Pacific, Suva, Fiji. Due to logistical constraints at the SMS aquaculture facilities, these tests were carried out using the rock oyster (*Saccostrea cucullata*). Larval growth comparisons for two different sieve designs were analyzed using a One–way Analysis of Variance using SPSS software version 13 (IBM Corporation, USA). It was found that on the third sampling day, the mean shell lengths using sieve design 1 (banjo sieve) were significantly greater than shell lengths using sieve design 2 (standpipe sieve), $F (d.f. 1,174) = 21.431, p < 0.001$. The average times until clogging for each of the four sieve sizes (32 µm, 50 µm, 75 µm, 120 µm) were compared using a One-Way ANOVA. It was observed that the larger mesh sieves clogged significantly more slowly than the small mesh sieves, (the largest mesh size of 120 µm did not clog within three days) $F (df = 3, 32) = 196.38, p < 0.001$. The results indicated that the standpipe sieve, also clogged significantly more slowly than the banjo sieve, $F (df = 1, 32) = 7.284, p = 0.011$ and that there was no significant interaction between the sieve mesh size and sieve design, $F (df = 3, 32) = 2.049, p = 0.127$. Finally, Constant clogging events were noticed in the banjo sieve design (treatment 1) compared to the standpipe sieves. Despite this high larval mortality was observed with the standpipe design (treatment 2 – 13,400 dead larvae). The inefficient water exchange method involved may have had a potentially deleterious effect on the larvae.

While there are recommended improvements regarding sieve design and overall larvae rearing, this study has shown the potential for the assimilation of flow-through systems into hatcheries around the small island countries where space and skilled labor are the main constraints faced. The use of flow-through technology may facilitate the establishment of pearl culture industries in the Pacific region.

**Keywords:**

Static system, Flow-through system, *Pteria penguin, Saccostrea cucullata*, clogging sieves, shell length, banjo sieve design, standpipe sieve design, survival, growth.
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CHAPTER ONE

INTRODUCTION

1.1 Aquaculture: global status and significance

Aquaculture is a rapidly expanding industry that has demonstrated substantial growth over the last 50 years, having an average annual growth rate of 8.3%, an annual production of 60 million tonnes of production (excluding aquatic plants and non-food products) valued at US$119 billion (FAO, 2010). It plays an important role in the global supply of food, providing approximately 64% of total world food fish supply in 2011 (Table 1.1), an increase from 50% in 2010 (FAO, 2012). As predicted by Prasad (2003) and Subasinghe and Hishamunda (2012), aquaculture production worldwide is expected to continue growing. Aquaculture production is continuing to grow to meet goals of food security for the world population, increased demand for fresh seafood products, aquatic animals for the aquarium trade, provide diversification of the primary industries, for the rehabilitation of over-exploited stocks and the production of, non-food items such as pearls from aquatic animals (Prasad, 2003). The expansion of aquaculture as a primary industry may provide low income food deficit countries (LIFDCs) with the means to address food supply, nutrition and livelihood problems while providing the means to alleviate poverty.

Fisheries and aquaculture provides an estimated 54.8 million people with livelihoods worldwide (FAO, 2012). The FAO (2012) reported a total of 190 countries raising aquatic organisms in captivity, with Asia accounting for more than 87% of the world total and Oceania with 0.3% (FAO, 2012). Growing employment rates in these two sectors have now exceeded that of agriculture activities, although there has been stagnation in the fisheries sector with the aquaculture sector continuing to expand (FAO, 2012). Data gathered from 1950-2008 relating to capture fisheries indicate a rapid exploitation of natural fish stocks and gradual decline in the catch rate per unit effort (Chiu Liao, 1996; Pillay, 1999; FAO, 2000, 2010). Numerous jobs are associated with aquaculture activities ranging from the primary production sectors to ancillary services such as processing, packaging, marketing and distribution, manufacturing of fish-processing equipment, net and gear making, ice production and supply, boat construction and maintenance, research and administration (FAO, 2012).
Table 1.1 World fisheries and aquaculture production and utilization. Source: FAO (2012).

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Notes: Excluding aquatic plants. Totals may not match due to rounding. Data for 2011 are provisional estimates.

The Oceania region contributes a small amount towards global aquaculture production, with much of its emphasis placed on marine molluscs (63.5%), and finfishes (31.9%) (FAO, 2012). Marine bivalves (including mussels, scallops and oysters) account for 65% of the region's total production, but there have been considerable developments in the finfish sector since the 1980’s (especially with Atlantic salmon in Australia and Chinook salmon in New Zealand) (FAO, 2012). The aquatic farming of marine bivalves in the Oceania region has considerable importance in the development of numerous small island states and thus highlights the need for further investment and scientific research to reach full production.
1.2 Molluscan aquaculture

Marine aquaculture accounts for about 29.2% of the world’s aquaculture production (FAO, 2012). Global marine aquaculture production has been dominated by marine molluscs (75.5%, 13.9 million tonnes, Fig. 1.1) (FAO, 2012). Although the share of molluscs (mostly bivalves e.g. oysters, mussels, clams, cockles, arkshells and scallops) has largely declined from the 1990’s (a turn down of 9.1%) to the present time, it is still the most widely practiced form of marine aquaculture (FAO, 2012). Molluscan aquaculture (63.5%) dominates aquaculture production in the Oceania region followed by finfish aquaculture (31.9 %) and crustacean aquaculture (3.7 %) (FAO, 2012).

![Figure 1.1 World aquaculture production compositions by culture environment (Marine Water)](image)

*Source: FAO (2012)*

The primary focus of mollusc aquaculture production in the Pacific Island Countries and Territories (PICT’s) is the farming of pearl oysters, which is an export commodity worth US$110 million per annum (Southgate, 2007). Sims and Sarver (1998) claimed that no other aquaculture industry presents the large potential for growth as the pearl industry does within the Oceania region. This is due to the lucrative markets that are already firmly established in Europe and private auctions in Japan, Hong Kong, United States, Myanmar (formerly Burma), and Tahiti. The dominance of a pearl producing island nation can be seen in Eastern Polynesia, with the production of round pearls from *Pinctada margaritifera* in the Tahitian islands (Kishore,
2010). Fiji, the Marshall Islands, Papua New Guinea, the Solomon Islands, Tonga, Kiribati and
the Federated States of Micronesia are still at various stages of commercialization of their pearl
culture efforts (Southgate, 2007). The success of pearl oyster culture in the South Pacific has
resulted in intensive research on the ecology and biology of tropical pearl oysters (Kimani and
Mavuti, 2002). Such research has also led to further ventures into pearl oyster culture, with the
introduction of blister or Mabé pearls produced from the winged pearl oyster, *Pteria penguin*
(Röding, 1798).

### 1.3 Aquaculture in the Pacific

The ‘Pacific’ is generally considered to be the region in the Pacific Ocean encompassing
Melanesia, Micronesia and Polynesia (Wright, 1993). The ocean is the main source of sustenance
for Pacific Island communities and seafood constitutes a large part of their diet, providing
valuable sources of protein and nutrients. In some islands, annual per capita consumption of
fisheries products can be as high as 250 kg (Wright, 1993). This may partially explain the slow
development of aquaculture within the Pacific, as there is still much dependence on capture
fisheries. The lack of trained personnel, appropriate technology and infrastructure has posed
problems for aquaculture development (Prasad, 2003). There have been attempts at new species
development (e.g. hatchery culture of sea cucumbers and *Trochus* at the Fiji Fisheries Division
Makogai hatchery facility), though only at a small scale and the commercialization potential of
these activities has yet to be determined (Prasad, 2003).
Table 1.2 Aquaculture production by region: quantity and age of world total production. *Source:* FAO (2012)

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<td>55,714,357</td>
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</table>

Notes: Data exclude aquatic plants and non-food products. Data for 2010 for some countries are provisional and subject to revisions. Production values for 1980 for Europe include the former Soviet Union.
The four main aquaculture practices in the Oceania region include pearl farming (using the blacklip pearl oyster *Pinctada margaritifera* and the winged pearl oyster *Pteria penguin*), tilapia farming, shrimp farming (*Penaeus* spp.) and seaweed culture (Adams et al., 2001). Though aquaculture has been practiced in PICT’s for some time now, many Pacific nations have no aquaculture tradition (Uwate and Kunatuba, 1984). The Food and Agriculture Organization (FAO) of the United Nations launched the South Pacific Aquaculture Development Project (SPADP) which looked to develop regional aquaculture (Tanaka H., 1997). Production levels of most farmed species grown in the South Pacific have been insignificant on a global scale (Prasad, 2003, Table 1.2) with only pearl oyster culture having a major influence in global markets (Sims, 1993; Fassler, 1995; Southgate, 1995).

Pearl oyster farming has existed in Fiji since 1966 (Gulick, 1990). With much technical and financial assistance being provided by the Australian Centre for International Agricultural Research (ACIAR) Pacific Island Pearl Oyster Resource Development project, FIS/1997/031 – ‘Pearl oyster resource development in the Western Pacific’ (Southgate, 1995, 1996b) . This project has demonstrated the potential for pearl oyster culture in this region (Prasad, 2003). There are a total of six established pearl farms in Fiji (Vilisoni, 2012), with the J. Hunter farm accounting for 96% of Fiji’s total pearl production with export earnings in excess of FJD $1 million per annum (Anonymous, 2007). Although pearl farming has demonstrated a promising future in this region, the continuous exploitation of wild stock may lead to very serious problems including decline in spat supply for grow-out and a potential decline in the genetic diversity of wild populations (Sims and Sarver, 1998). There is a need to have a ‘consistent supply’ of oyster juveniles or spat for the purpose of growing these animals and then implanting nuclei which will produce the cultured pearls upon harvest (Sims and Sarver, 1998). This constraint fundamentally requires research to optimize hatchery rearing techniques and spat collection to service the pearling industry (Vilisoni, 2012).

**1.4 Pearl Oyster farming in the Pacific**

**1.4.1 Cultured Pearls**

The three main forms of cultured pearls include composite or half pearls, non-nucleated pearls, and nucleated pearls (Taylor and Strack, 2008) Composite pearls, also referred to as Mabé pearls, are formed by the oyster’s ability to cover concentrations or protuberances on the inner
side of the shell with nacre (Southgate and Lucas, 2008). This is why they are sometimes called “blister pearls” and can be cultured from both *Pinctada* and *Pteria* species. Non–nucleated pearls also known as “Keshi” pearls are formed from the mantle sections being implanted from the donor oyster into the living mantle of the host oyster (Hänni, 2006). Southgate and Lucas (2008) state that marine production of keshi pearls is insignificant when compared to freshwater non–nucleated pearls, where a volume of over 1000 tonnes of pearls are produced annually and make up a vast majority of the global pearl production (Strack, 2006). Nucleated pearls are the most common form of cultured pearl production from marine oysters, and are generally produced in species belonging to the genus *Pinctada* (Southgate and Lucas, 2008). The technique involves the implantation of a nucleus with a section of mantle tissue removed from a donor oyster into the gonad of the host oyster (Southgate and Lucas, 2008). Pearls produced from this method include the South Sea and Akoya pearls.

### 1.4.2 Akoya Pearls

Pearls produced from the *Pinctada fucata*, *Pinctada martensii*, *Pinctada radiata* and *Pinctada imbricata* species are termed Akoya Pearls (Wada and Temkin, 2008) The culture of such pearls originated from Japan in 1916 and have since spread through Asia, India, The Americas, Australia, and the Arabian Gulf (Southgate and Lucas, 2008). Kokichi Mikimoto of Japan first developed the technique for cultured pearls using local Akoya pearl oysters, which had been known to produce pearls in that area for centuries (Southgate and Lucas, 2008). The development of the pearls was further improved by the Mise-Nishikawa method developed by Tokishi Nishikawa, a marine biologist, and Tatsuhei Mise, a Fisheries Inspector; which led to the regular production of cultured round pearls (Southgate *et al.*, 2008).

Japan and China are the leading countries in Akoya pearl production, although a large proportion of Akoya pearls being exported from Japan originate from China (Southgate and Lucas, 2008). This was not being made public to international markets until February 1992, when a Japanese company presented the first 10,000 strings on behalf of the “China Pearl, Diamond, Gem and Jewelry import and Export Corporation” (Southgate and Lucas, 2008, Fig 1.2).
India has also carried out research into various aspects of pearl oyster biology and culture, with commercial pearl farming being set up in 1983 (Chellam et al., 1991). Establishment of the practice on a large scale has yet to be made in India (Upare, 2001), being hampered by the quality of their pearls due to the ‘relatively’ thin shells of their native Akoya pearl oysters which restricts the size of the nuclei that can be implanted and results in much smaller pearl diameters (Victor et al., 2003; Mohamed et al., 2006). Vietnam has been known for its natural pearls for centuries and the production of cultured pearls has been on-going since the 1960s (Southgate and Lucas, 2008). The Atlantic Coast of South America, Australia, Korea and in the Arabian Gulf have also set up Akoya pearl oyster farms, although there has been limited pearl production from these areas (Southgate and Lucas, 2008).
1.4.3 South Sea Pearls

The “South Sea cultured pearl” as stated by Strack (2006) is the term used for pearls produced from marine pearl oysters south of Japan (Taylor and Strack, 2008). However, the name became synonymous with cultured pearls from both the Silver/Gold-lip Pearl Oyster *P. maxima* (Fig. 1.3) and the Black-lip Pearl Oyster *P. margaritifera* (Fig. 1.4) (Southgate and Lucas, 2008). Major South Sea pearl producing countries include Myanmar, Malaysia, Papua New Guinea, Philippines, Indonesia, and Australia (Henricus-Prematilleke, 2005).

**Figure 1.3** Keshi pearls from *P. maxima* (*Photo: David Jones*)
Indonesia and Australia at the moment are the leading countries in South Sea pearl production (Southgate and Lucas, 2008). “Black” South sea pearls produced from *P. margaritifera* oysters include overtones of purple, peacock, green and pinks, and are more highly prized than gray or brown appearing pearls (Southgate and Lucas, 2008)(Fig. 1.3, 1.5). Both the species are either cultured or harvested from their natural environment and are sold in assorted shapes and sizes (Southgate and Lucas, 2008). Shapes of the pearls include round, symmetrical and baroque and range from 7 – 20 mm averaging at 12 – 14 mm (Southgate and Lucas, 2008). The main markets for the “white” South Sea pearls are the American and European markets while the “black” South Sea pearls are sold to Japan and Hong Kong markets (Southgate and Lucas, 2008).
1.4.4 The Mabé Pearl

Composite cultured pearls commonly referred to as Mabé (Fig. 1.6) are formed by taking advantage of an oyster's ability to cover concentrations or protuberances on the inner side of the shell with nacre (Southgate and Lucas, 2008). In the commercial culture of Mabé pearls, a nucleus is adhered to the inner nacreous surface of the pearl oyster shell, over which the oyster covers layers of nacre to a thickness of about 1 mm (Southgate and Lucas, 2008).

Mabé pearls have been known to be the earliest form of cultured pearls for which the technology was developed in China over 2000 years ago (Dan, 2003). The traditional species used for Mabé pearl production is the Winged Pearl Oyster, *P. penguin* (Röding, 1798) (Southgate and Lucas, 2008). The number of Mabé that can be cultured from an individual oyster depends on the species used, however both *Pteria* species (*P. penguin* and *Pteria sterna*) may accommodate up to three Mabé pearl nuclei (Sauceño et al., 1998; Ruiz-Rubio et al., 2006).

The pearls are usually left for a period of 6 – 12 months to develop a nacre thickness of 0.7 – 2.5 mm (Shirai, 1981; McLaurin et al., 1997). Once the pearls have reached the appropriate
thickness, they are then removed using a diamond tipped hole-saw resulting in the death of the oyster (Fig. 1.6) (Southgate and Lucas, 2008).

Figure 1.6 Processed Mabé pearls produced from *Pteria sterna* (McLaurin *et al*., 1999) Source: Southgate and Lucas 2008, pg. 283.

Despite the economic importance of Mabé pearls, production declined significantly during the late 1980s, due to the rapid expansion of Chinese freshwater pearls and round marine pearls (Müller, 1997). However, developing nations still take an interest in its production because the production process is far simpler and less costly than for round pearl production (Ruiz-Rubio *et al*., 2006). Mabé pearl production also offers much needed opportunities for income generation in coastal communities (Southgate *et al*., 2006).

1.5 An overview of the biology of *Pteria penguin*

1.5.1 General Introduction
There are several oyster species that produce pearls and these belong to the genera *Pteria* and *Pinctada* within the Family Pteriidae (Southgate *et al*., 2008). Most of the pearls produced from the *Pteria* spp. include Mabé pearls, and only recently have round pearls been produced from these animals (*P. sterna*) (Kiefert *et al*., 2004) The *Pinctada* spp. are able to produce a wide range of assorted pearls from near spherical to ‘tear-drop’ shapes and to the Japanese made Keshi pearls (Figs. 1.3 and 1.4). *Pteria* and *Pinctada* spp. can produce Mabé pearls but the number of nuclei placed within the shell varies. Usually *Pteria* spp. can accommodate a total of three Mabé pearl nuclei (Saucedo *et al*., 1998; Ruiz-Rubio *et al*., 2006), *P. margaritifera* can hold 4 – 5 half pearl nuclei (Southgate *et al*., 2006) and *P. maxima* can have up to seven nuclei (Southgate and Lucas, 2008). However, the traditional species used for Mabé pearl culture is the winged pearl oyster, *Pteria penguin* (Southgate and Lucas, 2008), although not as ‘valuable’ as other pearls (nucleated and non-nucleated forms) produced by *Pinctada* spp.

