Environment, genes and the effects of genotype by environment (G x E) interactions, on the expression of commercially important traits in the Silver-lipped pearl oyster, *Pinctada maxima* (Jameson).

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Abstract

The silver-lipped pearl oyster, *Pinctada maxima,* is cultivated in Australia and throughout South East Asia for the production of "South Sea pearls". Pearls from *P. maxima* are the largest and most valuable pearl produced out of all pearl producing oyster species, with the industry being valued at ~US$ 412 million farm-gate globally. The commercial production of pearls from this species is well established and hatchery techniques have been developed to the stage where the life cycle is considered closed.

A standard commercial pearl production cycle takes 4 years from the time oysters are spawned in the hatchery to the harvesting of pearls. The first two years is a "pre-pearl" grow-out phase for the oyster that will become the host oyster, and then a further two years in a "pearl growth" phase whereby the oyster is 'seeded" and the pearl nacre is laid down. After this 4 year cycle only ~20% of pearls harvested fall within the category of being "gem quality", where the combination of the commercially important traits lustre, size, shape, weight, colour and complexion are all within the accepted standards. It is this long phase of production and the low proportion of gem quality pearls harvested that makes it very attractive for pearling companies to improve the consistency of production through the use of genetic breeding approaches. A current impediment to adoption of genetic approaches, however, is a lack of knowledge on the role additive genetic factors play in the realization of a pearl quality trait.

Another consideration for future breeding programs is that pearl companies generally have several farm sites situated in geographically distinct locations, with one hatchery supplying all the seedstock to these locations. As such, as well as understanding the genetic basis underpinning pearl traits, it is important for companies to determine the effect disparate environmental influences due to site location may have on both oyster growth and pearl quality, and to establish whether the realization of genetic potential in improved oysters will be affected by genotype by environment (G x E) interactions. Like that for genetic parameters governing pearl quality and growth traits, however, data on the potential impact G x E may have on selected oysters reared under different environmental conditions is lacking. In response to this critical missing information this thesis aimed to establish genetic parameters and estimate genotype by environment interactions for both growth traits and pearl quality in the silver-lipped pearl oyster.

Investigations commenced in this thesis by considering the effect long-line location (and perceived differences in micro-environment) at four sites *within* a pearl farm have on the realization of growth and pearl quality. Variance of these traits due to genetic differences as a consequence of using different cohorts of broodstock was also considered. In this experiment long-line site was shown to have a significant impact on the overall growth rate of oysters, with oysters reared on long-lines at the Sasanaflapo site growing significantly faster than those at the other three sites examined. These growth differences were also shown to manifest regardless of the age or genetic composition of the cohort evaluated. This shows that oyster growth is a trait that may be influenced by environmental parameters within farm locations, and that long-line location may override individual genetic effects on growth. The influence site has within a farm on pearl quality was, however, less conclusive. Only

differences in pearl colour overtones and lustre appeared to be modified by site effects *within* a farm. For example, pearl colour and lustre could be both partitioned using classification tree analyses by site, with the Duyef and Wulu sites producing on average more white pearls with pink overtones, while Maratlap and Sasanaflapo produced higher numbers of silver pearls with pink overtones. Likewise for lustre, splits in classification trees were related to the fact that the Wulu site produced more pearls exhibiting higher lustre grades. Despite these differences though no disparities were found in the economic value of pearls harvested from the various sites *within* the farm evaluated.

With the suggestion that local-scale environmental effects may modify oyster growth traits the thesis then goes on to test if genetic differences represented by oysters from different families could be easily modified through the manipulation of defined environmental parameters. Here the relative performance in shell growth of spat from five full-sib families when spat were communally reared at different salinities (29, 34 and 40 ppt), food availability (high, medium and low), food quality (high, medium and low), and in a hatchery vs. ocean environment for 43 days, were compared. In support of the first experiment, rearing environment was again found to significantly influence growth expression, with significant differences evident when spat were reared at different salinities, in the ocean instead of hatchery, or when fed algae of differing nutritional quality. Additionally, comparative family growth was also altered when the environment changed, with significant environment by family interactions (G x E) apparent under food quality, food availability and hatchery vs. ocean rearing conditions. These results indicate, that at least during early oyster growth phases, that growth and relative family performance in *P. maxima* may change dependent on local environmental conditions.