The Japanese refer to mabepearls as “gai”, which is an old Japanese dialect spoken in the Ryukyu Islands where the oysters which produce these pearls are farmed (Southgate and Lucas, 2008). Pearls produced by *Pteria* spp. were basically half–shaped pearls because of the morphological characteristics of the oysters, which restricted the pearl shape. It was not until 1995 when the successful culture of round–shaped pearls were produced from this genus, specifically *Pteria sterna* at the Instituto Tecnologico y de Estudios Superiores de Monterrey (ITESM) in Guaymas, Mexico (Kiefert *et al*., 2004). Although the sizes of the round pearls are much smaller than those produced by *Pinctada* spp., it still adds to a wide diversity of pearls. The production of Mabé pearls in the South Pacific Islands was started in the Kingdom of Tonga in 1975 by the Tasaki Pearl Company of Japan (Malimali, 1995; Finau, 2005). It was estimated that Tonga could support an annual capacity of about 750,000 pearls which would provide an annual revenue of ca. US$ 7.5 million (Finau, 2005). Fiji was also another candidate for Mabé pearl production, but there are no records of its success (Chand *et al*., in press) The species distribution, biology and other environmental parameters are vital in ensuring the success of a potential species for aquaculture, which in this case is the winged pearl oyster *P. penguin*.

### 1.5.2 Distribution

Most species of Pteriidae are tropical and subtropical, but the distributions of some species extend to higher latitudes (Southgate and Lucas, 2008). The distribution of *Pteria penguin* lies
within the tropical zone (23 degrees North and South of the equator), extending from eastern Africa and the Arabian Gulf, covering the entire zone of the Indian Ocean into the Asia-Pacific region and encompassing part of Oceania (Fig. 1.7). Geographic locations included in the distribution are the Red Sea and the Arabian Gulf, the tropical eastern Indo-Pacific and Southern Japan (Okinawa to Honshu), Southeast Asia, the Philippines, Queensland and northwest Australia, Thursday Island, Southern China and Taiwan (Prashad, 1932; Allan, 1959; Habe, 1977, 1981; Reid and Brand, 1985; Springsteen and Leobrera, 1986; Oliver, 1992; Bernard et al., 1993; Shirai, 1994; Burch, 1995; Lamprell and Healy, 1998; Higo et al., 1999; Hayami, 2000; Swennen et al., 2001; Hwang and Okutani, 2003).

![Figure 1.7 Approximate distributions of the winged pearl oysters, P. penguin (light-gray) and P. sterna (dark gray). Source: (Wada and Temkin, 2008)](image)

Southgate and Lucas (2008) have reported that species distributions of the genus *Pteria* are concentrated within the Indo-Pacific region, with 20 species being described from there, and with only four species being found within the Atlantic Coast of America.

### 1.5.3 Morphology
Morphology

Species belonging to the genus *Pteria* have a characteristic shell shape that places them apart from other species in the same family. The elongated hinge-line extending posteriorly from the dorsal end of the shell gives a ‘winged’ appearance, and is its distinctive feature and thus the animals are often called ‘winged–oysters’ (Lamprell and Healy, 1998).

The hinge of the shell is elongated, often being longer than the shell height in younger individuals, and forms an acute angle with the longest axis of the shell that extends backwards to meet the hinge line from the umbonal region (Virabhadra, 1960). The outer shell layer is composed of calcitic layers that typically abrade with age and a comarginal periostracal layer that is often colored black (Virabhadra, 1960; Southgate and Lucas, 2008; Wada and Temkin, 2008). The left valve is concave and deeper than the right valve, which fits over it like a lid (Virabhadra, 1960). The valves are fitted together at the hinge by an anterior sub-umbonal tooth in the left valve and a posterior sub-marginal oblique ridge in the right valve, with complementary sockets in the respective opposite valves (Wada and Temkin, 2008). The abductor muscle is oval in shape and is attached in the mid-postero-dorsal region of each of the shell halves (Virabhadra, 1960). The byssal threads are secreted by the byssal gland located ventrally to the short foot (Fougerouse *et al*., 2008).

The mantle is a film of tissue covering the visceral mass of the oyster and is divided into two layers (Southgate and Lucas, 2008). Separated anteriorly, ventrally, and posteriorly; the main function of the mantle lobes is to secrete shell valves and ensure their growth (Southgate and Lucas, 2008). The mantle on the dorsal side of the oyster is fused to the visceral mass and the abductor muscle along the hinge to form the isthmus (Fougerouse *et al*., 2008). Pigmentation is also present in this region and is often used to describe the species, for example the Black-lipped pearl oyster, *P. margaritifera* (Salmon and Roudnitska, 1986). There are four zones in the mantle region as shown below (Fig. 1.8). These include the isthmus, the central area, the distal area and the marginal area.
1.5.4 Nomenclature, classification and taxonomy

The genus *Pteria* Scopoli 1777, to which *P. penguin* belongs, is commonly known as the group of ‘winged-pearl oysters’. This relates to the elongated hinge that resembles a wing (Southgate and Lucas, 2008), and is a characteristic feature for the genus. There have been extensive synonymies compiled by Fischer-Piette (1982) on *Pteria* spp., although much needed revisions have not been made since then (Southgate and Lucas, 2008). The current taxonomic hierarchy for *P. penguin* is shown below in Figure 1.9.
The genus *Pteria* are defined by a number of morphological features including shell shape, pattern of the hinge teeth and shape of the posterior adductor muscle scars (Fougerouse et al., 2008) Anatomical features include the pattern of intestinal coiling and the relationship of the intestine and ventricle (Hayes, 1972; Mikkelsen et al., 2004). Extensive descriptions on morphological features have been provided in reports by Wada and Temkin (2008), Temkin (2006a), and Temkin (2004). There have however been many discussions on whether or not the Pteriidae family makes up a polyphyletic group from the analysis of 18S rDNA genetic data, but the relationships among the pteriod genera still remain uncertain (Wada and Temkin, 2008). Descriptions of morphological and anatomical features for the genus *Pteria* can be found in Southgate and Lucas (2008), Temkin (2006b), Hertlein and Cox (1969); Hayes (1972); Habe (1977); Oliver (1992); Lamprell and Healy (1998); Mikkelsen et al. (2004).

### 1.5.5 Habitat and Environmental Parameters

*P. penguin* occupies reef habitats within its distribution range. Larval settlements are often influenced by cues (Hayes, 1972) that indicate a suitable habitat. Such cues include;

1) Association with conspecifics. The presence of conspecifics or a different species for that matter would indicate habitat quality or an enhanced survival, as seen with pre-settling fish larvae that undergo settlement pressures (Levin, 1993; Booth, 1995).
2) The presence of suitable substrate to settle on. Features that promote larval settlement include a flat surface (Rose and Baker, 1994), a deep red color (Su et al., 2007) and a cryptic structure with shaded areas (Alagarswami et al., 1983a). The presence of other features unique to certain oyster species may also play a role in influencing settlement, such as the bright color of a gorgonian species being the preferred natural substrate for P. penguin larvae (Alagarswami et al., 1983b; Beer and Southgate, 2000).

3) The presence of microbial biofilms on the substrate surface. The surface of bio-films consists of bacteria, microalgae and detritus (Besley et al., 1998; Bao et al., 2007). The release of water soluble peptides from the bio-film attracts larvae towards the substrate (Zimmer-Faust and Tamburri, 1994), which is important in allowing initial settlement to occur.

Environmental parameters such as temperature, depth, salinity, substrate and silt load, current and pollution sources within the area are also important for a winged oyster population to thrive in.

**Temperature**

Optimal temperature ranges for most oyster species lies within 20 °C – 30 °C (Gervis and Sims, 1992). This can be seen in the distribution of the species, which fall within the tropical zones of the northern and southern hemispheres. Farmers choose to harvest the oysters in colder months as the luster and quality in pearls becomes more prominent due to the thin layer of nacre formed (Gervis and Sims, 1992). Settling in areas with optimal temperatures is very important as this also influences gonad development and spawning seasonality of the oysters (Lucas, 2008b).

**Depth**

Depths of settlement for most oyster species vary depending upon location. P. maxima has a depth range of 0 – 80 m (Gervis and Sims, 1992), P. margaritifera has a depth range of 0 – 40 m (Hynd, 1955; Intes, 1982; Intes and Coeroli, 1986; Intes et al., 1986; Sims, 1990)) and P. fucata a depth range of 0 – 30 m (Gervis and Sims, 1992). Depth has an effect on the growth of the oysters. Observations reported on P. maxima specimens taken from 73 to 82 m were seen to be of “smaller size” as compared to those taken from shallower depths (George, 1978). Such an observation could be explained by lower temperatures and a decrease in phytoplankton feed
availability (Gervis and Sims, 1992). Pearl quality and color is also affected by depth which is seen in *Pinctada fucata martensii* that produce high quality pinkish pearls below a depth of 5 m (Kafuku and Ikenoue, 1983).

**Salinity**

Oceanic salinity (32 – 35 ‰) is preferred by most pearl oysters (Gervis and Sims, 1992). Test salinities on Akoya oysters (*Pinctada fucata, martensii, radiata* and *imbricata* species complex), *P. margaritifera*, and *Pinctada maxima* oysters (larval, spat and adult forms) have shown the lower and limits of their salinity tolerance levels ranged from 18 – 45 ‰ for spat and adults (Lucas, 2008a) Adult salinity tolerance levels for the Akoya oyster vary with distribution. Akoya pearl oysters in Japan have been shown to tolerate a range of 18 – 30 ‰ (Katada, 1959), while the same species in India have a range of 17 – 45 ‰ (Alagarswami and Victor, 1976). Salinity stresses include stunted growth in individuals (Jeyabaskarn et al., 1983) and reduced heart rate (Numaguchi and Tanaka, 1986). This leaves the oyster vulnerable and prone to infection (Gervis and Sims, 1992), although such stresses cannot be entirely caused by extreme salinity conditions. Jeyabaskarn et al. (1983) noted that at times of high salinity, fouling increased and temperature was very often observed to be in the lower levels of oyster tolerance (< 20 °C).

**Turbidity**

Turbidity is measured by the density of suspended particulate matter (SPM) and affects filtering and pre-ingestive sorting processes in pearl oysters (Southgate and Lucas, 2008). For this reason, most oyster farms are located in areas with good water clarity and current. Being naturally an inter-tidal organism, oysters have a certain level of tolerance for turbid waters (Southgate and Lucas, 2008). Pearl oysters have been known to be non-specific feeders but if there is a high silt load in the water, feeding will be affected (Gervis and Sims, 1992). A study conducted on nucleated cultured Akoya oysters by Kripa et al. (2007) found that oyster mortalities were directly correlated with turbidity among the various other parameters measured in the study. For *P. penguin*, however, the juveniles have been found to thrive in areas with high levels of silt deposits. Milione and Southgate (2011) observed that juvenile growth parameters for the *P. penguin* oyster (dorsoventral measurement (DVM), anteroposterior measurement (APM), shell thickness and whole weight) increased significantly under high-turbidity conditions (*P* < 0.05). A
similar study by Milione and Southgate (2012) on *P. penguin* growth rates at three dissimilar sites in northeastern Australia also found that the oysters were able to tolerate and even thrive under a wide range of turbidity levels.

**Current**

Currents are very important in bringing food and oxygen to pearl oysters and carrying away their wastes (Gervis and Sims, 1992; Southgate and Lucas, 2008). The pearl oyster *Pinctada maxima* has been known to grow very well in areas of strong currents (Saville-Kent, 1890; Gervis and Sims, 1992), though poorer quality pearls are produced due to rapid nacre formation (Kafuku and Ikenoue, 1983). Recent studies have shown that strong currents may be deleterious to pearl oysters by increasing suspended inorganic matter, and interfering with filtering and pre-ingestive processes (Lucas, 2008a).

**Pollution**

Sources of pollution that affect pearl farms often include oil spills, fisheries activities, sewage, and chemical discharges (Lucas, 2008a). Studies show that pearl oysters are exceptional accumulators of heavy metals, so much so that levels measured are far above safe human consumption levels (Jacob et al., 1980; Shiber; Klumpp and Burdon-Jones, 1982; Ikuta, 1986a, 1986b). The 1975 Florida Keys oil spills caused mass mortalities of farmed and unfished Akoya oysters. This was attributed to the toxic water soluble component of the oil (Chan and Monney, 1977).

Oyster farms themselves can have a detrimental effect on oysters. Accumulation of organic substances from farmed oysters suspended in the water column resulted in the decline in productivity of oysters in Japanese farms (Ohwada and Uemoto, 1985). Reductions in dissolved oxygen and the formation of hydrogen sulphide are some of the conditions created by the organic buildup of bio-fouling organisms removed or cleaned-off the oysters themselves. Often left to accumulate at the bottom of farm site, if not ‘flushed’ out naturally by water currents, the decomposition would lead to the deteriorating water conditions (Hollyer, 1984; Ohwada and Uemoto, 1985).
1.6 Hatchery culture methods of Mabé producing pearl oysters (*Pteria penguin*)

The culture of *Pteria Penguin* larvae uses similar methods as designed for other pearl oyster species and have been proven to produce good results (Wassing and Southgate, 2011). *Pteria penguin* is widely cultured in Japan, Australia, the Phillipines, Indonesia, China, Thailand, Vietnam, and The Kingdom of Tonga (Southgate and Lucas, 2008). Despite this however, little research has been contributed towards its development as a major half-pearl producing species (Southgate, 2008)

**Spawning**

Spawning methods have varied little for all pearl oyster species through time with the same basic means of spawning inducement (thermal) being used consistently (Tanaka Y. *et al.*, 1970; Mizumoto, 1979; Alagarswami *et al.*, 1983a; Alagarswami *et al.*, 1983b; Alagarswami *et al.*, 1987; Gervis and Sims, 1992; Rose and Baker, 1994; Southgate and Beer, 1997; Choi and Chang, 2003; Southgate and Lucas, 2008). Thermal inductions have been used to trigger spawning in mature broodstock. Natural spawning is usually initiated by the male oysters with the sperm suspension stimulating the females to release their eggs (Alagarswami *et al.*, 1983b), however reports have observed that both stress and thermal induction are proven inducers of successful spawning (Tranter, 1958; Wada, 1976; Rose, 1990; Rose *et al.*, 1990). Temperature ‘shocks’ vary between species, with *Pinctada margaritifera* and *Pteria penguin* spawning at 5 – 6 °C above ambient seawater temperature (Gervis and Sims, 1992), and *Pinctada maxima* spawning at 2 – 5 °C above ambient water temperature with a maximum of 33 – 34 °C (Rose, 1990; Rose *et al.*, 1990) although temperature tolerations change with geographical locations.

Other means of spawning also include chemical stimulation (Southgate and Lucas, 2008). Since male oysters usually spawn first, it is a practice of pearl oyster hatcheries to simulate this by introducing a suspension of sperm obtained from a sacrificed male into the spawning tank (Southgate, 2008). Another chemical approach involves changing the seawater composition (Southgate and Lucas, 2008). *P. maxima* showed a spawning response when exposed to a weak
solution of hydrogen peroxide (0.006 %), following the adjustment of the holding water to pH 9 using ammonium hydroxide (NH₄OH), sodium hydroxide or tris buffer (Rose, 1990; Rose et al., 1990). Chellam et al. (1991) reported that a direct injection of 0.2 mL of 0.1 N NH₄OH solutions into the adductor muscle of the oysters stimulated a high (48 %) spawning response.

*Larval rearing*

Larval rearing methods for *P. penguin* are similar to those used for *P. maxima* and *P. margaritifera*, Table 1.3). Good feed quality and quantity, clean water and low larval densities are basically the same guidelines used to maintain rearing conditions for most bivalve larvae (Gervis and Sims, 1992).
**Table 1.3** Summary of larval rearing protocols for two cultured *Pinctada* species

*Source:* (Gervis and Sims, 1992; Rose and Baker, 1994; Southgate and Beer, 1997; Southgate, 2008)

<table>
<thead>
<tr>
<th></th>
<th><em>Pinctada maxima</em></th>
<th><em>Pinctada margaritifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal food tested (larvae to settlement)</td>
<td><em>Chaetoceros calcitrans, Chaetoceros gracilis, Tahitian Isochrysis galbana, Tetraselmis sp.</em>, <em>Nannochloris</em> (optional)</td>
<td><em>Tahitian Isochrysis galbana, Pavlova lutheri, Rhodomonas clavis</em></td>
</tr>
<tr>
<td>Algal food to post-settlement</td>
<td><em>Chaetoceros gracilis, Tahitian Isochrysis galbana, Tetraselmis sp.</em>, <em>Pavlova lutheri,</em></td>
<td><em>Tahitian Isochrysis galbana, Skelotonema sp., Nitzschia sp.</em></td>
</tr>
<tr>
<td>Algal density</td>
<td>100 – 25000 (cells/ind./day)</td>
<td>5 – 10 µl⁻¹</td>
</tr>
<tr>
<td>Day</td>
<td>1 – 30</td>
<td>1 – 28</td>
</tr>
<tr>
<td>Stocking density larvae per mL</td>
<td>10 – 1</td>
<td>1 – (unknown)</td>
</tr>
<tr>
<td>Filtration</td>
<td>5 or 1 µm</td>
<td>Sand filter and cotton wool</td>
</tr>
<tr>
<td>Water change</td>
<td>Daily</td>
<td>Daily</td>
</tr>
<tr>
<td>Survival rate</td>
<td>.0004 to .01% to days 15 - 23</td>
<td>6.3 % at day 28</td>
</tr>
</tbody>
</table>
There are a number of marine microalgal species used in the culture of pearl oysters, and these are usually fed in mixtures to ensure that a balanced supply of nutrients is made available for optimum growth (Webb and Chu, 1982; Gervis and Sims, 1992; Southgate, 2008). The three common genera used in the culture of pearl oysters are *Isochrysis*, *Pavlova* and *Chaetoceros*, although a variety of other marine species have also been tested (Southgate, 2008). The nutritional values of various marine algal species have been tested with the different commercial pearl oyster species, however this research has not been carried out with *P. penguin* to date.

*P. margaritifera* as observed by Martinez-Fernandez *et al.* (2006) showed good signs of growth in the D–stage and umbone larvae with the genus *Pavlova* (*Pavlova salina* and *Pavlova* sp.). Martinez-Fernandez *et al.* (2006) also noted that larval growth rate correlated well with carbohydrate, lipid, protein and Highly Unsaturated Fatty Acid (HUFA) content of the micro-algae fed. The diatom *Chaetoceros muelleri* was also recommended for *P. margaritifera* umbone larvae (Martinez-Fernandez and Southgate, 2007), although *P. salina* clearance rates and ingestion rates were five times higher than those for *Chaetoceros* spp. (Doroudi *et al*., 2003).

Minaur (1969) suggests that *P. maxima* larvae be fed with tropical micro-algae such as *Isochrysis galbana* and *Monochrysis lutheri*. *Chaetoceros calcitrans* and *Chaetoceros gracilis* were also considered to be nutritious (Rose, 1990), although *C. gracilis* was found to impede ingestion in small larvae (Rose and Baker, 1994). *Pteria sterna* (Gould, 1851) was fed with *Nannochloris* sp., *Tahitian Isochrysis galbana* (T–ISO) and *Pavlova lutheri* which were all ingested, however only T–ISO and *P. lutheri* were digested (Martinez-Fernandez *et al*., 2004). The brown alga *P. lutheri* has been questioned as a suitable food source for pearl oyster larvae, due to its potential change in physiology and morbidity rate when removed from its growing medium at 20°C and put into larval culture tanks at 28 – 30 °C (Tanaka Y. *et al*., 1970).

The ideal marine algal species for *P. penguin* larvae would thus have to be those that are highly nutritious and easily digestible. Algal species tested on the closest relative of *P. penguin*, *Pteria sterna*, show that the golden/brown algae species *Isochrysis* sp. and *P. lutheri* were the only algae to be successfully assimilated by the larvae (Martinez-Fernandez *et al*., 2004).
**Feeding density**

Ensuring that food is made available to all larvae in the larval rearing tanks is a priority during hatchery runs. This is to support high growth rates and the health of the larvae. Appropriate rations are also important in maintaining water quality (Southgate and Lucas, 2008). The densities fed are different for each of the commercial species.

**Table 1.4** Feeding schedule (cells/mL/day) used for larvae and early post larvae of some pearl oyster species. Values in parentheses are mean antero-posterior shell sizes of the larvae. *Source:* (Southgate, 2008).

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>P. maxima</th>
<th>P. margaritifera</th>
<th>P. jucata</th>
<th>P. mazatlanica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,000 (80)</td>
<td>1,000 (82)</td>
<td>5,000 (67.5)</td>
<td>10,000</td>
</tr>
<tr>
<td>2</td>
<td>6,000</td>
<td>1,000</td>
<td>5,000</td>
<td>10,000 (80)</td>
</tr>
<tr>
<td>3</td>
<td>7,000</td>
<td>1,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>4</td>
<td>8,000</td>
<td>1,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>5</td>
<td>10,000 (98)</td>
<td>2,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>6</td>
<td>12,000</td>
<td>2,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>7</td>
<td>14,000</td>
<td>2,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>8</td>
<td>18,000</td>
<td>4,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>9</td>
<td>20,000</td>
<td>8,000</td>
<td>5,000</td>
<td>10,000</td>
</tr>
<tr>
<td>10</td>
<td>25,000 (115)</td>
<td>8,000</td>
<td>5,000 (135)</td>
<td>10,000</td>
</tr>
<tr>
<td>11</td>
<td>28,000</td>
<td>8,000 (138)</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>12</td>
<td>30,000</td>
<td>8,000</td>
<td>10,000</td>
<td>10,000 (139)</td>
</tr>
<tr>
<td>13</td>
<td>32,000</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>14</td>
<td>34,000</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>15</td>
<td>38,000 (144)</td>
<td>12,000</td>
<td>10,000 (180)</td>
<td>10,000</td>
</tr>
<tr>
<td>16</td>
<td>40,000</td>
<td>12,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>17</td>
<td>42,000</td>
<td>12,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>18</td>
<td>44,000 (165)</td>
<td>12,000</td>
<td>10,000 (200)</td>
<td>10,000</td>
</tr>
<tr>
<td>19</td>
<td>48,000</td>
<td>12,000</td>
<td>15,000</td>
<td>15,000</td>
</tr>
<tr>
<td>20</td>
<td>50,000 (185)</td>
<td>10,000 (214)</td>
<td>15,000 (220)</td>
<td>15,000 (193)</td>
</tr>
<tr>
<td>21</td>
<td>50,000</td>
<td>10,000</td>
<td>50,000</td>
<td>15,000</td>
</tr>
<tr>
<td>22</td>
<td>50,000</td>
<td>10,000</td>
<td>50,000</td>
<td>15,000</td>
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<tr>
<td>23</td>
<td>50,000</td>
<td>10,000</td>
<td>50,000</td>
<td>15,000</td>
</tr>
<tr>
<td>24</td>
<td>50,000</td>
<td>10,000</td>
<td>50,000 (300)</td>
<td>15,000 (223)</td>
</tr>
<tr>
<td>25</td>
<td>50,000 (237)</td>
<td>10,000</td>
<td>50,000</td>
<td>15,000</td>
</tr>
<tr>
<td>26</td>
<td>50,000</td>
<td>12,000</td>
<td>50,000</td>
<td>15,000</td>
</tr>
<tr>
<td>27</td>
<td>50,000</td>
<td>12,000</td>
<td>50,000</td>
<td>30,000</td>
</tr>
<tr>
<td>28</td>
<td>50,000</td>
<td>18,000</td>
<td>50,000</td>
<td>30,000</td>
</tr>
<tr>
<td>29</td>
<td>50,000</td>
<td>18,000</td>
<td>50,000</td>
<td>30,000</td>
</tr>
<tr>
<td>30</td>
<td>50,000 (281)</td>
<td>25,000</td>
<td>50,000</td>
<td>30,000</td>
</tr>
</tbody>
</table>


*a50,000 cells/day/ spat.

*a Diet composed of Isochrysis (T – ISO), Chaetoceros gracilis and Nanochloropsis oculata (~ 5–10%).
b Diet composed of *Isochrysis* (T – ISO), *Pavlova salina* and *Chaetoceros simplex*.

c Diet composed of *I. galbana* only to 15 days after settlement when *Chaetoceros* is added to the diet.

d Diet composed of 1:1 mixture of *Isochrysis* (T – ISO) and *Pavlova lutheri*.

The findings of Doroudi *et al.* (1999) state that optimal algal rations vary according to larval age, with higher algal densities yielding optimal growth rates, though within tolerable limits of the rearing system. There is also a close relationship between algal density and larval density (Doroudi *et al.*, 1999). Doroudi and Southgate (2000) suggest that *P. margaritifera* larvae up to 8 days of age should be fed an algal ration of 8,000 cells/mL in an initial larval density of 3 larvae/mL, while 13 – 20 day old larvae be fed an algal ration of 25,000 cells/mL and with a larval density of < 2 larvae/mL (Table 1.4).

While there may be slight differences in the way the larvae of different pearl oyster species are reared, the method is essentially the same; from broodstock spawning to spat settlement. The culture systems used in pearl oyster hatcheries vary only by the operator’s preference and the suitability of the system to the hatchery setup and the area being 'farmed' on.

1.7 Pearl oyster culture systems

The traditional culture system used by many pearl oyster hatcheries is the static system. This system involves the rearing of larvae in large tanks (up to 50,000 litres in volume) at low densities (< 10 larvae/mL) (Sarkis, 2011a), where the water is completely exchanged every 1 to 2 days (Sims, 1996). This method involves labor intensive, time consuming procedures with consistent handling of the larvae (Sims (1996); Southgate and Ito (1998); Sarkis *et al.* (2006); Rico-Villa *et al.* (2008); Braley (1992); Andersen *et al.* (2000)). Much research has been carried out towards simpler methods with minimal human interaction, yet ensuring a steady supply of spat for pearl production (Sims, 1996).

Flow-through systems have been used in the culture of giant clams, scallops, and oysters. They have also been used for marine finfish (Shields, 2001) and lobster (Nicosia and Lavalli, 1999) larval rearing. Flow-through systems are better suited for regions that have problems with space requirements (tankage capacity for larval rearing) and high labor costs (Sarkis *et al.*, 2006). As stated by Southgate and Ito (1998), the development of simplified hatchery techniques (“Flow-
through system”) is more appropriate for Pacific nations, which in turn would facilitate the establishment of pearl culture industries around the region.

Flow-through systems involve the usage of 150 - 200 liter conical tanks and have a constant flow of seawater through the system (Sarkis (2011b); Rico-Villa et al.. (2008). In doing so, the density of larvae being reared can be increased to as much as 100 larvae/mL (Rico-Villa et al., 2008), without worrying about bacterial or protozoan proliferation (Sims, 1996). Since there is a constant exchange of seawater, algae have to be fed into the system continuously. This form of feeding is more effective than batch feeding because the suspended algae in the water column cause the larvae to actively seek its food keeping them away from the bacterially loaded tank surfaces (Whitford, 2005b).

The preference of flow-through systems over static systems is yet to be fully established, with much research still needed in areas dealing with flow-rate, feeding rate and larval stocking density (Sims, 1996). Some hatcheries prefer the use of both systems, with the static system used to rear D-stage larvae and a partial flow-through system to rear the pediveliger larvae during their settlement phase (Whitford, 2005a).

1.7.1 Flow-through System Designs

1) Partial-flow through systems

The partial-flow through system has been used in studies on rearing the pearl oyster larvae, *P. margaritifera* (Southgate and Ito, 1998). The design included a central standpipe, from which a replaceable nylon mesh cone and float were attached (see Figure 1.10). The large surface area of the mesh cone along with the flexible aeration tubing around the base of the cone reduced suction pressure and the likelihood of the larvae becoming entrapped (Southgate and Ito, 1998).
Figure 1.10 Partial flow-through system used to culture pearl oyster larvae showing A) Floatation device B) Nylon mesh covering C) Flexible aeration tubing. FSW, 1 µm filtered sea water. Source: Southgate and Ito (1998).

The workings of this system involve the exchange of water over a specified period of time, in this case a 12 hour “on-off” period (Southgate and Ito, 1998). With a tank volume of 500 liters, the flow rate of water to get a 100% water exchange was 50 L / hour. However, as in static systems, a complete drainage and re-fill of the tanks is needed due to the build-up of debris on the tank bottom, and Southgate and Ito (1998) stated that this was done on days 7, 14 and 21 of culture.
In comparison with static systems on larval survival and growth for the same species, Southgate and Ito (1998) reported 5% survival (compared to 6.3% for a study by Alagarswami et al. (1989)), reaching pediveliger stage on day 20 of culture which was also seen by Kakazu (1988) and Alagarswami et al. (1989). Although the results obtained were comparable, the partial-flow through system is preferred because of the reduced manual labor involved (with resulting reduced hatchery operational costs), better water quality and reduced interaction with the larvae (Southgate and Ito, 1998).

2) *Recirculation aquaculture systems*

The recirculation aquaculture system (RAS) is not termed an “open” or “flow through” system, but neither is it a completely “closed” system. This is because, to a certain degree, water is lost to the surroundings through evaporation and periodical water exchange (Helfrich and Libey, 1991). The RAS has been stated by Helfrich and Libey (1991) to be a suitable system for rearing a certain number of species including oysters and clams. However, this system is better suited for rearing fish indoors at high densities.

The functional parts of the RAS design include 1) growing tank, 2) sump to house a particulate removal device, 3) biofilter, 4) oxygen injection with U-tube aeration and 5) water circulation pump (Helfrich and Libey, 1991) (Fig. 1.11). This system is useful for farmers that face a limited supply of water and land (Helfrich and Libey, 1991). However, since pearl oyster hatcheries are typically located close to the sea, it would also be useful to hatcheries that experience large variations in tidal level throughout the day. It is known that static systems are commonly used for pearl oyster larval rearing, and over the course of the larval lifecycle, bacteria and protozoan proliferation can be a problem for this system. With the RAS, water is continuously flowing and being filtered (though certain modifications may be in place to suit oyster rearing), and in turn bacteria and protozoans are kept in check and the handling of larvae is kept to a minimum (despite it occurring when static tanks need to be drained).

The operation of the RAS requires constant supervision and skilled technical support. This is to maintain and manage the circulation, aeration and bio-filter systems along with constantly performing water quality analyses (Helfrich and Libey, 1991). The systems are also relatively expensive to develop and because they rely so heavily on the running of essential components
such as pumps, aeration, heating, and lighting, if mechanical or electrical failures occur, the success of the hatchery run is at stake (Helfrich and Libey, 1991). This could be a possible system to trial on pearl oyster larvae but its suitability in PICTs is still to be determined.

Figure 1.11 Recirculating culture system showing the primary and secondary filtration systems. Source: Serfling et al. (1974).

3) Continuous flow-through systems

Continuous flow-through systems were developed by the late John Bayne in the 1980’s for the Pacific Oyster, *Crassostrea gigas* (Whitford, 2005a). It has been tested in the commercial production of *P. maxima* oysters and has proven to yield good results (Whitford, 2005a).

The success of the system lies with the continuous flow-through of water in and out of the rearing tanks. Continuous water exchange allows larval rearing densities to be increased (up to 100 larvae/mL) and tank sizes to be reduced (from 10 000 L to 250 L). This permits time and labor spent on each tank to be shortened and made easier to manage, as draining and refilling can take up to 3 hours in static tanks compared to 20 minutes for the flow-through tanks. It thus reduces the time for the larvae spent outside the tanks during such procedures. Bacteria and protozoan contamination is controlled with fresh seawater continuously being supplied and the
larvae are suspended in the water column by strong circulatory effects resulting in higher survival (Creswell (2011); Reiner (2011); Rico-Villa et al. (2008); Southgate and Lucas (2008); Sarkis et al. (2006); Whitford (2005a); Andersen et al. (2000); Southgate and Ito (1998); Perry et al. (1997); Southgate and Beer (1997); Southgate (1996a); Sims (1996); Braley (1992)).

There are many design types for this system, each depending on the production capability of the hatcheries and the organism involved. Scallop, giant clam and pearl oyster hatcheries basically use the same design in terms of the tanks used for larval rearing and the overall system set-up. The tanks involved (Fig. 1.12), depending on the size of the operation and the available building space, range from 200 L – 4500 L in volume and are typically conical in shape. The water inlet flow is usually from a hose suspended in the tank or positioned at the bottom where it is joined with the aeration pipe. Water outlet valves are situated close to the top end of the tank to which the ‘banjo’ sieves are attached. The banjo sieves enable water to flow through the tank and at the same time, ensure that the larvae are retained in the tank. Banjo sieves are available in different mesh sizes and are used at different growth stages of larvae during the hatchery run. Basically, smaller mesh sizes are used for smaller larvae and larger mesh sizes for larger larvae. Because water is continuously being exchanged over a time period, feeding must also be continuous, as the algae tend to flow out with the water leaving the tank.
Figure 1.12 Schematic for high density / low volume larval culture tanks for culture of pearl oyster larvae. *Source:* Whitford (2005a).

This is also the cause of much of the problems faced with this system. With the continuous feeding of algae into the tank, the sieves are more prone to becoming clogged, which would eventually lead to water overflowing. There are measures taken in case there is an overflow, which includes a t-joint being attached to the outlet pipe that ensures the overflow does not reach the tank surface (Fig. 1.11). Also, banjo sieves are cleaned at least twice a day to remove the algae and other substances, which are clogging the surface of the sieve.
Lobster and fish larval rearing hatcheries have the same system in place but the larval rearing tanks are configured differently when compared to those used in bivalve flow-through hatcheries. The tanks used are essentially the same with conical bottoms, but the sieve designs and out-flow pipes are modified to suit the type of larvae being reared (Fig. 1.13). Unlike the bivalve flow-through system, water is re-circulated back into the system in these designs, much like the RAS systems used to culture fish larvae at high densities.

1.8 Common issue involved with the operation of the flow-through system tanks

The continuous flow-through system, as mentioned previously, has been designed to allow for a continuous exchange of water in and out of the tanks over a period of time. In the case of rearing pearl oysters, this exchange requires a constant supply of algae to be fed into the system for the larvae. This constant supply of algae is a major issue of concern for the system performance.
because over time, the sieves become clogged. This clogging event can be problematic because the tanks overflow with the continuous supply of water into the system and the reduced outflow of water. Fail-safes have been implemented into the tank designs (overflow valve) but the stress being placed on the larvae would affect larval health. Therefore it is necessary to address this problem for the flow-through system since its potential here in small island hatcheries has yet to be fully exploited.

1.9 History of flow-through systems in the PICTs

Whitford (2005a) has described a major reason for the slow uptake of the ‘flow-through’ method in pearl oyster hatcheries in PICTs as being due to the amount of investment already in place in ‘static culture’ equipment and facilities, along with the subsequent unwillingness of companies to write it off. The Australian Centre for International Agricultural Research (ACIAR) has funded projects on simplifying larval and nursery rearing methods for pearl oysters with James Cook University in Australia, that has been commissioned to carry out the research (Southgate, 1996a). Much of this research was first initially tested on giant clams (Braley, 1992), scallops (Rico-Villa et al., 2008), table oysters (Southgate, 1996a) and greenshell mussels (www.cawthron.org.nz), and has only been recently tested in pearl oyster hatcheries (Southgate and Ito, 1998). Commercial pearl oyster hatcheries that have used the system include those culturing *Pinctada mazatlanica* and *Pinctada margaritifera* (Southgate and Beer, 1997; Martinez-Fernandez et al., 2003).

This study will look into streamlining this culture method by addressing some of the common problems associated with these systems. Removing such issues would enable a more efficient production rate, thus making it more appealing to PICT pearl oyster hatcheries.

1.10 Research objectives

The research described in this thesis examines the engineering and improvement of the high density flow-through rearing system, so that advancements in rearing techniques and technology obtained may be transferred to PICT hatcheries for the improvement of pearl oyster larviculture efforts. The only phase of the study that involved live larvae was a hatchery run comparing the
flow-through system with conventional ‘static’ culture rearing of *P. penguin* larvae conducted at the Tongan mariculture facility in Sopu, Nuku’alofa. The primary objectives of this thesis were to:

- compare an experimental high density flow-through system with the traditional static culture system in terms of growth and mortality performance, during a commercial hatchery run using, *P. penguin* larvae;
- evaluate and solve the banjo sieve clogging problem associated with the experimental high density flow-through system by trialing a new banjo sieve design against the original design.
- Determine trial design performance in terms of resistance to clogging by algal cells and particulate matter; and
- test the new sieve designs in a hatchery run with the use of live larvae.
CHAPTER TWO

ASSESSMENT OF THE FLOW-THROUGH SYSTEM VERSUS THE TRADITIONAL STATIC SYSTEM IN A HATCHERY RUN WITH LIVE P penguin (RÖDING, 1798) LARVAE

2.1 Introduction

2.1.1 Previous comparative studies

Previous comparative studies of the flow-through culture system against the static culture system for bivalve hatcheries have been based on larval survival to pediveliger stage (settlement phase), and shell growth throughout the larval life-stage (Sarkis et al., 2006).