To further examine the effect environment and genetics has on oyster growth, spat were produced for a large commercial scale trial using broodstock originating from three distinct Indonesian populations (Bali, West Papua, Aru Islands). These spat from different genetic backgrounds were communally on-grown to adult sizes for two years at each of two Indonesian commercial farming locations (Bali and Lombok). Microsatellite based parentage determination analyses were used to retrospectively sort out oysters to both their family and population of origin. Significant size differences were observed in all shell growth traits measured (dorsal-ventral measurement DVM, anterior-posterior measurement APM, shell width SW and wet weight WW), with oysters originating from Bali and West Papua (DVM (mm) = 103.7±0.9 and 101.0±0.6 respectively) growing faster than those from the Aru population (93.5±0.5) at both grow-out locations. Family level differences within these populations were also present for shell traits, indicating a large amount of genetic variability present for potential breeding programs. However, although there were significant familial size differences for shell traits, unlike the earlier spat growth experiment, genetic correlation analyses showed little evidence for re-ranking of family performance among the two culture sites (rg = 0.89–0.99). This implies that under the commercial conditions oysters were evaluated that insignificant genotype by environment deviations among sites were evident. Heritability analyses based on these families were also conducted for shell traits, with DVM and APM found to be moderately heritable (0.15 ±0.00 (DVM), 0.23 ±0.03 (APM)). Thus selection for faster growing host oysters should be possible which would advantage the industry by

decreasing the amount of time it takes to grow oysters to a size suitable for nucleus implantation.

The final experiment undertaken in this thesis estimated for the first time genetic parameters and G x E for pearl quality traits when multiple families were again reared at Bali and Lombok. Here significant differences in the size and value of pearls produced at the two locations were observed, with pearls produced at Lombok generally bigger and more valuable than their Bali counterparts. Comparisons of pearls produced by the various families jointly reared at these two sites also indicated adverse genetic correlations for size (r_g = -0.22), colour (r_g = 0.28) and weight (r_g = 0.38), and less so shape (r_g = 0.56) and lustre (r_g = 0.59); thus the occurrence of genotype by environment modifications for these pearl quality traits needs to be factored into improvement programs. Heritability analyses based on the donor-oyster additive genetic contribution showed that all pearl traits except that of shape exhibited low to moderate heritabilities (size h^2 = 0.13, lustre h^2 = 0.14, weight h^2 = 0.15, colour h^2 = 0.15 and complexion h^2 = 0.25). As a consequence these traits could be improved through the practice of selection.

The findings of this thesis have substantially advanced our knowledge of the respective role genetics and the environment play in the realization of commercially important traits in the pearl oyster *P. maxima*. Pearl quality and oyster growth traits have been shown to have a heritable basis, thereby making them amiable to improvement through selection approaches. Results also have shown that when designing future breeding programs considerations of large-scale site induced environmental effects and associated genotype by environment modifications will need to be factored. Through implementation of the information gathered in this thesis the *P. maxima* pearling industry now has a sound basis for the future design of efficient selection programs aimed at improving the productivity and profitability of their industry.

Table of Contents

List of figures

Chapter 2

Chapter 3

Chapter 4

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 1. General introduction

A fundamental requirement in the design and conduct of efficient selective breeding programs is an understanding of the genetic basis of traits under selection. In aquaculture, acquisition of genetic knowledge over the last three decades has permitted the development of industrial-scale breeding programs in several commercial species, including Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), marine shrimp (family Penaeidae) and tilapia (tilapiine cichlids) (Gjedrem, 2005). However, for many other aquaculture species, targeted improvement programs are still impeded by a basic paucity of data on the quantitative genetic basis of traits. One such aquaculture industry that is interested in applying selection to improve profitability, but for which there are currently gaps in genetic understanding of traits, is that of South Sea pearl farming, an industry based on the culture and harvest of pearls from the silver-lipped pearl oyster, *Pinctada maxima*.

1.1 Prerequisites of Selective Breeding

Historically, pearl production of the silver-lipped pearl oyster, *Pinctada maxima*, was based on the harvest of oysters from the wild and their subsequent rearing on commercial long-lines (Gervis and Sims, 1992). It is only in the last 20 years that hatchery techniques have been developed to the point where the life-cycle could be considered closed and reliable hatchery production of seedstock has occurred. Closure of the life-cycle has allowed the possibility for selective breeding programs to be instigated and recently there has been increased interest from pearling companies in the application of quantitative genetic breeding methodologies to the improvement of

traits of economic interest, such as survival, growth rate and pearl quality (Knauer, et al., 2007; Rose and Baker, 1994; Wada and Jerry, 2008).

To enable *P. maxima* pearlers to exploit the improvement potential that selective breeding can bring there is an urgent need to understand the fundamental genetic mechanisms determining commercially important pearl and shell traits. In particular there is a requirement to determine the heritability and genetic correlations of important pearl quality traits, as well as providing species-specific heritability estimates for shell growth characters. This information is essential since there is no way of determining beforehand whether phenotypic variability observed in a trait is a result of heritable gene effects (ie. additive genetic variation), non-heritable genetic interactions such as dominance and/or epistasis, or a dissimilar environment. Also of interest is whether the genetic potential of an individual will be realized under disparate local environmental conditions (so called genotype by environment (G x E) interactions).