Results of such a study conducted by Andersen et al. (2000) on great scallop larvae, Pecten maximus (Linnaeus, 1758), showed no significant difference in larval shell growth between the two rearing systems (flow-through and static), although the highest survival (on day 22 at 52%) was obtained using a large flow-through system with a volume of 4700 L. Torkildsen and Magnesen (2004) also carried out rearing experiments with Pecten maximus larvae in flow-through and static culture, both treated and untreated with the antibiotic chloramphenicol. It was reported that larvae reared in the large-scale flow-through tanks (4700 L) showed better survival (8.6%), than larvae reared in the 300 L untreated static system tanks (7.3%) (Torkildsen and Magnesen, 2004), though it could be perceived that the tank sizes played a significant role in the difference.

Similar comparisons were also observed between flow-through and static rearing systems for growth and settlement rates during the rearing of the Atlantic calico scallop, Argopecten gibbus (Linnaeus, 1758) (Sarkis et al., 2006). Results showed the shell growth for flow-through reared larvae being similar or significantly higher than in the static system (P < 0.01) (Sarkis et al., 2006). A preliminary study carried out on the Pacific oyster, Crassostrea gigas, for basic data collection on larval performance (survival, growth and competence) in a flow-through system, involved a run against a static rearing system (Rico-Villa et al., 2008). There was a significant difference for shell length between culture systems with those from the flow-through treatment (295.8±45.0 µm), being greater than those from the static treatment (262.5±58.4 µm). The
competency of the systems in rearing larvae was also found to be more successful with the flow-through system than in the static system (71 % vs. 55 % survival), although survival rates were not significant (p > 0.05) (Rico-Villa et al., 2008).

Grow-out of hatchery reared juveniles of the marine gastropod, *Babylonia areolata* (Link 1807), using a flow-through system compared to a recirculating sea-water systems also showed no significant differences in terms of the survival (p > 0.05) (Chaitanawisuti et al., 2005). The intensive rearing of sea bass *Dicentrarchus labrax* (Linnaeus, 1758), and sea bream *Sparus aurata* (Linnaeus, 1758), using the flow-through system showed that survival of the finfish larvae (with yolk sacs) at Day 30 and Day 40 to be 30 % and 50 % respectively (Shields, 2001). The yolk-sac larvae were reared in 1 – 20 m³ cylindro-conical tanks with upwelling currents at a density of 50 – 100 / L (Shields, 2001). The yolk-sac larvae feed off their endogenous reserves when feeding mechanisms are yet to be formed, which allows for the high density rearing of the larvae (Shields, 2001).

These studies have shown that the various systems used to culture the larvae are similar though there are differences in system performance, though there may be other factors. However successful the rearing systems, much of the benefit to be gained in rearing efficiencies would depend upon its suitability to the hatchery. This is advocated by Southgate (1996a) and Southgate and Ito (1998) on the use of static system tanks in PICTs, and the reasoning that such a system was not necessarily appropriate for the majority island hatcheries because of the infrastructure and labour required to run the production.

2.1.2 Dietary Studies

The use of THA, PAV, T – ISO and TET sp. as feed for Pteria penguin larvae stems from the knowledge that these are all species which have been evaluated for use in pearl oyster hatcheries around the region (Alagarawasmi et al., 1987, 1989; Gervis and Sims, 1992; Southgate and Lucas, 2008; Wassnig and Southgate, 2011). Although there have yet to be any nutritional studies carried out on the palatability and digestibility of these algae (Whitford, 2012), their nutritional effects on the larvae during this experiment seemed to be as expected. A nutrition and digestibility study conducted by Martinez-Fernandez et al. (2004) with Pt. sterna which is a close relative of Pteria penguin on the ingestion and digestion of 10 species of microalgae produced
interesting results. The 10 species of microalgae tested included Phaeodactylum tricornutum (PHA), Chaetoceros muelleri (CHM), Chaetoceros calcitrans (CHC), Thalassiosira weissflogii (THA), Dunaliella salina (DUN), Tetraselmis tetrathele (TET), Tetraselmis suecica (TES), Isochrysis aff. galbana (T–ISO), Nannochloris sp. (NAN) and Pavlova lutheri (PAV). The results showed that of the 10 species fed to the Pt. sterna larvae, T–ISO, NAN and PAV were ingested, and of these, the larvae digested only T–ISO and PAV.

It was of note that the larvae did not ingest the diatom THA along with CHC, CHM and PHA, given that they are rich in the polyunsaturated fatty acids. It was suggested by Martinez-Fernandez et al. (2004) that the reason for the inability of the larvae to ingest the diatoms was due to the physical dimensions (form and size) of the alga. This was seen also in a study on larval and spat culture of P. maxima in Western Australia, where the elongated spines of the diatom Chaetoceros gracilis was observed to have hampered ingestion by smaller larvae (75 – 85 µm) (Rose and Baker, 1994). Despite this, irrespective of the effect that form and size of the diatom may have on larval growth, the use of such organisms appears to be essential for larval health and growth and has been used in many bivalve hatcheries in enhancing the diets offered.

TET sp. has been noted to have high concentrations of ascorbic acid (1 – 16 mg g-1 dry weight) and riboflavin (20 – 40 µg g-1), but is deficient in polyunsaturated fatty acids (0 – 3 %) (Brown et al., 1997), which was supplemented by the diatom THA. The flagellates T–ISO and PAV that were initially fed to the larvae in the early stages of development were rich in Omega 3 (20:5 and 22:6) fatty acids 5 – 20 %) (Brown et al., 1997). This mixing of the algal diet would have ensured a balance in the nutritional requirements for the winged – oyster during larval development.

2.1.3 Research objectives

The primary objective of this experiment was to compare the performance of a high density flow-through rearing system with the static culture system in terms of the growth and mortality of P. penguin larvae cultured in them. To achieve this, the specific aims of this chapter were to:

(i) measure the larval growth and survival rates of P. penguin larvae reared in static and flow-through tanks;
(ii) compare the performance of the flow-through system against the static system; and make recommendations about the suitability of the flow-through system in PICT hatcheries.

2.2 Methodology

The first phase of the experiment was carried out in the Tongan Fisheries aquaculture facility, Sopu, Tonga from April 1 – 30 2012. The following sections will describe the setting up of the flow-through and static culture systems, the preparation of algal feeds and the sampling procedures involved.

2.2.1 Flow-through system set-up

2.2.1.1 Tanks

The tanks that were used for the trial runs in this study were 150 L transparent cylindro-conical tanks, which were remodeled from their intended design for use in algae culture to suit the rearing of *P. penguin* larvae (Fig. 2.1a). Such remodeling included the fitting of a 13 mm valve socket thread/barb, which was inserted at the base of the cone-shaped tank to allow for air-inflow to the tank (Fig. 2.1b). Water delivery into the system was through a simple manifold that is described below (Section 2.2.1.2). Wastewater exited the system through the special ‘banjo’ sieve mounted onto the drainage plumbing. The drainage plumbing included an overflow T-joint fitting when clogging problems occurred, to prevent water from spilling over the sides of the tanks (Fig. 2.1). All necessary plumbing fittings were purchased from a local hardware store.

The draining of the tanks was carried out from the aeration inlet pipe at the base of each tank. This allowed water to be completely drained and also ensured easy and safe collection of the larvae. The collection of the larvae was carried out into a basin covered with the respective sieve size (32 µm, 50 µm, 75 µm, 120 µm), depending on how large the larvae were during a particular point in the culture cycle. This was also where the grading of the larvae was carried out, where slow growing, sick or deformed oysters were removed from the tank to maintain uniformity in larval size (Fig. 2.2) (Alagarswami *et al*., 1987; Southgate and Lucas, 2008).
Figure 2.1 The 150 L transparent cylindro-conical tanks fitted-out and trialed as flow-through larval culture tanks in this study showing (a) over-flow T-joint fitting (indicated by arrow) and (b) aeration / drainage inlet pipe at the base of the tank.

Figure 2.2 Drainage of the experimental flow-through larval culture system showing a) draining of tank carried out with tube (indicated by arrow) placed in a sieve for larval collection and grading, and b) outlet pipe draining into screen mesh used as a safety measure to capture larvae.
2.2.1.2 Water and Aeration Inlet plumbing

The supply of air and seawater into the system was delivered via two separate pressure PVC pipes (50 mm diameter) cut into suitable lengths (1.5 m). Running along the aeration and seawater pipes, holes were drilled to insert inlet barbs (13 mm) that allowed seawater and air to be supplied into the tanks (Fig. 2.3). The 50 mm PVC manifold was connected to the main aeration and seawater supply. Connected to the inlet barbs was 13 mm clear vinyl tubing which directed water and air into the tanks.

Figure 2.3 Aeration and seawater inlet plumbing of the experimental flow-through larval culture system with 13 mm barbs feeding the 50 mm PVC manifold (indicated by arrows).
Valves were fitted along the tubing that enabled controlled water and airflow. The ends of the seawater inlet tubing were attached with a ‘restrictor’ that reduced pipe diameter to induce a ‘jet flow’ of seawater into the tanks (Fig. 2.4). The force of water entering the tanks allowed a spin tumbling effect on the larvae that would see the non-swimming larvae travel down the outer-edge of the tank only to be re-suspended by the aeration coming up the center of the tank.

![Restrictor valve (indicated by arrow) placed at end of tube to create ‘jet-flow’ of incoming seawater in the experimental flow-through larval culture tanks.](image)

**Figure 2.4** Restrictor valve (indicated by arrow) placed at end of tube to create ‘jet-flow’ of incoming seawater in the experimental flow-through larval culture tanks.

Aeration fittings were simple with the tubing being connected to a barb (13 mm) fitted at the base of the tank (refer to Fig. 2.1b).

### 2.2.1.3 Banjo screens

The banjo screens were made of four different mesh sizes, 32 µm, 50 µm, 75 µm and 120 µm. The varying mesh sizes accounted for the growth and increase in size of the larvae over the
hatchery run. As the larvae increased in size, the banjo mesh size increased correspondingly, to keep larvae from flowing out with the water exiting the tank and maintaining a continuous flow. PVC pressure pipes, 200 mm in diameter, were used to frame the screens, which offered a large surface area to reduce clogging by the algal feed. The construction of the sieves were adopted from the method described by Whitford (2005a), with a jig made of plywood backing plate, a plywood ring of relevant diameter (a 200 mm ring was used in this experiment) and six G-clamps (Fig 2.5). The screens were glued to the circumference of the pipe with PVC glue and then cut flush with a scalpel to remove the excess mesh (Fig. 2.6). The screens were constructed in an air-conditioned room as suggested by Whitford (2005a), to allow shrinkage of the pipes and upon subsequent exposure to ambient air temperatures when in use the expansion of the pipe would enable a tighter fit of the mesh to the pipe.

Figure 2.5 The jig used in constructing the banjo sieves with a screen being fitted onto the PVC pipe.
Figure 2.6 Completed Banjo sieves with PVC glue used to attach the screen mesh to the PVC frame of the banjo.

2.2.1.4 Algae mix tank

Establishing the algae mix tank involved the use of a 200 L transparent cylindro–conical tank, a submersible pump, and a container with a tap to control the drip rate of algae into the mix tank (Fig. 2.7). A ballcock was also used to maintain seawater level in the mix tank. Adjusting the tap nozzle for the algae feed container allowed a controlled drip rate to last over a 24 hour period. This ensured that the larvae were kept constantly mobile and suspended in the water column away from the tank walls. Placement of the algae mix tank within the overall flow-through larval culture tank set-up is show in Fig. 2.8.
Figure 2.7 Plastic container holding algae stock culture and tap to control drip rate of algae suspension into mix tank.
**Figure 2.8** Algae mix tank as part of the overall flow-through larval culture tank system set-up (indicated by arrow).

As seawater flowed into the mix tank through the ballcock valve, algae dripped into the tank from the algae container and the submersible pump transported the ‘mixed’ seawater into the larval rearing tanks to feed the larvae (Fig. 2.8). The advantage of this method is the equal distribution of algae into the rearing tanks and a reduction in cost of expensive equipment, such as the peristaltic pump, which would otherwise be required to distribute the algae to the tanks. The only disadvantage of this method is that the scale of the flow-through system is limited by the size of the submersible pump being used, due to the high flow rates required (Whitford, 2005a).

**2.2.2 Static culture system**

**2.2.2.1 The system**

Two 5 tonne black cylindrical tanks were used to rear larvae in this experiment (Fig. 2.9). This is usually the standard tank size that is available in most pearl oyster hatcheries around the region. There is only one outlet valve situated on the bottom of the tank where draining and larval culling are carried out. Such tanks require at least 3 hours of drain time for a complete water exchange. This is carried out at least two to three times a week (Braley, 1992; Sarkis et al., 2006; Southgate, 1996; Torkildsen and Magnesen, 2004; Southgate and Beer, 1997; Andersen et al., 2000; Southgate and Ito, 1998; Gervis and Sims, 1992; Tanaka et al., 1970; Alagarswwami et al., 1989).
Rearing of larvae in these tanks becomes tedious and time consuming especially when there is a
need for complete water exchanges (Rico-Villa et al., 2008; Reiner, 2011; Southgate and Ito,
1997). The tanks take up a lot of space and hatcheries with limited infrastructure would find it
difficult or expensive to house them.

The development of appropriate low-technology methods for hatchery and nursery culture was
an objective for the Pacific Island Pearl Oyster Resource Development Project (Southgate,
1996a). The projects greatly improve hatchery running costs, productivity and would enable the
establishment of pearl culture industries within the Pacific Islands region (Southgate and Ito,
1998).

Aeration is kept at a minimum in the static tanks, as the aim is more to create currents to keep the
larvae afloat and away from the bacterially loaded tank surfaces than to provide oxygen. In fact it
has been reported that aeration may affect the growth of pearl oyster larvae and lead to greater larval mortality (Alagarswami et al., 1987). However, a minimum amount of aeration helps in the uniform distribution of algal feed around the tank (Alagarswami et al., 1987).

Seawater was passed through a series of filter cartridges (5 μm, 3 μm and 1 μm) before being pumped into the tanks. This was to ensure that organic or non–organic materials taken in by the seawater pump are prevented from contaminating the hatchery tanks that could potentially lead to high larval mortalities. Water temperature was kept at ambient room temperature (28 ± 2 °C) throughout the hatchery run. Protocols involved in maintaining these tanks included a weekly and a day-to-day procedure. Daily protocols involved taking water and room temperature readings. Samples of larvae were taken from the tanks to check on overall health and growth of larvae as well as feeding activity (gut color). Feeding was carried out twice daily, at 09:00 h and 16:00 h, with the algal species *P. lutherii*, *T. iso*, and *Tetraselmis* sp. (see section 2.2.3).

Weekly protocols involved performing complete water exchanges, and carrying out larval sampling procedures. This was carried out twice a week until the settlement phase of the larvae was reached. The static tanks were ‘washed down’ to remove debris and excess feed. The larvae would also be drained through a mesh screen of the appropriate size (32 μm, 50 μm, 75 μm or 120 μm) depending on the size of the larvae. The sampling procedures are explained further in this chapter (see Section 2.2.4). The whole procedure of draining, cleaning and culling, sampling and filling the static tanks took a total of 6 – 7 hours, with over half of the time spent on draining and filling the tanks.

### 2.2.3 Algae feed preparation

Algal concentrates or algal pastes were being used as the sole larval food source in this experiment. This protocol has been used successfully for culture of *P. penguin* larvae in the Tonga Fisheries hatchery facility for at least the past six years. These commercially available pastes included *Isochrysis galbana* (T – ISO), *Pavlova* species (PAV), *Thalassiosira weissflogii* (THA), and *Tetraselmis* species (TET sp.), which were purchased from Reed Mariculture Incorporated, San Jose, California, USA. D–stage larvae (the larval stage after a period of 20–22 hours post fertilization that forms a ‘D’ shaped shell) were fed a mixture of 1:1 T–ISO and PAV until Day 8 in both the flow-through and static rearing systems, then THA and TET sp. were
incorporated into the flow-through and static system diet ratios respectively (Table 2.1). Feeding rates for the flow-through system began at 10,000 cells/mL for Day 1, increased to 15,000 cells/mL at Day 6 and on to 20,000 cells/mL for Day 7, but was decreased to 15,000 cells/mL at Day 9 onwards because of the clogging problems it caused to the system sieves. However for traditional low-density tanks, feeding rates gradually increased from 1,000 cells/mL on Day 1; 10,000 cells/mL at Day 7 and finally 17,000 cells/mL on Day 14 untill the larvae reached settlement phase (Table 2.1). The algal densities were prepared using the concentration formula (Adopted from Whitford (2005a)):

\[ D_1 V_1 = D_2 V_2 \]

Where:

\( D_1 \) = stock algal density counted by haemocytometer (cells/mL)

\( V_1 \) = volume of stock algae required (liters)

\( D_2 \) = feeding rate required in culture water (i.e. in tanks) (cells/mL)

\( V_2 \) = volume of flow-through water
Table 2.1 Feeding schedule (cells/mL/day) used for larvae and early post larvae of *P. penguin* reared in flow-through and static systems.

<table>
<thead>
<tr>
<th>Culture Day</th>
<th>Algal feeding density (Flow-through system) cells / mL / day</th>
<th>Algal feeding density (Static system) cells / mL / day</th>
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<tbody>
<tr>
<td>1</td>
<td>5,000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,000&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2</td>
<td>5,000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,000&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5,000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,000&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>11,000&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>15,000&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>15</td>
<td>15,000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17,000&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> PAV AND T – ISO (1:1) algal diet.
b PAV, T – ISO AND TW (1:1:1) algal diet.

c PAV, ISO AND TET (1:1:1) algal diet.

The flow-through system feeding protocol involved a continuous supply of algae into the tanks over a 24-hour period, compared to the batch-feeding regime used for the static system tanks. Since there is a continuous flow of water into and out of the system, algae would also have to be supplied in the same way. There is an advantage of this type of feeding over the traditional ‘batch’ feeding method mentioned by Whitford (2005). In batch feeding the larvae are fed at least two times a day, generally in the morning and evening. When there is no feeding, the larvae tend to stop swimming in search for food. This causes the larvae to settle on bacterially loaded tank surfaces and excess feed ‘debris’ that could affect their health or entangle the larvae potentially leading to mortalities. When there is a continuous supply of algae into the system as required with the flow-through tanks, the larvae are continuously feeding and moving thus keeping them suspended in the water column and away from the potential dangers of excessive contact with bacterial biofilms.

2.2.4 Broodstock spawning

The brooders were cleaned of fouling and boring organisms before spawning was carried out. These included sponges, tunicates, polychaetes, other molluscs, barnacles and bryozoans. It prevented any of the fouling organisms from spawning (Whitford, 2012) when thermal stimulations were carried out and also allowed a sterile environment for the spawning to take place. The effect of fouling and boring organisms on the overall health of adult oysters usually has a negative effect on growth and pearl formation (Gervis and Sims, 1992). Removal or cleaning procedures are carried out on a regular basis, either on land or in water on site (Gervis and Sims, 1992). Several methods of carrying out these procedures include mechanical or manual scrubbing with stiff brushes, use of high-pressure water jets, submersion in a brine solution for 15–40 minutes, brushing with 1% formalin then leaving to dry out in the sun for 15 minutes, or submerging in freshwater for 6–10 hours (Shirai, 1970; Gervis and Sims, 1992). There are alternative methods researched by Gervis and Sims (1992) dealing with the use of
antifouling agents such as DDT, BHC and compounds of chlorine, copper sulphate, ferric chloride, pentachlorophenol, mercury, arsenate, blueing agents, and naphthalene.

The spawning of the broodstock was carried out in rectangular spawning tanks at a water temperature of 27 °C (Fig. 2.10). This was gradually increased to 34 °C, held there for a half hour period and then brought down again, to induce spawning through thermal stimulation. Forty-four brood oysters were used. It was not determined which individuals and how many were male and female until spawning had occurred. No spawning occurred on Day 1 and Day 2, even though gonad extractions from a sacrificed male were injected into the water column to induce a spawning reaction (Fig. 2.11). Day 3 saw the brooders being transferred to an outdoor raceway after another failed attempt (probably caused by insufficient sunlight intensity which is reported to be an important factor in inducing giant clams to spawn (Braley, 1992)). The oysters were fed with Shellfish diet 1800, which is a combination of PAV, ISO, TET and TW species, to be conditioned for a three–day period till the next attempt.

After the conditioning period, another spawning was attempted in the lab with the same method of thermal stimulation and gonad extractions. The attempt was unsuccessful again. It was then decided that the spawning should be attempted in the outside raceways (Fig. 2.12). This was carried out the following day at 13:25 h. Male oysters were the first to spawn one hour later at a water temperature of 31 °C. Females spawned two hours later (14:25 h). Fertilization was then carried out at 16:00 h and the eggs stocked into incubation tanks at 18:00 h (Fig. 2.13). Thus a total attempted spawning period of 7 days resulted before success on Day 8.
Figure 2.10 Initial spawning tanks for *P. penguin* broodstock used indoors with heaters submerged.
Figure 2.11 Introducing sperm from a sacrificed male oyster into the inhalant current of *P. penguin* brooders.
**Figure 2.12** *P. penguin* broodstock transferred to the outside raceway in the third attempt to spawn.

**Figure 2.13** Incubation tank prepared to stock fertilized eggs for development into D–stage larvae.

### 2.2.5 Sampling procedure for larvae

Larval growth measurements and estimates of larval mortality for *P. penguin* larvae cultured in both the flow-through and static culture tanks were taken every two days during water exchanges until the settlement phase was reached. Larvae were sieved or graded during the draining and placed in 20 L buckets for each of the tanks. A perforated plunger was used to gently stir contents in the bucket so that an equal distribution of larvae was maintained throughout the water column. A 1 mL automatic pipette was used to remove larval samples from each of the buckets. Three replicates were used to get an average for the larval mortality sampling and a total of thirty
larvae were used for the growth measurements from each of the rearing tanks. A Sedgewick–Rafter Chamber was used to count the larvae under the microscope (Fig. 2.14). A light green to golden brown gut color was looked for during this time to indicate that larvae were feeding along with larval activity and mobility under a microscope with the 100× magnification. Counting was aided with the use of formalin (10% formaldehyde in sea water) that acted as a fixative for the larvae.

Figure 2.14 Instruments used to sample and observe larvae Source: (O’Connor et al., 2008)

The overall health check of the larvae included observations that they were actively swimming, the velum (bilobed ciliated organ used for swimming and food particle capture) remained attached, that they possessed good gut coloration and the amount of lipid globules (energy reserves) present within the tissues of older larvae. Bacterial or prolific infestations of protozoans were monitored in the rearing systems and were usually associated with the accumulation of biofloc material in the tanks.

2.2.6 Statistical analysis
A t-test was carried out to compare larval growth (as shell length) between the larvae reared in the flow-through and static systems using the SPSS program. Rates of survival were estimated by comparing the initial number of D-larvae stocked, to the number that survived to the pediveliger stage (Day 16).

2.3 Results

2.3.1 Larval growth in flow-through and static systems

The tanks operated with the different rearing systems are referred to as Low Density Tank (LDT) and High Density Tank (HDT) for the static system and flow-through systems, respectively. Average shell length (± SE) on Day 10 for the LDT larvae was 100.8 (± 1.85) μm compared to 89.8 (±0.73) μm for HDT larvae. A T-test confirmed that larvae reared in the static water LDT tanks were significantly larger than those reared in the HDT tanks, t = 3.0591, df = 38, p value = 0.004056. Despite this, more consistent growth rates were measured throughout the rearing in the HDTs.

The sampling for LDT 2 was stopped at Day 10 and grading procedures were carried out. The larger larvae (> 55 μm) were stocked into the LDT 1 and smaller larvae (< 55μm) were discarded.
Figure 2.15 Graph showing changes in the mean shell length of larvae cultured in high density flow-through (HDT) and low density static (LDT) systems over the 10 day period of this study.

2.3.2 Sieves clogging
The main problems that were faced with the flow-through system in this study, and which would have been a determining factor in its overall performance, was the problem of sieve clogging. The sieve retains the larvae in culture tanks while allowing excess water, uneaten algae and metabolic wastes to exit. During a clogging event pores in the ‘banjo’ sieve become blocked or obstructed with algae filaments, which are caught up in the fine mesh; this reduces outflow and raises the water level in the tanks. There are measures in place that counteract such an event, such as the fitting of a t-joint on the outlet pipe to direct water back into the pipe. Also a mesh screen placed at the end of the pipe to capture any larvae that would have escaped counteracts this (see Plate 2.1b). The banjo sieves of the flow-through system in this experiment began clogging on Day 6 at a feeding rate of 20,000 cells/mL, after which the feeding density was brought down to 15,000 cells/mL when the sieves were seen to be clogging continuously despite persistent and diligent cleaning. Larger meshed banjo sieves were fitted into the tanks once the larvae had grown to appropriate sizes, but in spite of this, the sieves had to be cleaned at least twice a day. This increased handling time and the continuous procedure involved in removing the banjo sieves would have placed unnecessary stress on the larvae.

2.3.3 Larval Mortality

Over the first six days of the hatchery run, the larval populations in both treatments saw a steady decline, with larvae in the HDTs showing the highest mortality on Day 2. This eventually evened out with the LDTs on Day 6. The larval mortality rates in both systems continued to decline to Day 10. Larval population in the LDT 2 was affected the most because of the Day 10 grading procedures, which saw larger larvae separated into the LDT1. The transfer of larvae into LDT1 affected the population mortality rate, which seemed to have flattened-out in sample Days 8 and 10 in the LDT 1 (refer to Appendix 2.1 for data).
2.16 Changes in larval mortality in LDT and HDT systems during the first 10 days of larval culture.

Larval mortality in the HDTs continued to decline until larval density was too low to provide an accurate count and recordings were thus discontinued after Day 10. HDT count on Day 10 was 740,000 larvae / 150 L.  

2.4 Discussion

2.4.1 Spawning success

The *P. penguin* broodstock used for the spawning were two years of age. They were selected from a batch of individuals that were reared from larvae in the hatchery. *P. penguin* is known to be multiple spawner, as observed by Arjarasirikoon *et al.* (2004) in Thai waters. The study noted that there are two spawning peaks for males (March–June and December–January), and one spawning peak for females (June–July). The sister-species *P. sterna* observed in Mexican waters showed a different pattern in its spawning peaks, which were within the winter months of December–February, April–May and August, with breeding seasons from October through to May (Arizemendi-Castillo, 1996; Hernández-Díaz and Bückle-Ramírez, 1996; Saucedo and
Monteforte, 1997; Vite-Garcia, 2005). The spawning of the brooders in this experiment took place in the first half of April, which is the start of the cooler months for Tonga. Average seawater temperatures around Nukuʻalofa within the winter months (April–December) are generally between 17 °C and 22 °C (WWCI, 2013).

Spawning stimulation included thermal and chemical induction. The most common form of stimulation is through the constant fluctuation of water temperature in the spawning tanks (Alagarswami et al., 1983a; Alagarswami et al., 1983b; Alagarswami et al., 1989; Chellam et al., 1991; Gervis and Sims, 1992; Rose and Baker, 1994; Southgate and Beer, 1997; Southgate and Lucas, 2008). Gervis and Sims (1992) observed that Australian broodstock of *P. maxima* taken from the wild spawn in the transportation tanks while on their way to the hatchery. The main goal in spawning induction is to stress the oysters into releasing their gametes instead of allowing it to occur naturally.

The oysters were conditioned with ‘Shellfish Diet 1800’© (Reed Mariculture, USA) (a combination of PAV, ISO, TET and TW) that provided polyunsaturated acids, ascorbic acid, riboflavin and omega 3 fatty acids, which would have ensured proper gonadal development in the broodstock oysters. However there have been difficulties faced with the reproductive conditioning of pearl oysters including *P. penguin*, (Rose and Baker, 1989; Lucas, 2008b). Providing large volumes of micro-algae to the broodstock are essential to proper gonadal development, but adds considerable expense to operation of the hatchery. This is mentioned by Yukihira et al.. (1998) who observed that pearl oysters have a greater filtering capacity compared to other commercial bivalve species, and hence require a healthy production of algae to sustain them. However, despite failed attempts with the larger pearl oyster species, Akoya pearl oysters have been successfully conditioned outside the normal breeding season. This was carried out by Hayashi and Seko (1986), who were able to show gonadal development and maturation by increasing water temperature from 18 °C to 24 °C and maintaining 24 °C for a period of three weeks. Increases in temperature have a positive influence on the ingestion rates of the pearl oyster (Numaguchi, 1994; Yukihira et al., 1998), as the high water temperature facilitates greater ingestion by improving respiration and the rate at which the energy is used up (Kobayashi and Tobata, 1949; Lucas, 2008b)
For the broodstock used in this experiment, the temperature was raised 6–7 °C above the ambient water temperature, held there for 30 minutes and then brought down again. This was carried out several times over a period of four days. It was observed that the oysters were not responding as expected to the temperature fluctuations being induced. After placing the oysters in chilled water (18 °C) overnight and then ‘shocking’ them in heated water (32 °C) the following morning, a spawning event was still not seen. The oysters were then conditioned in raceways outside the hatchery. They were fed with an algal diet of ISO and PAV (1:1) (‘Shellfish Diet 1800’© (Reed Mariculture, USA)) for a period of four days. After the conditioning period, another spawning was attempted but was unsuccessful. A few of the reasons for failed spawning attempts mentioned earlier could be due to the lack of sunlight intensity when inductions were conducted indoors, and this was proven true when inducement was conducted outdoors under bright sunlight. This is a common yet important method used in giant clam hatcheries to ensure successful spawning. Brooders are placed dry under the sun for a period of 30–60 minutes before being placed back in the raceway, after which spawning immediately occurs (Braley, 1992).

2.4.2 Larval growth in flow-through and static systems

The stocking density of both the static and flow-through rearing systems was 7.5/mL in this experiment. Stocking densities for static rearing systems usually range from 1–2/mL, 3/mL and 1–8/mL for the oyster species *P. margaritifera*, *P. mazatlanica* and *P. maxima* respectively (Alagarswami *et al*., 1989; Rose and Baker, 1994; Southgate and Beer, 1997; Martinez-Fernandez *et al*., 2003). Flow-through rearing densities can be increased up to as much as 15–100/mL (Reiner, 2011), however it was not possible to stock larvae at such high densities as there was a limited number of eggs produced by the brooders in our trial. It has been mentioned by Southgate (2008) that oyster larval density has significant impacts on food availability and water quality within the tank environment, which leads to stunted growth rates and high mortalities.

The larvae cultured in the HDTs showed a very slow growth rate over the 13-day culture period with shell lengths recorded between 80 μm and 90 μm, which were exceeded only on Day 13. The shell lengths for the larvae in the static systems however continued on a steady increase, with a sudden increase on Days 12 and 13. The average shell length measurements taken on Day
8 for larvae in the LDT replicate 2 showed an increase to just over 110 µm and then back down to under a 100 µm the following day. This showed the irregularity in larval sizes within the tank system (possibly reflecting mortality of larger larvae) and because of this, grading procedures were carried out to allow for faster growth rates, and for a more homogenous population size-wise (Alagarswami et al., 1987; Southgate, 2008). In-order to have a continuous flow-through of water; a banjo screen of appropriate size (32 µm, 50 µm, 75 µm, 120 µm) was placed on the outflow pipe. This allowed water along with excess feed and metabolic wastes to exit the tank. This is also the reason such a high density of larvae was able to be stocked into the tanks, without compromising tank health and deteriorating water quality.

Considering the ‘improvements’ that the flow-through system has over the static system, the larval growth rates were comparable. A significant difference (p < 0.05) was detected with the static system supporting the growth of larger larvae. However, the flow-through system was only introduced into the Tongan hatchery in this study, and a lot more development and testing is needed to optimise the method.

The methodology and day-to-day operations of the static system was more established than that of the HDT system, with general maintenance of the system and larval handling being more fluent and routine than that used for the pioneering flow-through system, which was often run carefully and slowly, during the hatchery phase. This may have had an influential role in the faster growth rates, without taking into account the size grading carried out on Day 10. However in a report by Andersen et al. (2000) that looked into the performance of different rearing systems (static vs. flow-through), it was observed that flow-through systems had the lowest bacterial counts and an increased larval survival rate but interestingly, growth rates were not affected by the different rearing systems. It was also observed by Sarkis et al. (2006) that the growth rates of calico scallop, Argopecten gibbus, larvae were comparable between the flow-through and static system or significantly higher than in the flow-through system. In consideration of the fact that the static system would be expected to have higher bacterial counts (Andersen et al., 2000), and be much more difficult to work with, the larval growth rates obtained are often comparable with the flow-through system and, in the case of this experiment, significantly higher. This revisits the suggestion that operational protocols for the two systems were significant in determining larval success, and that a more standardized method would need
to be developed for the flow-through system in the Tongan hatchery to improve larval performance.

2.4.3 Larval Mortality

The success of a hatchery run is often measured by the amount of larvae that can be transferred to the nursery stage (Magnesen et al., 2006). Day 2 of larval rearing showed that, in fact, it had the highest mortality rates, and this continued to decline until stabilising on Day 6, as was seen in the static system also. There is often a close association of static systems and use of antibiotics during a hatchery run (Southgate and Ito, 1998; Andersen et al., 2000; Magnesen et al., 2006; Sarkis et al., 2006), due to the problem of bacterial proliferation. There are procedures in place to control this such as regular water exchanges, standardized feeding times and quantities, grading procedures, and the consistent control of water parameters within the tank environment such as dissolved oxygen (DO), temperature and salinity. However, such proliferations only need a slight change in environmental conditions to produce negative effects, thus making the use of antibiotics in the system necessary, or at least beneficial. Despite the fact that no antibiotics were used in either system during this experiment, larval survival seemed to be fairly stable from Day 6 onwards despite larval survival in the flow-through system continuing to decline until Day 12. Successful larval development has also been noted to be correlated with initial larval density, algal concentration and season (Magnesen et al., 2006). Since both the rearing systems were stocked with equal larval densities during the same spawning season (7.5 larvae/mL in the winter months of April–December), algal concentration could have affected mortality rates. The larvae in the flow-through system were fed with algal concentrations four times greater than those in the static system, with feeding rates of 5,000 cells/mL, 15,000 cells/mL and 20,000 cells/mL during the course of the hatchery run. This was possible because of the continuous supply of fresh seawater being brought into the system, but was enough to speculate that the high algal concentrations may have had a contributing effect to larval mortality. Magnesen et al. (2006) made an important observation concerning the control of algal concentrations in flow-through larval culture tanks. It was seen that a negative correlation existed between larval survival and feeding densities. This is an important factor to consider for successful larval rearing.
It was also possible that reduced larval survival within the flow-through system could have been influenced by the detrimental effects of bacteria and/or dissolved compounds from algal cultures (Magnesen et al., 2006), although this may have been mitigated by the continuous water exchange.