Quantitative genetic theory predicts that phenotypic variation within a population is determined largely by both genetic and environmental factors according to the equation; $V_P = V_G + V_E + V_{GE}$, where V_P is the total phenotypic variation for a trait within a population, V_G is the amount of phenotypic variation attributable to genetic potential of an individual, V_E is the modification of genetic potential due to environmental variation, and V_{GE} is the variation attributable to environment specific interactions between the genotype of an animal and its local environment (G x E interactions) (Dunham, 2004). Most selection programs aim to improve the contribution of the V_G component of phenotypic expression through targeted breeding of individuals possessing favourable genes for a trait of interest. Commonly, given

infrastructure requirements, this selection will proceed in a single environment and often possible modifications of genetic potential by local culture environments (ie G x E interactions) are ignored. As such it is important to obtain important genetic information both in determining heritability of important pearl oyster growth and pearl traits and also to estimate the effect of genotype by environment interaction for the species under selection.

1.1.1 Heritability

Heritability is the proportion of phenotypic variation in a population that is due to additive genetic variation between individuals (i.e V_G) (Falconer and Mackay, 1996). Most heritability studies conducted in aquaculture species have been with fish (Gjedrem, 2005), with reports of heritability estimates for growth rate ranging from 0.10 to 0.20 (Gjedrem and Baranski, 2009).

Although no heritability studies have been conducted with the silver-lipped pearl oyster, *Pinctada maxima*, a selective breeding study in the Akoya pearl oysters (*Pinctada fucata*), found realized heritability of two shell traits, shell width and convexity, to be 0.47 and 0.35, respectively (Wada, 1986). Velayudhan et. al., (1996) estimated rather higher realized heritability of the same traits in Akoya pearl oysters from India over four generations as did He et. al., (2008) (0.71 ± 0.21). Heritability estimates for growth traits in closely related pearl oyster species to that of *P. maxima* highlight the potential genetic gains that can be realized for shell growth traits through selective breeding. However, as heritability dictates what breeding scheme would be most effective in a selective breeding program (Falconer and Mackay, 1996) heritability for shell traits of interest in *P. maxima* will need to be estimated before breeding programs can be commenced in this species. At present there are no heritability estimates for pearl quality in any pearl producing oyster species.

1.1.2 Gene by environment interaction

Genotype by environment (G x E) interactions occur when genotypes of the cultured species express their phenotypes differently when reared under diverse environments (Sheridan, 1997), i.e. the genetic potential of the animal is modified depending on the specific genotype of the animal and its response to the environmental influence exerted on it - most commonly as a consequence of differing selective pressures (Dunham, et al., 1990). Numerous studies from terrestrial animal industries and several aquaculture species demonstrate that G x E effects commonly are present and that the genetic potential of an individual is not always realized when animals are reared under different environments. As an example, Wohlfarth et. al., (1983) found that Chinese and European carp each have adapted to the conditions in which they were initially domesticated. For the trait growth rate, in a good environment (moderate stocking density and good diet) the European carp was dominant over the Chinese carp. Conversely, in a poor environment (high stocking density and poor diet) the Chinese strain produced a superior growth rate over the European carp (Wohlfart, et al., 1983). Significant genotype by environment interactions have also been identified in bivalves by Newkirk (1978), who found significant differences in the growth rates of four different populations of *Crassostrea virginica* larvae reared in different salinities (Newkirk, 1978, 1980).

There are two different types of G x E interactions that can be detected in aquaculture situations (Dunham, 2004). The first type of G x E interaction is observed when the relative performance rank of two or more genotypes changes when compared in two

or more environments, as in the above two examples of carp and *C. virginica*. The other G x E effect observed which is not as severe in terms of impact on breeding programs, is not where ranking of genotypes change in the disparate environments, but is due to magnitude effects among genotypes (Dunham, 2004). Here superior performing families may still be superior for the trait of interest, but the difference between their performances compared to other families may be much smaller or greater due to the particular environmental challenge.

In aquaculture species, most studies on G x E interaction have been with high production fish species (i.e. salmonids, tilapia, catfish and some marine finfish) with G x E interactions found to vary in importance. Several studies found evidence for low G x E interactions (Eknath, et al., 1993; Gjerde, et al., 1994; Kolstad, et al., 2006; Ponzoni, et al., 2008; Saillant, et al., 2006; Sylven, et al., 1991), whilst other studies have conversely found significant G x E effects (Dunham, et al., 1990; Dupont-Nivet, et al., 2008; Imsland, et al., 2005; Imsland, et al., 2000; Iwamoto, et al., 1986; Wohlfart, et al., 1983). In pearl aquaculture the determination of possible disruption of genetic performance of families due to G x E interactions is of major importance to breeding programs, as pearling companies often rely on a few hatcheries to produce seedstock which are subsequently reared at several geographically distinct grow-out sites. Thus, the information on whether selectively bred oysters genetically improved at one location will produce offspring that perform well at other locations is of great consequence. Sheridan, (1997) states that there is considerable experimental evidence that the growth rate of oysters is sensitive to apparently small environmental differences. If this is true than an understanding of potential G x E impacts are essential before targeting breeding begins.