2.5 Conclusion

Flow-through larval culture systems have been trialed in a variety of hatcheries which rear scallop, lobster, mussel, shrimp, and several species of fish (Reiner, 2011). This system has a number of advantages over the traditionally used static system. Being small compared to static system design, the flow-through system can be scaled into any infrastructure with limited space and yet still have the ability to produce a high number of settling larvae. These characteristics have great appeal for application to oyster hatcheries in PICTs, however extensive testing is required to determine their suitability. The small size of the whole set-up also enables very easy and fast handling times when doing complete water exchanges, which is important so that larvae are not removed from the tank environment for too long. However, despite obvious design improvements over the traditional static system, the larvae in the flow-through system during this experiment showed slower growth rates and higher mortalities. This was seen with a significant difference detected in larval growth rates (p < 0.05) between the two systems. A major concern and a contributing factor to the inferior performance of larvae in the flow-through tanks, is the problem of sieves clogging. This is a major problem with the system and is closely related to the feeding density. As observed on days 1–6, when algal densities were increased from 5,000 cells/mL to 20,000 cells/mL, clogging rates increased drastically over time. Measures taken to control clogging times such as increasing sieve sizes (as appropriate to larval size) and reducing water flow rates proved largely ineffective. The flow-through system is fed high algal densities as water is continuously flowing out of the system and taking the algae along with it. Feeding density calculations are based on the amount of water flowing in and out of the tank over a period of 24 hours. The volume of algae to be used is thus measured accordingly and slowly delivered into the rearing tanks over a 24-hour period. This continuous delivery eventually causes the clogging problem. Another practice that may have led to a better overall performance in the static system is the grading procedure carried out on Day 10. The grading of larvae is an essential and routine practice in many hatchery operations, and can enhance larval production in
flow-through systems by removing sick and slow growing larvae as observed during the experiment.

The issues mentioned above which have negative effects on flow-through system performance such as sieve clogging and feeding density need to be addressed if this system is to be introduced successfully into hatcheries in PICTs. The static rearing method of oyster larvae has been firmly established in many small island hatcheries in the Pacific, and much investment has been made into these developments. Considering this, the flow-through system a design developed for the convenience of small hatcheries, which warrants extensive testing to determine its suitability for facilities in the Pacific. It can also be argued that the advantages of this system outweigh its production performance in comparison to the traditional static system. Since this is a pioneering system in small island hatcheries, much development and research needs to be carried out in order to optimise operations and establish them in these locations. This is crucial if the flow-through system is to be made successful within the PICTs.
CHAPTER THREE

EVALUATING AND ATTEMPTING TO SOLVE THE SIEVE CLOGGING PROBLEM ASSOCIATED WITH THE HIGH DENSITY FLOW-THROUGH SYSTEM WITHOUT THE USE OF OYSTER LARVAE

3.1 Introduction

The high density flow-through system is a system that has been trialed in many hatcheries alongside traditionally used low density static systems, and has proven to be an effective and efficient means of larval rearing in large scale productions (Southgate and Ito, 1998; Andersen et al., 2000; Bergh and Strand, 2001; Torkildsen and Magnesen, 2004; Whitford, 2005a; Magnesen et al., 2006; Sarkis et al., 2006; Rico-Villa et al., 2008; Reiner, 2011; Sarkis, 2011a). The flow-through system trialed in this study however has shown to be less successful when compared to a static rearing system, but has the potential to offer much more towards the continuous development of larval rearing techniques and production levels in small island hatcheries.

Larval mortality with the experiment conducted in Tonga (Chapter 2) experienced a steady decline and crash in the flow-through system. Fortunately for the hatchery, the system was still in its testing phase and the larvae lost had no impact on production for the season. The problem of sieve clogging was an issue identified that seemed to be tied in with other problems associated with the system such as the larval feeding concentrations. Sieves in the flow-through system used in Chapter 2 were cleaned by manual removal of the sieve followed by gently scrubbing to remove algae from the micromesh. Other methods used to reduce clogging rates included the regulation of airflow over the banjo sieves. These methods did reduce clogging on the sieves but with the compromises made, such as reducing feeding densities, a negative impact on the growth of larvae may have resulted. It would be much more convenient if no such precautions were taken to reduce clogging rates, and if the problem of clogging could be eliminated altogether to enable more efficient running of the system with little or no handling by personnel. Ideally, the
system itself would have been built at a relatively low cost. Thus a new method was examined using a revised sieve design, which reduced clogging rates, without having to decrease feeding concentrations or even regulating airflow, and could be run without having to continuously monitor the water level in the tanks.

Various flow-through designs have been used, and are often optimized to suit the organism being reared (Hughes et al., 1974; Serfling et al., 1974; Braley, 1992; Nicosia and Lavalli, 1999; Robert and Gerard, 1999; Shields et al., 1999; Andersen et al., 2000; Shields, 2001; Hirokazu and Taisuke, 2010; Stefano et al., 2012). Examples include the “Hughes Pot” or “kreisel” (Hughes et al., 1974), which is a tank that had been developed for lobster larvae culture. Its function is essentially the same as the flow-through system tanks in that a continuous exchange of water occurs, with the use of sieves to allow for the removal of excess feed and metabolic wastes, and the ability for high density rearing. However, it incorporates a central standpipe to allow water to enter and exit the system. A similar example would be the partial flow-through system set up by Southgate and Ito (1998) (see Figure 1.8). The usual way to prevent the sieves in flow-through systems from clogging is to increase airflow over the sieves; this is often carried out with giant clam larvae (Braley, 1992). An interesting observation by Alagarswami et al. (1987) which bears consideration suggested that pearl oyster larvae are affected by the rate of airflow within the tanks, which is even more pronounced in smaller rearing tanks. The main goal in designing any rearing system is to make the rearing operation simpler. Easy handling and time saving procedures are some of the reasons that flow-through systems are a preferred method to the static rearing method.

This led to the idea that if a standpipe were fitted into a banjo sieve, then not only would the aeration supplied have a reduced effect on larval growth, it would also reduce the clogging of the sieves since the air supply would be bubbling through the sieve. Thus the idea of developing and trialing a new sieve design combining the standpipe method with the banjo screens may present an approach towards solving the clogging problem.
3.2 Research Objectives

The primary objective of this chapter was to evaluate and attempt to solve the clogging problem associated with the experimental high density flow-through culture system by trialing a new banjo sieve design and determining design performance in terms of resistance to clogging by algal cells and particulate matter. To achieve this, the specific aims of this chapter were to:

(i) construct a new sieve design for the flow-through tanks that solves the problem of sieve clogging;
(ii) determine its performance in terms of rate of clogging against the original sieve design; and,
(iii) compare its suitability in an actual hatchery setting against the original sieve design in terms of maintenance and handling in the absence of larvae.

3.3 Methodology

3.3.1 Flow-through system setup in the USP aquaculture lab

In order to carry this experiment out, a complete system of plumbing was constructed. This was similar to the Tongan experiment setup (Fig. 2.7), but on a much larger scale. The plumbing fittings and pipes that were used in the construction were purchased from a local hardware store. Special instruments such as glass water heaters and a peristaltic pump were purchased online from overseas stores. A schematic representation of the system is shown in Fig. 3.1. Seawater and aeration supply were delivered into the respective manifolds via the hatchery water and air pumps. From the manifolds, seawater and air were distributed into the treatment tanks. However unlike air supply that is connected to the main air lines of the hatchery (due to plumbing difficulties experienced), the seawater was first filled into a mix tank (300 L) before being mixed with concentrated algae, which was delivered from an algal container (20 L) via a peristaltic pump (Fig. 3.1). This mixture was then pumped into the seawater manifold to be distributed to the treatment tanks.
Figure 3.1 System design used to assess a new banjo design for use in flow-through larval culture systems.

3.3.1.1 Tanks

Sixty Litre cylindro-conical tanks were used for this experiment. A total of six tanks were prepared, with three replicate tanks per treatment. As mentioned above, such tank type has been used in other flow-through system setup thus was appropriately chosen for this experiment.

Each tank was fitted with an outlet pipe, located near the top end of the tank along with an aeration inlet fitting, attached at the base of the tank (Fig. 3.2). The water inlet manifold was fixed overhead, with the 13 mm inlet hoses feeding off the manifold into the treatment tanks.
Each hose was fitted with valves to control the inflow of seawater and to induce a ‘jet flow’ for circulation within the tank column.

**Figure 3.2** Cylindro-conical tanks used during the clogging trial with the seawater inlet hoses hanging into the tanks.

Tanks were cleaned during each sampling day (every second day), where the tanks and pipefittings were disinfected with a dilution of commercial hypochlorite solution (4%). Tanks were then filled up and left ‘running’ with fresh seawater (28Ω) flowing into and out of the system. This allowed time to remove any hypochlorite solution residue that may have been left over from cleaning.

3.3.1.2 Aeration and Water manifolds
Two 6 m manifolds constructed from 50 mm PVC pipe delivered seawater and air to the flow through culture tanks. At regular intervals on the pipes, holes were drilled to fit 13 mm valve socket threads/barbs. These were then fitted with 13 mm clear vinyl tubing at appropriate lengths. This was used to feed the seawater and air to the tanks. Aeration was connected to the main airlines of the hatchery, while a submersible pump had to be setup to supply the seawater into the tanks (Fig.3.3). Flow rates for both air and seawater were not measured but were controlled manually to allow for optimum rearing conditions. The submersible pump used in transporting seawater was placed into the algal mix tank, which pumped seawater to the manifold overhead to be distributed into the treatment tanks.

**Figure 3.3** Seawater (bottom white arrow) and aeration (upper white arrows) manifolds with 13 mm transparent hoses.
3.3.1.3 Banjo sieve designs

The construction of a new sieve design was examined by combining the standpipe used in the Hughes kriesel (Hughes et al., 1974) and the banjo screen developed by the late John Bayne in the 1980’s (Whitford, 2005a). This design was chosen to determine whether the flow of air through the standpipe would play a significant role in reducing the clogging rate of the sieves. In the original banjo design (see Chapter 2), aeration provided bubbles that flowed over the outside of the screens, preventing larvae and algae from being sucked into the screen mesh. However with the assessment carried out in Tonga (Chapter 2), it was noticed that once high algal concentrations (20,000 cells/mL) were supplied to the tank, aeration could not prevent clogging of the banjo mesh.

The design was kept simple, using the same method of constructing a banjo sieve as described above (Fig. 2.5), with a 20 mm PVC pipe being fitted directly below the outlet fitting on top of the sieve. Improvised plumbing fittings were made to enable the pipe to be inserted into the banjo screen (Fig. 3.4 and 3.5).

![Figure 3.4 Banjo sieve design with standpipe.](image-url)
Since screen clogging was initiated by the algae being caught within the fine mesh and is further aggravated by suction created from the outflow of water, the aeration flowing through the centre of the sieve should have reduced such a problem. Holes of 1 mm diameter were drilled at the base of the standpipe to allow air to escape, so that the larval and algal distribution would be maintained in the tank.

### 3.3.1.4 Algal mix tank

A 300 L cylindro-conical tank (Fig. 3.6) was used for mixing seawater and algae before being pumped into the tanks via the overhead seawater manifold. A microalgae combination of T–ISO and PAV (‘Shellfish Diet 1800’© (Reed Mariculture, USA)) was introduced into the mix tank at a ratio of 1:1, with the use of a peristaltic pump (Fig. 3.7) (1.3 L/h), which drew its supply from a pre-mixed 20 L container. There was a continuous supply of fresh seawater into the mix tank daily. The seawater used had been filtered through a sand filter system and then
passed through a 5 µm then 1 µm filter cartridges. This ensured that the seawater was kept relatively free of suspended particulate matter, so that if clogging occurred it would have been as a result of the algal feed. The filter cartridges were cleaned (three times a week) to allow for a regular flow rate.

Figure 3.6 Algal mix tank with 5 µm and 1 µm filter cartridges (1 L).
3.3.1.5 Plumbing Equipment
As mentioned above, the majority of the materials used for the hatchery setup were purchased from local hardware stores. However, a few of the fittings such as the 13 mm inlet barbs along with the valves were purchased online, though improvised fittings could be made. Each replicate tank for both treatments was fitted with a series of plumbing items and fittings in order for that tank to operate.

Seawater Outlet Plumbing
The outlet pipe plumbing, where excess water, algae and wastes flowed out of tank was constructed with two 25 mm valve sockets with a male thread/slip, one 25 mm 90° elbow thread/slip, one 25 mm 90° elbow, a 25 mm 90° T-joint, silicon sealant and 3 meters of 25 mm PVC pipe. The 3-meter PVC pipe was cut into the required lengths which attached the fittings together. The longest length however was the pipe extending down from the tank into the overflow mesh screen.

As mentioned previously, the T-joint is fitted to direct the overflow of water back into the outlet plumbing. A 25 mm valve socket male thread/slip was inserted into a hole drilled into the banjo screen and glued with silicon sealant. The male thread/slip socket valve and the 25 mm 90°
elbow thread/slip were then used to secure the plumbing inside of the tank to the plumbing outside the tank (Figs. 3.8 and 3.9).

Figure 3.8 Outlet plumbing showing the 90° elbow joined to the T-joint which is connected to the male thread/slip socket

Figure 3.9 Outlet plumbing on the outside of the tank leading down into the overflow mesh screen with the 25 mm, 90° elbow thread/slip.
The whole outlet plumbing was fitted at least 5 cm below the tank surface to allow the overflow of water into the T-joint and not over the tank sides.

**Air and seawater manifolds**

The air and seawater inlet plumbing was constructed with two 6 m, 50 mm PVC pressure pipes, a fourteen 13 mm valve socket thread/barb, 30 m of 13 mm clear vinyl tubing, six 13 mm barbed taps, and four 50 mm PVC caps to block the ends of the manifold pipes (Fig. 3.10). The manifolds were tied overhead because the air and seawater supply pipes were located overhead also, thus making it easier to make the connections. Along the manifolds, 13 mm holes were drilled and evenly spaced out into which the valve socket thread/barbs were inserted. The barbs were attached with the clear vinyl tubing that was hung into each tank. At the end of the tubing were fitted barbed taps that controlled water inflow.

![Figure 3.10](image)

*Figure 3.10* Seawater (bottom white arrow) and aeration (upper white arrow) manifolds with 13 mm valve socket thread/barbs inserted into the 50 mm caps.
The manifold ends were capped and sealed shut, however one of the caps for each manifold was drilled to insert a 13 mm valve socket thread/barb (Fig. 3.10). This was to allow seawater and air into the system via separate manifolds.

Aeration Plumbing

Aeration inlet plumbing on the tanks (Fig. 3.11) was connected bearing in mind the size of the cone at the base of the tank. This caused a bit of difficulty because the tanks had been previously modified. However, this was resolved with improvisation that included a 25 mm valve socket thread/slip, a 25 mm to 13 mm reducer and a 13 mm valve socket thread/barb.

Figure 3.11 Aeration inlet modification at the base of the cylindro-conical tank with the 25 mm valve socket thread/slip, a 25 mm to 13 mm reducer and a 13 mm valve socket thread/barb being attached at the end.
Air was pumped into the tank from the bottom-up that allowed for better circulation of the water and the upwelling effect created maintained the homogenous distribution of larvae in the tank. Like the seawater hoses, aeration was controlled using barbed taps.

3.3.1.6 Criteria for sieve clogging
A few basic observations were used to determine the condition of a sieve during the experiment. They were:

1) the color of the sieve mesh - most clogged sieves have a darker green color than unclogged sieves - the darker the mesh, the more clogged it is;
2) the degree of inward ‘curving’ of the mesh, caused by the suction pressure of water rushing out of the tank; and
3) the rise in water level of the tanks caused by algal obstruction of the mesh where inflow is greater than outflow.

3.3.1.7 Sampling Procedures
The data collected in this experiment was based on time taken until the sieve clogged. Four sieve mesh sizes were tested - 32 µm, 50 µm, 75 µm and 120 µm. For each sieve size, four different algal concentrations (10,000, 20,000, 30,000 and 50,000 cells/tank) were trialed. Each mesh size was tested during five runs at the same algal density to produce an average clogging time. The sieves were given a test-period of three days before being designated as ‘Not Clogging’ (NC). The system was run continuously over a three-day period (time-taken for larvae to outgrow current sieve mesh size) until a clogging event occurred. When this happened, time of clogging was taken and entered, after which the tanks were then drained and the sieves cleaned to prepare for the next replicate run. As anticipated, the smaller mesh (32 µm and 50 µm) sizes showed quicker clogging times than the larger sizes (75 µm and 120 µm). The whole experiment including the setup, initial testing, equipment calibration and running of the trials were carried out over a 12 month period.

3.3.1.8 Algal preparation
The standard method of algal preparation in an aquaculture laboratory was followed although no live oyster larvae were used in this experiment. A combination of T–ISO and PAV (1:1)
Shellfish Diet 1800© (Reed Mariculture, USA) algal pastes was used. Unfortunately, TW and TET species could not be imported into the country, as there were biosecurity issues.

The concentrations for each tank were calculated using the method described in Whitford (2005a). Algal concentrations were increased when each of the four mesh sizes had completed the five replicate runs. The algae was prepared into a 20 L container and pumped into the mix tank via a peristaltic pump, which was then distributed into the treatment tanks with a submersible pump.

3.4 Results

The mean times required for clogging of two sieve designs made with four different mesh sizes are shown in Fig. 3.12. The average times until clogging for each of the four sieve sizes (32 µm, 50 µm, 75 µm, 120 µm) were compared using a One-Way ANOVA. The larger mesh sieves clogged significantly more slowly than the small mesh sieves, (the largest mesh size of 120 µm did not clog within three days) F (df = 3, 32) = 196.38, p < 0.001.

The results indicated that the new sieve design (sieve type 2), also clogged significantly more slowly than the old sieve design (sieve type 1) in all sieve sizes, F (df = 1, 32) = 7.284, p = 0.011 and that there was no significant interaction between the sieve mesh size and sieve design, F (df = 3, 32) = 2.049, p = 0.127.
Figure 3.12 The mean ± 95% confidence intervals for clogging time for two sieve designs and four mesh sizes.