Commonly the magnitude of G x E interaction increases with genetic distance between genotypes and disparity in environments. When G x E interactions are considerable, a breeding program should be developed for each of the different environments in which the animals are to be reared (Gjedrem, 2005). The development of several breeding lines generally involves more labour and cost for the industry, however, their presence might not be all bad. Rawson and Hilbish, (1991) surmised that if a G x E interaction is significant it should preserve any heritable variation associated with the growth trait in their experimental animal the hard clam *Mercenaria mercenaria,* and they also indicated that G x E might lead to the development of phenotypic plasticity for growth (Rawson and Hilbish, 1991). This indicates that G x E interactions can act to maintain or help against the removal of genetic variation.

1.2 Pearl culture and aquaculture

The silver-lipped pearl oyster, *Pinctada maxima*, is the largest of the Pteriidae pearl oysters and is cultured throughout south-east Asia and Australia for the production of South Sea Pearls, with the industry worldwide valued at US$412 million (Wada and Jerry, 2008). Cultured pearl production began in full-scale for this species in Australia in 1949 based on the "seeding" of wild adult oysters. Pearl oysters of an adequate size were collected by divers from the seafloor using hard-hat diving suits and then implanted with a small bead nucleus and placed into nets suspended from long-lines. For the next 40 years cultured pearls were produced this way based solely on harvest of wild stocks. However, over-exploitation of oysters and the consequent instigation

of strict heavily regulated quotas led in the early 1980's to the development of hatchery culture techniques. Nowadays the majority of cultured pearls are derived from hatchery sources. Modern cultured pearl production is consequently divided into three distinct culture phases, each requiring different production technologies and expertise. The first phase, that of hatchery or larval rearing, is the most demanding, requiring cultivation of algae as feed for the larvae and providing substrate for the spat to settle on. For *P. maxima*, Tanaka and Kumeta, (1981) first published accounts of successful spat production as early as 1981, while the Western Australian Department of Fisheries and Wildlife (FIRTA) developed and published a hatchery manual nine years later (Rose and Baker, 1994; Tanaka and Kumeta, 1981). However, fears of over-production resulted in the manuals limited distribution and routine production of *P. maxima* spat in commercial hatcheries really only became commonplace in the early 2000's (Minaur, 1996). Since these early days in hatchery production, a gradual change of attitude has developed and there are now a number of commercial hatcheries producing *P. maxima* spat.

Several studies has been conducted on optimal larval conditions for *P. maxima* and the other two main commercial pearl producing species *P. margaritifera* and *P. fucata* (Doroudi and Southgate, 2000; Doroudi, et al., 1999a; O'Connor and Lawler, 2002, 2004; Rose and Baker, 1994; Taylor, 1999; Taylor, et al., 2004; Taylor, et al., 1997). These studies described optimal food density, larval density, salinity and temperature for the hatchery phase. In research studies into the hatchery culture of pearl oysters survival has been looked at as an important factor in assessing different culture conditions, however, growth seems to be the predominant factor when evaluating physiological condition (Doroudi and Southgate, 2000). Studies have found that

larvae fed high food rations had lower growth (Doroudi, et al., 1999b, c; Riisgard, 1991). This has been explained with an increase in microbial activity and resulting poor water quality, but another explanation is that the reduction in clearance rate was associated with reaching maximum gut retention, leading to valve closure and reduced metabolism (Riisgard, 1991). Rose and Baker, (1994) pointed out in the hatchery protocol that *P. maxima* larvae should not be overfed and algal densities should be adjusted based on their clearance rate to maximize growth and survival. Live food is one of the major costs in running a hatchery and optimizing the algal density is key for both health of the animal and keeping the running costs down. Stocking densities of the larvae is another important factor, and the different species of *Pinctada* seem to vary a lot in their ability to tolerate high stocking densities, with *P. maxima* needing to be reared at lower larvae densities than *P. fucata* (Doroudi and Southgate, 2000; Rose and Baker, 1994). If the stocking densities are too high it might decrease the water quality causing low survival and growth of the larvae, however, if the larvae density is too low it might increase the running cost making the hatchery unprofitable (Doroudi and Southgate, 2000). Despite the difference in the ability to tolerate high larval densities, studies found that these three species, *P. maxima*, *P. margaritifera* and *P. fucata,* settle at the same time (20-23 days after fertilization) and at the same size (230-266 μm) (Alagarswami, et al., 1989). Temperature seems to be major factor in survival with the highest survival being at lower temperatures, however, this is not the best condition for increased growth (Doroudi, et al., 1999b). The success in producing *P. maxima* spat means the life-cycle is closed for this species and a selective breeding program is possible to establish.