3.5 Discussion

The modified standpipe sieve was shown to clog significantly slower than the banjo sieve used in culture trials in Tonga (Chapter 2). As stated above, sieve sizes of 32 μm and 50 μm clogged the fastest at the higher algae densities (30,000 and 50,000 cells/tank) with this result being consistent across both sieve types. No significant interaction was detected between sieve mesh size and sieve design $F_{(df = 3, 32)} = 2.049$, $p = 0.127$. However, although the standpipe sieve performed better in terms of reduced time taken to clog, it lacked in the area of practicality and ease of use. This was realized during complete water exchanges and cleaning of the sieves when it had to be removed (see Appendix 5.1). With the banjo type sieve, whenever a sieve clogged, the procedure carried out to clean it was simple. It involved draining the tank to a level just below the banjo sieve, then removing the sieve by turning the fittings upwards. Complete drainage was not necessary unless it was required to carry out sampling and tank cleaning. However, cleaning the standpipe sieve design involved completely draining the culture tanks. Additionally, removal of the pipe from the tanks took a bit of tugging, which is ideally avoided.
in order to reduce larval stress. The whole procedure required to remove the sieve for cleaning was tedious and time consuming, and would also increase the time larvae spend outside of the tank, further increasing susceptibility to stress and potential mortality. Though the new sieves clogged slower, 3 days is the limit before cleaning is to be carried out. A simple modification to enable complete draining with the standpipe sieve is a ‘quick release’ between the banjo screen and the standpipe so it is only drained below the screen as in the original banjo screen.

At times, the rate of water exiting the tank of the standpipe sieve was influenced by the airflow running through the banjo sieve. This would often determine the time taken for the sieve to clog, and thus saw the standpipe sieve clog faster than the banjo sieve in one of the replicates when airflow had been reduced. When airflow was increased however, the flow of water exiting the tank was also increased and some suction was noticed with the sieve mesh curving inwards. This would almost certainly lead to larvae becoming pinned against the mesh, and certainly aggregate algae onto the sieves, further increasing clogging rates.

The airflow and water flow rates were maintained as consistently as possible throughout the trials. There were a few hiccups during the trials that saw whole system shut down due to power cuts or when back-wash procedures carried out with the primary sand filter supplying seawater to the laboratory complex. When such an event occurred, the algae that were already in the tanks would settle onto the mesh screens and more easily cause clogging. When water flow resumed, clogging would occur almost immediately. However, this was mostly observed with the banjo design, when the aeration supplied was not strong enough to remove excess algae from the screens. On the other hand, the standpipe sieve with the aeration going straight through the ‘banjo’ would have certainly unclogged the system and got it running smoothly again. This would have prevented unnecessary stress to the larvae and also to hatchery personnel during an actual hatchery run.

Concerning the homogenous distribution of algae in the tanks, the aeration coming up from the center would have ensured this. For the standpipe sieve design, holes were drilled at the base of the standpipe to allow bubbles to escape and to equally distribute algae within the tanks. However, due to the lack of pressure being built up within the pipe, air instead travelled straight up into the banjo. This often resulted in algae settling at the base of the tank, although the water jet induced by the water-inlet hose created a circulation effect by suspending the algae into the
water column to be removed via the sieve. However, this was insufficient to keep most of the algae suspended and debris was still noticed at the base of the tanks. If the screen could filter throughout the water column, this would reduce the collection of debris at the bottom of the tanks. The standpipe itself could be turned into a screen filter, allowing for filtering through the water column.

3.6 Conclusion

The standpipe sieve clogged significantly slower than the banjo sieve, and has proven to be an avenue worth exploring during the further development of flow-through culture systems. Since most of the problems relating to the flow-through system are linked to sieve clogging, the development of a clogging-resistant sieve would be a great advancement.

The standpipe sieve out-performed the banjo sieve by having significantly slower clogging rates, which was the objective of this experiment. However, its performance is to be compared again to determine the effects that clogging rates have on live larvae in terms of mortality and growth. This is critical if the design is to be proven successful in the commercial environment.

Technical difficulties for the procurement of *Pteria penguin* larvae and its broodstock for the next experiment were proven impractical and posed logistical difficulties. Among the problems seen, a major drawback was the ‘low’ quality of seawater being used by the University of the South Pacific (USP) aquaculture laboratory, which is pumped from the jetty located within the campus site at the Nasese foreshore, Suva. Since the pump is near to the foreshore and at a shallow depth off the jetty (< 2 m), there are variations in salinity and temperature during heavy rainfall (32 ‰ – 24 ‰) and run-off from the land introduce harmful chemicals into the seawater environment. This would have led to high larval mortalities for *P. penguin* larvae since high quality seawater is required for rearing. On this basis, the local rock oyster *S. cucullata* (von Born, 1778) was used to supply larvae used in the next experiment. The larval life cycle of this species is similar to that of *P. penguin* with 20 days of development before settlement and importantly the larvae are also very similar in size and growth rates. Since the broodstock would be collected from the Nasese foreshore, the oysters would be more tolerant towards the seawater environment that will be used in the USP laboratories.
The results of the experiment will shed further light on the success rate of the two sieve designs in terms of larval growth and mortality when carried out in an actual production run.
4.1 Introduction
The major constraint of the flow-through system and one that has been discussed and evaluated thoroughly in this study is the problem of the culture vessel outlet sieves clogging. There have been a number of measures taken in other studies to address this including: (1) increasing sieve mesh sizes in accordance with larval growth rate (Rico-Villa et al., 2008); (2) standardization of hatchery protocols including larval rearing densities and the water flow–rates (Reiner, 2011); (3) construction of larger sieves to provide for a wider surface area (Sarkis et al., 2006); (4) controlling algal concentrations fed into the system Magnesen et al. (2006); and (5) simple basic methods such as directing airflow over the sieves (Southgate and Ito, 1998).

These measures were implemented in the experimental protocol while carrying out the clogging trials on the different sieve designs (Chapter 3). The success of the standpipe sieve design has shown that clogging rates can be reduced for a period of up to three days or until the larvae are big enough for the next sieve change. However, the true success of such a design can only be determined in a realistic hatchery setting involving the rearing of live larvae.

In the previous experiment carried out in Tonga (Chapter 2), larvae of the winged pearl oyster, P. penguin were used for the performance comparison between the flow-through and static rearing systems. This was only possible due to the readily available supply of broodstock. However, because the experiment reported in this chapter is carried out in Fiji, the procurement of broodstock for spawning purposes or even larvae from a pearl hatchery posed logistical difficulties. The seawater being used in the USP hatchery laboratory where the experiment would be carried out was also not deemed suitable for culturing larvae of P. penguin. On this basis, the
rock oyster *S. cucullata*, which proliferates around the Nasese foreshore located besides the USP campus and is presumably well adapted to local water conditions, and was considered a more suitable source of larvae to be used in this experiment. The growth rate and sizes of the larvae of *S. cucullata* are also very similar to those of *P. penguin*, as is the life cycle and larval duration of around 20 days.

Flow-through systems have been used in the rearing of rock oysters (O’Connor *et al.*, 2008), and one of the various advantages of the flow-through system is its ability to be scaled according to the space requirements and size of the operation, as well as the availability of material used in its construction (O’Connor *et al.*, 2008; King *et al.*, 2010). This was shown with the system used by O’Connor *et al.* (2008) in rearing the rock oyster spat. The material used included conical based bottles of approximately 1000 mL (1 L bottles compared to the 250 L tanks used in most flow-through setups) in volume with water being delivered via glass tubing extended into the base of the bottle and flexible silicon tubing fitted onto its end that is connected to the water manifold. The continuous flow of water into the bottle suspends spat (300 µm) and metabolic wastes in the water column, from which wastes are removed via an outlet near the top end of the bottle. A 200 µm screen is fitted to prevent spat from flowing out with the wastes, and water flow rates are controlled with adjustable clamps placed on the silicon tubing (O’Connor *et al.*, 2008) (refer to Appendix 4.1). It was noticed however that no direct aeration was included in the system, although this could have been supplied by the continuous inflow of oxygenated water.

The distribution of the rock oyster *S. cucullata* is widespread in the Indo–West Pacific, from East and South Africa, including Madagascar, the Red Sea and the Persian Gulf, to eastern Polynesia; north to Japan and south to New South Wales and New Zealand (Carpenter and Niem, 1998). Being found attached to hard substrates in dense colonies in the marine, estuarine and mangrove areas, the oyster is known to be a major commercial species in many tropical western Pacific countries (Carpenter and Niem, 1998), although Fiji is not one of them.

Larval development up to the plantigrade stage (settlement phase) of the rock oyster occurs over a period of 20 days (Sukumar and Mohan Joseph, 1988), which is similar to many oviparous oyster species which require a duration of 18–24 days (Wassnig and Southgate, 2011). The
hatchery rearing method of the rock oyster as described by O’Connor. et al. (2008) is one that can be compared to the pearl oyster hatchery rearing protocols described by Alagarswami et al. (1983b), Alagarswami et al. (1987), Alagarswami et al. (1989), Beer (1999), Chellam et al. (1991), Gervis and Sims (1992), Martinez-Fernandez et al. (2003), Southgate and Lucas (2008) and Torkildsen and Magnesen (2004).

The use of the rock oyster *S. cucullata* larvae with the flow-through system should be manageable, given the similarities in rearing practices. Since the larvae settle at approximately the same time as pearl oysters, the larvae of this species can be easily integrated into the existing system specifically designed for rearing pearl oysters.

### 4.2 Research Objectives

The primary objective of this chapter was to compare the performance of rock oyster *S. cucullata* larvae between the banjo sieve and standpipe sieve designs developed in the work carried out for the previous chapter (Chapter 3). To achieve this, the specific aims of this chapter were:

(i) to measure the larval growth and survival rates of *S. cucullata* larvae reared in tanks equipped with both the banjo sieve and standpipe sieve designs;

(ii) to evaluate which is the better sieve design for optimal larval growth and survival performance and;

(iii) to make recommendations about the suitability of the superior sieve design for its application to PICT hatcheries.

### 4.3 Methodology

#### 4.3.1 Broodstock Collection

Adult individuals of the rock oyster *S. cucullata* were collected from the Nasese foreshore, Laucala Bay, Suva (lat. 18° 9’2. 91" S; long. 178° 27’ 15. 80" E) during the months of March and April 2013. Oysters were taken from the natural populations at various locations along the foreshore and examined for gonad maturation (Fig 4.1), before a site to collect the broodstock for this study was selected. Once a site was chosen, a total of 80 oysters were then removed for use as broodstock.
The oysters were first cleaned of fouling organisms before introduction into the laboratory environment. This was carried out by leaving the oysters out of seawater for 2 h and then scrubbing their shells with a stiff brush to remove fouling/burrowing organisms and algae. The procedure removed most of the epibionts and this process was reapeated for another day until the oysters were clean. Povidone–Iodine antiseptic was not used as suggested by O’Connor. et al. (2008) in sterilizing the oyster broodstock. Instead, the oysters were wiped with a 10 % formalin solution (10% v/v, formaldehyde in seawater) to achieve disinfection. The oysters were then placed into 50 L aquariums and conditioned by feeding with T-ISO and PAV microalgae (1:1) (‘Shellfish Diet 1800’© (Reed Mariculture, USA)) for a period of 5 days to ensure proper gonad maturation. Feeding was performed twice a day, one during the morning and one during the evening. The water temperature was maintained at 22 °C to 26 °C during this time.
4.3.2 Broodstock spawning

4.3.2.1 Strip spawning and Fertilization procedure

A total of 12 sexually mature oysters were used to obtain eggs and sperm for artificial fertilization. After identifying the sex of the oyster through visual inspection of gonad colour (male white and female yellow), 6 males and 6 females were selected. To obtain the gonads, the oysters were carefully opened to prevent damage to the gonad. Once opened, a small incision into the gonad was made with a surgical blade, and the gametes were washed from the opening using sterile seawater, which was passed through a 75 µm sieve into 1 L jugs (Figs 4.2 and 4.3). The sterile seawater used had been autoclaved at a temperature of 115 °C and then cooled to a room temperature of 22 °C; salinity was maintained at 30 °C. The sieve was used to remove shell debris that may have fallen in with the gametes while strip spawning the oysters. Eggs of the female oysters were kept separate from the sperm and examined using a microscope (100× objective) to evaluate the total percentage of eggs undergoing water hardening (eggs swelling as they absorb water, Fig. 4.4). The process of water hardening allows the maturity of the egg-batch to be assessed and its current health state. An evaluation or estimate could be made on the fecundity of the egg-batch being used (O’Connor. et al., 2008). Sperm was also examined for spermatozoid mobility and shape to ensure that live and healthy gametes were used.

Water hardening for the eggs took at least 18–20 minutes. The sperm were then pooled together into a 1 L jug, with approximately 160 mL of sperm contributed by each male oyster. The contribution of sperm made by each male oyster ensures a diversification of phenotypic traits. The sperm are then added to the eggs at a ratio of one sperm visible per egg and fertilisation is conducted. The embryos were stocked into each tank at <1 embryo/mL.
Figure 4.2 Stripped gametes from *S. cucullata* being sieved through a 75 μm mesh to remove debris material.
Figure 4.3 Jugs (1 L) containing stripped gametes from *S. cucullata*

Figure 4.4 Freshly released eggs for *S. cucullata* illustrating the “water hardening” process.
4.3.3 Rearing and Sampling protocols

4.3.3.1 Egg incubation

Following fertilization, the eggs were placed into two 60 L cylindro–conical tanks, at equal quantities (three jugs (3 L)/tank). The tank water was heated to a temperature of 26 °C prior to stocking and the heater units removed once the eggs were ready for incubation. Aeration was gently introduced to keep a steady mixing effect and to maintain the eggs in suspension within the tank. Salinity was maintained at 30 ‰ while the seawater in the tanks was filtered to 1 µm. No antibiotic was used to treat the tank.

The tanks were left over a 24–hour period to allow for proper development of the larvae to the veliger stage (Fig. 4.5). A general rule for first feeding described by O’Connor. et al. (2008) involves the addition of 10 % of algal feed ration into the tanks immediately the following morning. This is also carried out when complete water exchanges are done. The purpose of this is to ensure that the larvae are not left starving throughout the draining and sampling procedures.

The larvae had developed into the D–shaped veliger stage over an 18–hour period but were left for a whole 24–hour period before tank draining procedures were carried out to perform initial larval counts. This was to ensure a high percentage of D–stage veliger larvae being collected (as some larvae would have developed later than others), and to ensure the larvae were robust enough to be handled on a mesh screen.
Draining was carried out gently over a 32 µm sieve to retain the larvae and the sieve containing the larvae suspended in a basin of seawater while this was done (see Chapter 2, Figure 2.2 for a similar example). The collected veligers were then pooled into a 20 L circular-based bucket. An initial sampling of the larval population was carried out (see Section 4.2.3.2 for the procedure). A total of six tanks were prepared, with three replicates per treatment for the rearing trials. After the larval population was determined, the larvae in the buckets were then equally distributed into each of the respective tanks/treatments.

4.3.3.2 Larval culture
The method followed in rearing the *S. cucullata* larvae were identical to the one followed for rearing the *P. penguin* larvae in Tonga. Each day involved feeding, monitoring water parameters

![Figure 4.5 General life cycle of oysters](http://www.barnegatshellfish.org)
(DO, salinity and temperature), checking larval health in terms of gut colour, movement, and progressive growth; and ensuring the smooth running of the systems. Sampling days were carried out every four days when complete water exchanges were performed. This involved the complete draining of water in the tanks and collecting the larvae in sieves. After sampling, the tanks were then refilled; the banjo screens cleaned, inspected for damage and then fitted again to resume operation.

The larvae were reared in two treatments: 1) Banjo sieve design; 2) Stand-pipe sieve design. The banjo sieve (treatment 1) is the design used in current flow-through systems for larval rearing. The stand-pipe sieve design (treatment 2) is the design developed to aid in reducing the clogging problem faced with the current sieve.

Each treatment had 3 replicate tanks: 1) Banjo sieve design- T1, T4, T5; 2) Stand-pipe sieve design- T2, T3, T6.

4.3.3.2.1 Feeding

The larvae were fed with the algal pastes *P. lutheri* and *Isochrysis* species (‘Shellfish Diet 1800’© (Reed Mariculture, USA)). These were imported from Reed Mariculture Inc., California, USA. The algal feed was prepared into a 20 L container (Fig. 4.6) before being pumped into the main algal mix tank that supplied a constant flow of seawater into the treatment tanks. A peristaltic pump was used for delivering the algae into the mix tank (Fig. 4.6b). Feeding calculations were followed using the method described by Whitford (2005) (see Section 2.2.3), and the larvae were fed at a ratio of 1:1. The following apparatus was used in the feed preparation:

- Measuring cylinder 1000 mL
- Graduated pipette 10 mL and
- Plunger
The volume of algae to be fed was determined by the size of the larvae and gut colouration when inspected during sampling, and calculated using the concentration formula $D_1V_1 = D_2V_2$. Once the volume to be fed was determined, a graduated pipette was used to extract the required volume of algal paste, which was then placed in the measuring cylinder. The measuring cylinder was then filled with the required volume of filtered seawater before its contents were poured into the 20 L container that was also topped up with seawater. The peristaltic pump supplied algae into the mix tanks at 1.3 L/h and over a 24–h period a total of 15 L of algae was supplied into the mix tanks (algal concentrations being fed were 10,000 cells/mL, 15,000 cells/mL and 20,000 cells/mL, over the course of the run).

### 4.3.3.2.2 Water parameters

Water parameters included salinity (%), dissolved oxygen (mg/L) and temperature (°C) were measured daily using a YSI model 85 meter.

### 4.3.3.2.3 Larval sampling

The sampling of larvae was carried out every four days during routine water exchanges. This comparatively longer sampling interval was possible as the flow-through system allowed for a complete exchange of water on an hourly basis for each tank, and thus tank health was
maintained over a longer period requiring a lesser need for complete drainage. Sampling protocols involved the following pieces of equipment:

- Sieve mesh strainer (35 µm, 50 µm, 75 µm, 120 µm) of the appropriate size to retain the larvae.
- Large basin to submerge the sieve in while draining (keeps the larvae suspended while out of the tank).
- 20 L circular based buckets (x 6).
- A perforated plunger to evenly distribute the larvae in the water column.
- A dropper (1 mL).
- A binocular microscope.
- A Sedgewick–rafter slide.
- A counter.
- Fixative solution (10 % formalin).