The second production phase, the grow-out phase is a relatively simple procedure since the animals are filter feeders, with pearl oysters able to filter water at rates up to 25 $l\,h^{-1}\,g^{-1}$ of dry wt. tissue (Gifford, et al., 2004). Hence, no artificial feeding is required with their main diet being phytoplankton and particulate matter (Lucas, 2003). In the grow-out phase oysters are simply placed into pockets of panel nets suspended from long-lines. However, it takes almost two years until oysters are large enough for pearl nuclei seeding and during this time they have to be regularly cleaned to maintain them free of biofouling organisms. Biofouling control throughout the growth phase is not only labor intensive, but one of the major costs to commercial operators. For example, in Indonesia the cost of undertaking these activities equates to ~US$0.40/month/oyster (Joseph Taylor, unpublished data). A typical large pearling farm may carry 300,000+ pre-implantation oysters. Therefore reducing the time taken for oysters to grow to a size suitable for nuclei implantation by as little as one month will provide significant cost savings to companies. Genetic selection for fast growth is one option available to reduce the time farmers have to grow oysters till implantation size.

Nuclei implantation and the subsequent growth of the pearls comprise the third and final culture phase. A piece of mantle tissue from a donor (or saibo) oyster is inserted into the gonad of a host oyster along with a bead nucleus honed from the shell of Mississippi mud clam. The donor oysters are selected for their nacre colour and are sacrificed and the mantle tissue (nacre producing organ of the oyster) are dissected out and cut into smaller pieces. The host oysters are gently opened up, and an incision is made into the gonad and a bead nucleus is implanted together with a piece of the donor mantle tissue. If the grafting surgery is successful the piece of mantle tissue will

grow around the nucleus and form what is called a pearl sac where the tissue will start to lay down nacre, creating the pearl. As such the formation of cultured South Sea pearls result from a complex man-made process incorporating two different oysters to produce a single pearl.

The value of the pearls produced are based on five quality traits or virtues; size, shape, colour, lustre and surface complexion. Although there is no world recognized standard for the grading of pearls; the larger, rounder, smoother and brighter the pearl is the higher its value (Taylor and Strack, 2008).

Unlike other pearl producing species such as *P. fucata*, silver-lipped pearl oysters are usually only seeded with a single nucleus. However, the overall larger size of the silver-lipped oyster means that a larger nucleus can be implanted resulting in pearls between 10 mm and 20 mm (average of 13 mm) in diameter. A single host oyster can be seeded at least three times during its productive life, producing a pearl on average every two years. Repetitive seeding of adult oysters is a way companies compensate for the long initial grow-out phase to seeding size, however, usually after the 2nd pearl has been produced nacre quality deteriorates due to the age of the oyster (Dr Joseph Taylor pers. com.).

1.3 Thesis aims and structure

This thesis was undertaken as part of the Australian Research Council Linkage Project LP0560298; "*Towards selective breeding of the Silver-lipped pearl oyster, Pinctada maxima*", and was a research collaboration with the commercial pearling company

Atlas South Sea Pearl Ltd. The main objective of this study was to produce essential genetic information to allow the company to commence a genetic selection program for *P. maxima*. For this to be successful, the program needed to establish for the first time the genetic basis of oyster growth and pearl quality traits, as well as how the phenotypic variance in these traits varied due to population and/or location of culture (i.e. G x E).

Chapter 2 provides an insight into how site selection *within* a pearl oyster farm can primarily affect host pearl oyster growth and variation in pearl quality. The chapter highlights the role of multiple environmental factors and determines whether pearl quality and value were significantly influenced by grow-out location within a pearl farm when a selective breeding program was not instigated. From this starting point the subsequent aim of the thesis was to estimate genetic parameters for important traits through the various phases of the production cycle (from spat to harvest of pearls). As highlighted earlier there are three distinct stages in pearl aquaculture (1) the hatchery phase, 2) pearl oyster grow-out to seeding and 3) pearl production. Chapter 3-5 incorporates each of these stages and looks at heritability estimates and G x E interaction for families produced from three distinct Indonesian populations when reared at two commercial sites.

Chapter 3 examined whether the environment significantly influenced growth differences among pearl oyster families in the final stages of hatchery production and whether there is any evidence of genotype by environment effects operating on growth early in production. It achieved this aim by assessing the relative performance

in oyster spat shell growth traits in five full-sib families communally reared under different salinities, algae diet and hatchery vs. ocean environment for 43 days.

Chapter 4 follows 32 full sib and 80 half-sib families produced from broodstock originating from three populations from spat until they reach the required size for seeding. This grow-out period takes up to two years and the industry currently faces problems with different growth rates and the amount of time it takes until the animals are ready to be used for seeding. This chapter estimated heritability and G x E of important pearl oyster shell growth traits and lays the fundamentals for commencing a selective breeding program for increased host oyster growth.

Chapter 5 unravels in a pearl producing species for the first time the complexity of pearl formation and provides valuable information on genetic parameters of pearl quality traits. Heritability and the effect of genotype x environment interactions are estimated and the interplay between the host and donor oyster and their contribution to pearl formation are highlighted. The importance of estimating genetic correlations between pearl quality traits and especially to determine how traits of interest are correlated is of critical importance before setting breeding goals for this industry.

Together, these chapters provide important genetic information on all three production stages in pearl oyster aquaculture and will aid in the development of a selective breeding program for the silver-lipped pearl oyster, *Pinctada maxima*.

Each of the data chapters in the thesis contains a stand-alone Introduction, Materials & Methods, Results and Discussion section formatted for scientific journal

publication. At the time of thesis submission, three of these chapters have been accepted for publication in peer-reviewed journals and are presented with minimal modification and minor re-formatting as they were published.

Chapter 2. The effect of site selection on shell growth and pearl quality traits of the silver/gold-lipped pearl oyster, *Pinctada maxima*.

2.1 Introduction

The silver/gold-lip pearl oyster, *Pinctada maxima*, is cultured throughout south-east Asia and Australia for the production of South sea pearls. In *P. maxima,* as in other mollusks, the shell is a comprehensive product of biomineralization formed through the deposition of aragonite $CaCO_3$ crystals by the oyster mantle tissue (Dix, 1972; Gong, et al., 2008a; Gong, et al., 2008b; Miyamoto, et al., 1996). The production of a cultured pearl occurs through the same biomineralization process, except in this instance a piece of mantle tissue from a donor oyster is inserted into the gonad of a host oyster along with a "seed" nucleus. Over time, this implanted mantle tissue grows to encompass the seeded nuclei in what is referred to as a pearl sac and lays down a nacreous covering comprising aragonite to form a pearl.

The physical composition of nacre is mostly comprised of biomineralized $CaCO_3$ (91.50%), with traces of organic substances (3.83%), water (3.97%) and finally residual elements (0.01%) (ie. Ba, Mg, P, Mn, Fe, Al, Cu, Zn, Ag, Hg, Li and Sr) (Taylor & Strack, 2008). Recent evidence from the freshwater bivalve, *Elliptio complanata,* suggests that micro-environmental factors like variation in water trace element levels can influence the mineral composition of pearl nacre, with levels of Mn, Sr and Ba in the water correlating with levels of these same elements observed in the nacre (Carroll and Romanek, 2008). Whether or not these changes in microchemistry lead to quantifiable differences in pearl quality traits, or pearl value, is not known however. In fact there is a paucity of data relating to culture site effects

on pearl quality, with the reported effects restricted to industry manuals and other grey literature which state that site factors such as disparate temperature and food availability impact on the rate of nacre deposition, see (Saucedo and Southgate, 2008).

Studies which have examined site effects in pearl oyster culture have been restricted to examining impact on growth. For instance, mathematical modeling of *P. maxima* growth was performed to determine the growth requirements of pearl oysters during the various production stages (ie. spat, juvenile and seeded oysters) (Lee, et al., 2008). Lee, et al., (2008) found that control over environmental factors that influence growth of the pearl oyster (water temperature, salinity and food availability) can be somewhat exerted through careful selection of culture sites within a farm. However, the role that the site, and thus the change in multiple environmental factors, play in determining the quality of the pearls harvested has not been explored. In response to this gap in our understanding, a study was instigated whereby pearl oysters from two genetically differentiated spawnings were each reared at four geographically isolated sites within a large *P. maxima* pearl farm in Indonesia. Data were collected on oyster shell growth traits, along with pearl size and quality traits, to determine if pearl quality and value were significantly influenced by grow-out location within a pearl farm.

2.2 Materials and Methods

2.2.1 Experimental animals

Mass spawnings involving 50 broodstock oysters were conducted in October 2003 and March 2004 in a commercial hatchery resulting in the production of two separate genetic cohorts of *P. maxima*. The broodstock for these two cohorts were selected for

their silver/white nacre colour (Atlas South Sea Pearl, Bali, Indonesia). Before spawning, all broodstock were individually tagged using Dymo™ labels with an identification number and the sex of the animal. To encourage spawning, the broodstock were initially placed in the sun for ~20 min, then positioned upright in racks situated in a 600 L spawning tank. This tank was then filled and aerated for 30 min for acclimation, alternately drained, and refilled. The animals were allowed to mass-spawn and the fertilized eggs were passed through a 200 µm screen and collected in 10 L buckets and then stocked into separate 400 L larval tanks.