Water was drained from the tanks gently into a basin through a sieve (Fig. 4.7). The sieve collected the larvae for sampling and allowed the algal debris to pass through. The larvae were then transferred into the 20 L buckets filled with seawater. From the buckets, the larvae would be then sampled for viewing under the microscope. Sampling involved a perforated plunger fitted with a circular head. The head was drilled to make holes of various sizes (Fig. 4.8). This allowed for the equal distribution of larvae within the water column that would enable random sampling. The plunger was moved three times down and up in the bucket before a 1 mL sample was taken with a dropper. The sample was then transferred onto the Sedgewick–rafter slide to be viewed under the microscope (Fig. 4.9). A general larval health was performed which included mobility, gut colour, shape and size of shell, and the presence of protozoans or foreign organisms. Following the general check-up, the larval count and larval measuring was carried out. A fixative (10 % formalin) was used to kill the larvae so that accurate measurements of larval size and larval counts could be made. The larval count was done using a hand-held counter and the divisions provided on the slide. This aided in the counts which were entered into an excel worksheet. Larval measurement was carried out using an eyepiece graticule that allowed measurement of the larvae (Fig. 4.10). The shell height (SH) and the shell length (SL) of the larvae were measured. SH was measured from the umbone or the straight hinge line (in the case
of D–veligers), to the opposing end and SL was measured perpendicular from the height line (Fig. 4.11).

Figure 4.7 Sieve being used to retain the larvae during draining of culture tanks.
Figure 4.8 Plunger used to evenly distribute *S. cucullata* larvae before being sampled with a dropper.
**Figure 4.9** Sedgewick – rafter used in counting *S. cucullata* larvae in replicate sub-samples to calculate survival.

**Figure 4.10** Larva of *S. cucullata* viewed under a microscope at 10 X with an eyepiece graticule showing the two valves of the shell.
Figure 4.11 Measuring S. cucullata larva using a microscope and eyepiece graticule: shell height (SH) (indicated by green arrow) and shell length (SL) (indicated by blue arrow) are indicated.

4.3.3.4 Tank and sieve equipment cleaning

Tank and sieve equipment cleaning was carried out during water exchange days while the larvae were being sampled. The sieves were removed and sprayed with a jet of water to clean the algae off. A 50 L aquarium filled with freshwater mixed with hypochlorite solution (at a 1:5 ratio) was used to soak the sieves and to disinfect them before further use. Tanks were also disinfected by scrubbing with hypochlorite solution. Water pipes supplying seawater to the larval rearing tanks were first flushed with freshwater and then with seawater before the larvae were placed back into the tanks. The freshwater in the system was used to kill organisms that would have passed through the filter bags. Flushing with freshwater also controls bacterial growth such as the ‘pink–spot’ bacteria which are often found at tank bottoms where dead larvae and algae collect (Whitford, 2012). The filter cartridges were replaced at least three times a week to prevent clogging.

4.3.3.3 Statistical analysis

A One–way ANOVA was used to compare the two different sieve designs using SPSS software version 13 (IBM Corporation, USA). Mean shell growth rates and standard deviations were calculated for the two treatments. Survival rates were determined from the initial number of D–veliger larvae hatched to larvae that reached day 16 of culture.

4.4 Results

4.4.1 Larval growth

Changes in shell dimensions (growth) of larvae were measured from day 1 to day 16 with four day intervals, totalling 4 sampling periods in both the treatments. Growth of larvae in treatments 1 and 2 increased steadily during the culture period (Fig. 4.12 and 4.13).

Larvae in treatment 1 (banjo sieve) showed a decrease in shell length on day 4 of the experiment. This was also seen on day 3 for treatment 2. Larvae were seen to be variable in size when observed during sampling. This was due to the fact that no grading procedures were carried out.
during the course of the experiment. This indicated that the larger larvae were having higher mortality rates further into the experiment.

**Figure 4.12** Changes in the mean (±SD) shell length of *Saccostrea cucullata* larvae sampled every 4 days for a 16 day culture period in flow-through tanks equipped with banjo sieves.

**Figure 4.13** Changes in the mean (±SD) shell length of *Saccostrea cucullata* larvae sampled every 4 days for a 16 day culture period in flow-through tanks equipped with standpipe sieves.
Comparison tests on the performance of the sieve designs in terms of larval growth were analysed using a One-way Analysis of Variance in the SPSS software version 13 (IBM Corporation, USA). It was found that on the third sampling day, the mean shell lengths using sieve design 1 (banjo sieve) were significantly greater than shell lengths using sieve design 2 (standpipe sieve), $F$ (d.f. 1,174) = 21.431, $p < 0.001$.

4.4.2 Larval mortality

The decrease in larval populations in both treatments occurred steadily over the culture period (Fig. 4.15). Large mortalities were noticed on day 1 of the experiment with treatment 2 (standpipe design) having the highest mortality (13,400 dead larvae). Despite this, the highest mortality was seen with the standpipe sieve-equipped tanks. On day 16 of the experiment, counting of the surviving larval population was difficult to carry out. Thus, larvae had to be concentrated into a 1
L container in order to be sampled. Sampling was discontinued on the 5th sampling interval (20 d), larval counts were undertaken and the experiment terminated.

Figure 4.15 Changes in the mean (±SE) numbers of *S. cucullata* larvae sampled every 4 days for a 16 day culture period in flow-through tanks equipped with either banjo sieves (◆) or standpipe sieves (■).

4.4.3 Larval rearing salinity, temperature and dissolved oxygen

The use of seawater from the local area was not a problem for this experiment in terms of larval rearing (Johnson Seeto, pers. comm., 2014). Changes in water quality parameters during this experiment are shown in Fig. 4.15. The broodstock used were originally from the vicinity and so had adapted to the water conditions. Temperature and dissolved oxygen (DO) remained stable throughout the rearing period. Salinity was observed to fluctuate, although by marginal amounts and remained within tolerance limits (Fig. 4.16) (O’Connor. A. and Lawler, 2004).
Figure 4.16 Changes in the major water quality parameters salinity, temperature and dissolved oxygen (DO) during the experiment.

4.5 Discussion
4.5.1 Larval growth

The complete rearing of the *S. cucullata* larvae to the plantigrade stage in a hatchery occurs over a period of 20 days (Sukumar and Mohan Joseph, 1988). Larval rearing unfortunately was only possible until day 16 in the present study due to the high mortality rate and the lack of larvae available to sample by the end of the experiment. The measurement of larvae over the whole 16-day period in both the treatments saw slow growth rates that stayed within the ranges of 65 µm – 90 µm. This is considered to be very slow when compared to growth rates observed by Sukumar and Mohan Joseph (1988) on days 12 and 18 which were 109 µm and 143 µm respectively for *S. cucullata*. The data suggests that it was not primarily due to screen design but associated with a failure to meet one or more of the larval requirements of this species. The growth rates of a close relative *Saccostrea glomerata* (the Sydney rock oyster) indicated 220 µm and 280 µm for day 12 and 16 respectively (O’Connor *et al*., 2008). The growth of larvae in the banjo sieve treatment showed a decrease in mean shell lengths in all the three tanks (T1, T4, T5) on day 4. This was also seen in treatment 2 for Tank 6 on sample day 3.
The decrease in average larval size measurements further into the experiment is possibly caused by the lack of homogeneity in larval sizes within the tanks. This inconsistency allowed size recordings to vary on each experimental day as observed in T6 with treatment 2, although it may not be a likely assumption with measurements seen in the treatment 1 tanks (T1, T4, T5). In these tanks, it was noticed that as the larvae developed, it was becoming more difficult to find larger larvae compared to individuals, which were measured in the previous sampling (4 days prior). In fact it seemed that the larvae stayed at approximately the same length over the sampling interval. It was determined that the larger larvae were dying off in the tanks and the slower developing larvae remained, which was confirmed when many of the dead larvae seen or empty shells collected when removing larvae to sample had shell lengths of $>100 \ \mu m$. An alternative interpretation of the data is that the larvae perished when reaching a certain size or age, possibly because of inappropriate culture conditions such as limited availability of key nutrients.

Size grading is a common protocol followed in commercial hatcheries whereby smaller and slower growing larvae are separated by sieving, to allow for a more homogenous population (Alagarswami et al., 1987; Gervis and Sims, 1992; Southgate and Lucas, 2008). This in turn allows for faster settlement and higher growth rates of the larvae which are selected to be on-grown (Southgate and Beer, 1997). Gervis and Sims (1992) observed that grading procedures enhance larval survival to post–settlement stage without influencing larval growth.

In this experiment, grading was not carried out for the tanks. There were a number of reasons for this, the main element being a lack of larvae to run the experiment with. Starting densities depending on rearing method used for hatcheries range from 1–12 larvae/mL for static tanks (Hayashi and Seko, 1986; Alagarswami et al., 1987, 1989; Rose and Baker, 1989) and 15–100 larvae/mL for flow-through tanks (Loosanoff and Davis, 1963; Castagna et al., 1996; Helm et al., 2004; Rico-Villa et al., 2008). The stocking density for this experiment in both the treatments was $<1$ larvae/mL at 45,533 larvae/60 L tank. The removal of smaller or slower growing larvae would have greatly affected the final outcome and may have led to a shorter experimental run with the larval density used.
The flow-through tank environment is constructed such that a constant flow of seawater is maintained in and out of the system. This design also creates an upwelling effect within the tank that prevents the larvae from settling at the bottom (Southgate and Ito, 1998; Reiner, 2011) with the algal debris. Although such a construct is made to decrease larval mortality for pearl oysters, it may have had an effect on the *S. cucullata* larvae used. The natural environment from which the broodstock were collected from is generally characterised by calm waters with little wave and current action. It is a possibility that the tank environment was not suitable for rearing the rock oyster larvae for the reason that the upsetting and constant motion of the tank water had a negative effect on larval survival.

It is also of note that the full dietary requirements for the rock oyster species used was likely not provided for by the algal species being fed to the larvae. Algal species used in the study by Sukumar and Mohan Joseph (1988) on *S. cucullata* larvae included *Tetraselmis gracilis* (TETRA) and T – ISO. The *Tetraselmis* sp. provide high concentrations of ascorbic acid (1 – 16 mg g⁻¹ dry weight) and riboflavin (20 – 40 μg g⁻¹); and T – ISO is rich in Omega 3 (20:5 and 22:6) fatty acids (≥ 5 – 20 %) (Brown et al., 1997). In this experiment the algal species used in the rearing were T – ISO and *P. lutheri* (PAV) (which is also rich in Omega 3 fatty acids). This combination was also used with the rearing of a close relative the Sydney rock oyster *S. glomerata* in an oyster hatchery in NSW, Australia, although the diatoms *C. calcitrans* and *C. muelleri* were also introduced into the feeding regime at 50 % of the feed ration (O’Connor et al., 2008).

Alagarswami *et al.* (1987) stated that the growth of larvae is dependent upon the right type of diet and when nutritional requirements are not provided for, larval development can be stalled for as much as 40 days. The use of PAV in replacing TETRA may have had a contributing factor in the stagnation of growth of the larvae over the rearing period, even though the nutritional content of the *Tetraselmis* sp. was supplemented by the T – ISO diet. Diatoms form an essential part of bivalve diets and often make up a large part in the algal diet ratio (O’Connor. *et al*., 2008; Southgate and Lucas, 2008). The lack of diatoms in the rearing of *S. cucullata* larvae in this experiment may have contributed to be the main cause of the slow growth rates and eventual total mortality seen with the larvae.
4.5.2 Larval mortality

Observations made by Reiner (2011) on the build-up of metabolic wastes, in the Eastern oyster *Crassostrea virginica* larval rearing flow-through system, saw that the accumulation of metabolic wastes in the tanks increased bacteria numbers, alter pH levels and negatively affected the growth of larvae leading to mass mortality. Though flow rates in this experiment had been adequate to keep metabolic waste build-up in check, days when sieves were clogged would have been a problem for the larvae. This allowed the water flow rate to slow down and algae being fed to the larvae to settle on the tank walls. Complete water drainage and tank cleaning were carried out every two days as part of hatchery protocol. Once feeding density was increased, the frequency of sieves being clogged was hourly. It was noticed that tank water turbidity increased each time this occurred, since feeding was continuous, and excess algae were accumulating on the tank floor. During sampling days, it was observed that larvae were seen tangled amongst the algal debris when viewed under a microscope.

The practicality of the sieve design in this experiment was also a problem, especially when it had to be tested with live larvae. Having a design that incorporated both the standpipe and banjo sieve was observed to clog significantly slower than the original banjo sieve design on its own (P < 0.05). However when used in rearing *S. cucullata* larvae, handling was a major issue and a possible cause for high mortality. When a standpipe sieve had to be removed due to clogging, the process likely induced more stress than the original banjo sieve design might have, due to the amount of manipulation required to pull the sieve assembly out of the tank. Although this was not carried out very frequently throughout the experiment, a more delicate method in design should be considered to address the issue.

The problem of sieve clogging is still a major issue for the flow-through system. The standpipe design offers a new direction for solving this problem, though much optimization is still needed before being successfully introduced in PICT pearl oyster hatcheries. The fact that standpipe sieve out-performed the banjo sieve by having significantly slower clogging rates as seen in Chapter 3 results, shows that aeration flowing through the sieve does have a significant impact in reducing clogging times.

4.6 Conclusion
The standpipe design (treatment 2) proved its resistance to clogging but was unable to perform in other areas that are essential in larval rearing. Despite being more clog-resistant than the banjo sieve, the standpipe sieve did eventually clog. When this happened the effort involved in cleaning out the sieves was comparatively more difficult and potentially deleterious to the larvae. The reason behind this involved the standpipe being fitted in the centre of the tanks, and to remove the pipe required a bit of tugging that saw the water being stirred, suspending the dead algae at the bottom of the tanks. This was difficult to avoid even if gentle pressure was applied to remove the pipe assembly. The water is then drained completely, the sieves are cleaned and then refitted before the tank is filled up and the larvae put back in. Although this was not carried out as often as with the banjo sieve design at any of the algal densities, it is not possible to remove the standpipe sieve in its current configuration without disturbing the larvae.
CHAPTER FIVE
GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Review of Objectives

The major objectives of this thesis and the extent to which they were achieved are summarized below. For detailed descriptions of the investigations carried out to achieve the following objectives, please refer to the relevant chapters.

i) To compare an experimental high density flow-through system with the traditional static culture system in terms of growth and mortality performance, during a commercial hatchery run using, *P. penguin* larvae.

The investigations described in Chapter 2 of this thesis detail the comparison of the experimental high density flow-through system against the traditional static culture system in terms of growth and mortality performance. The flow-through system was also investigated of the sieve clogging problem associated with its operation.

ii) To evaluate and solve the sieve clogging problem associated with the experimental high density flow-through system by trialing a new banjo sieve design against the original banjo sieve, and determine trial design performance in terms of resistance to clogging by algal cells and particulate matter.

The investigations described in Chapter 3 of this thesis detail the construction of a new sieve design to address the problem of sieve clogging. The new sieve design was also trialed against the original banjo design to determine the rate of clogging and was shown to have much better performances.

iii) Test the new sieve designs in a hatchery run with the use of live larvae.

The investigations described in Chapter 4 of this thesis detail the testing of the successful new design in an operation with live larvae and determine performance in terms of growth and mortality. The design though proven to have a slower rate of clogging, did not outperform the original banjo sieve design with significantly greater larval growth rates supported by the banjo
sieve design \( F(\text{d.f.} 1,174) = 21.431, p < 0.001 \) and lower mortality experienced over the 16 day culture period.

The general implications of the findings detailed in this thesis, and recommendations for further research into the development of the flow-through system in PICT pearl oyster hatcheries are discussed in the following sections.

5.2 Study constraints and limitations

A major constraint faced with the experiments carried out in Tonga and Fiji, would have been the procurement of a large number of larvae for the experiments. Such a constraint limited the operational capability the flow-through systems both in Tonga and Fiji and led to shorter rearing periods than anticipated.

A secondary constraint would have been the location of the USP aquaculture laboratory in Suva, Fiji. This prevented the rearing of pearl oyster larvae for the final phase of experiments due to the poor seawater quality experienced in the area. It eventually led to the use of local rock oysters \( (S. cucullata) \) to carry out and complete the experiment.

Since much of the flow-through system had to be built for the University aquaculture lab, a few of the important pieces could not be found in local hardware stores. This increased budget costs and a few of the items had to be improvised with local fittings, which may have affected system performance.

Due to the nature of the experiment and the window of opening given (spawning season from March to April) to carry out the rearing trials with the two sieve designs on the use of live larvae \( (S. cuculata) \), replications of the final experiment was hampered. The final phase experiment (phase 3) was discontinued after the larval numbers dropped to zero in both the static and flow-through systems.

5.3 Recommendations for future research

The problem of sieve clogging is still a major issue for the flow-through system. The standpipe design offers a new direction for solving this problem, though much optimization is still needed before being successfully introduced in PICT pearl oyster hatcheries. The fact that standpipe
sieve out-performed the banjo sieve by having significantly slower clogging rates as seen in Chapter 3 results, shows that aeration flowing through the sieve does have a significant impact in reducing clogging times. However the standpipe design proved to be an inconvenience during handling and removal procedures in comparison with the banjo sieve. This was seen as a problem in the chapter 4 experiment with live larvae. Clogging as expected was handled well by the standpipe sieve but higher mortality and slower growth rates were observed in these tanks. The main problem was the removal of the standpipe sieve during cleaning and/or sieve changing procedures. However there are areas that were identified during the clogging trials with both the original and standpipe sieve designs, which need be further improved to ensure an effective and efficient production of oyster larvae in a hatchery. These would include; standardizing feeding protocols for the larvae; the effect of airflow in the tanks on the larvae; best pump type and flow rate used in transporting mixed algae from the mix tank into the rearing tanks; type of algal diets used in feeding to ensure nutritional requirements are met; and the improvement of the sieve design that will continue addressing the clogging problem.
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Appendix 2.1 Fluidised spat bottle system. Inset shows close-up of spat in the base of the bottle.
Appendix 4.1 Diagram illustrating the removal of the sieve types with the Banjo sieve (upper half) and the Standpipe sieve (bottom half).
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<td>7,400,000</td>
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**Appendix 5.1** Data showing the larval mortality in the Static and Flow-through tanks over the rearing period of 12 Days.