After 48 hours, tanks were drained, larvae collected onto 45 µm screens and transferred into 3000 L rearing tanks where they were reared under standard commercial conditions until settlement. Once the larvae approached plantigrade metamorphosis, polypropylene ropes were placed into tanks to provide a substrate for settlement. The spat were then transferred to long-lines until they reached the required size for seeding.

2.2.2 Experimental design and site selection

Host oysters were selected to be a similar size at seeding, while donor (saibo) oysters were selected based on their white nacre coloration. It is important to condition the gonad of host oysters prior to seeding so that they are not in an active phase of gametogenesis, this will lessen the incidence of rejection after the pearl nuclei are inserted. Consequently, host oysters were covered with mesh-cloth 3 weeks prior to seeding operations. Oysters were seeded with pearl nuclei during two operation periods. The first pearl seeding was undertaken in September 2005 using the fastest

growing oysters from the 2003 spawning (Cohort 1a, n=959). Pearl nuclei sizes (and weight in brackets) used for this seeding were 6.1 mm (0.33 g), 6.7 mm (0.44 g) and 7.3 mm (0.57 g). The second pearl seeding was conducted in March 2006 using both the remaining oysters from the 2003 spawning (Cohort 1b, n=1113) and those spawned in 2004 (Cohort 2, n=1076). Nucleus sizes utilized for seeding in these two oyster cohorts were 7.6 mm (0.65 g), 7.9 mm (0.73 g) and 8.2 mm (0.82 g) for cohort 1b and 7.3 mm (0.57 g), 7.6 mm (0.65 g) and 7.9 mm (0.73 g) for cohort 2. As the surgical technique and hygiene used by grafting technicians may influence pearl quality, all seeding operations were undertaken using only two technicians. Oysters seeded by each technician made up half of each 16 pocket net panel to minimize bias due to technician experience.

Once all oysters had been seeded they were transported to a single recovery site for 10 weeks post-operation. After this time they were randomly split into four groups (average 400 animals per cohort per site) and relocated to their respective grow-out sites within a single farm located in West Papua. The four sites utilized in this experiment were; Duyef (0°11. 607'S, 130°15.288'E), Maratlap (0°09.450'S, 130°17.410'E), Sasanaflapo (0°10.514'S, 130°16.468'E), and Wulu (0°11.995'S, 130°18.804'E). Sites were located 3-7 kms apart with water depths ranging from 35-45 m. Sites varied in water current and surrounding topography (Figure 2.1), however, environmental parameters were not specifically measured during this trial. Once established in their grow-out locations the seeded oysters were placed in 32 pocket-panels and cleaned according to commercial farm practices until pearls were harvested 22 months later. The animals were moved into larger 16 pocket-panels are they grew.

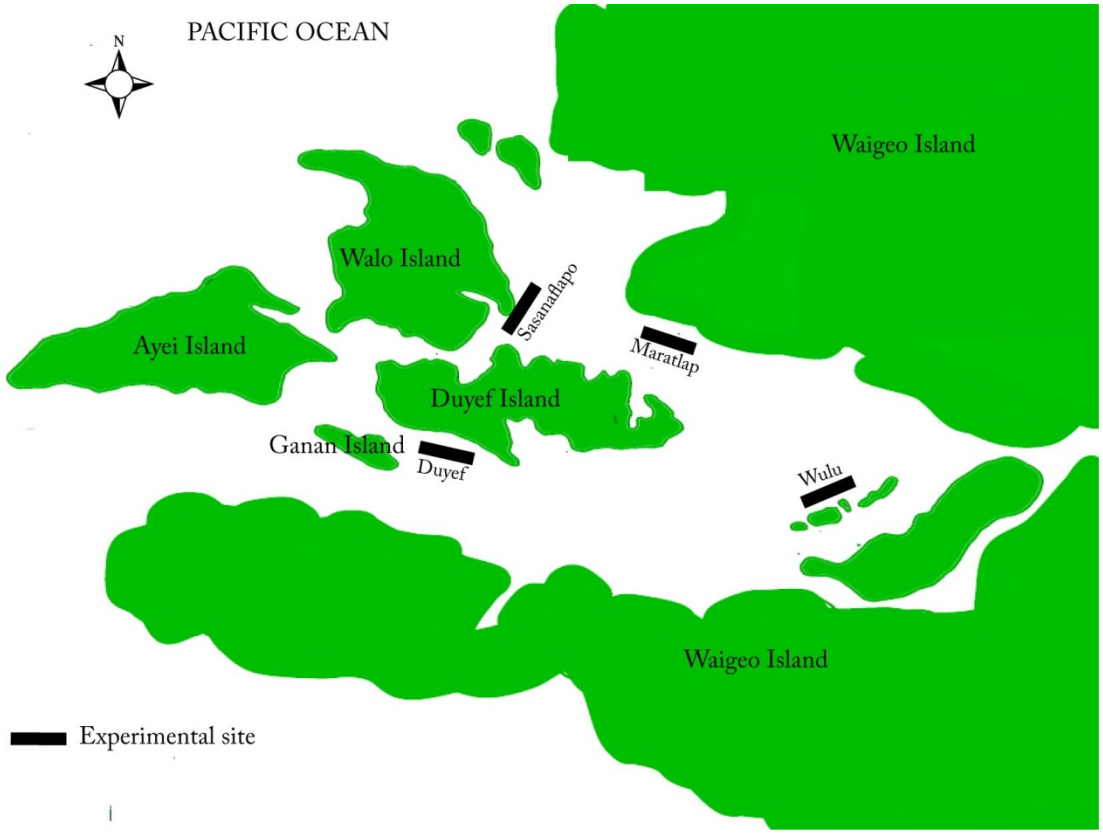


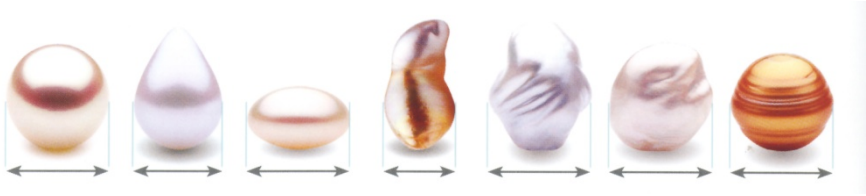
Figure 2.1. Location of the experimental sites within the *P. maxima* pearl farm site in West Papua. Modified from Lee et al., (2008).

2.2.3 Effects of site selection on pearl quality

At harvest, anterior posterior (APM) and dorsal ventral (DVM) shell growth measurements were taken from the host oyster and the host shell nacre colour recorded. Pearls, if present, were harvested and placed into an individually labeled bag for grading. If hosts did not contain a pearl, or if a keshi pearl had formed (a small irregular shaped pearl resulting after nuclei have been rejected), this was also recorded. After cleaning, pearl size (mm), weight (g), shape, lustre, surface complexion and colour were measured and commercially graded according to the criteria outlined in Table 2.1. As six different nucleus sizes were utilized for this experiment, harvested pearl size and weight were initially adjusted to determine if

actual nacre deposition values differed among pearls seeded with similar sized nuclei. However, analyses showed that the trends of these two data sets did not vary with or without inclusion of initial nucleus information. The value of each individual pearl was calculated from a proprietary formula which simultaneously evaluated all pearl quality traits (Atlas South Sea Pearl, unpublished data).

Table 2.1 Grading system for *P. maxima* pearls. A summary version from Atlas South Sea Pearls Inc. (Taylor 2007)

Characteristics	Grading description
Size: How a pearl is measured is dependent on shape	The position for measuring pearl diameter relative to the pearl shape 
Shape: In order to assist in pearl classification and valuation some broad shape categories are utilized	Round, Near Round, Semi Round, Oval, Drop, Semi Drop, High Button, Flat Button, Semi Button, Semi Baroque, Baroque, Circle
Colour: like shape, colour can only be broadly defined	White, White with silver overtone, White with pink overtone, White with fancy overtone, Silver, Silver with pink overtone, Silver with fancy overtone, Pink, Cream, Cream with pink overtone, Cream with fancy overtone, Yellow, Light Gold, Metallic Gold, Metallic Gold with fancy overtone, Fancy colour; i.e. apricot, purple, chocolate and platinum
Complexion: A blemish is an imperfection in the surface of a pearl, the cleaner the complexion, the higher the grade	A1=no blemishes or 1 small blemish, B1=1-3 very small blemishes, B2=3 or more blemishes, C1=minor blemishes all over the pearls surface, C2=blemished but still demonstrating lustre that is not appropriate for retail sale, D1=blemished low quality pearl
Lustre: a combination of reflection and refraction of light from the surface of a pearl	1=gem/brilliant: pearls appear very bright, light appears to reflect from within the pearl, 2=excellent: pearls are bright and have a good inner reflection, 3=high: pearls are bright on the surface but have minimal inner reflection, 4=modest: reflection is not clear and the pearls appear slightly opaque, 5=poor: opaque appearing “milky”

2.2.4 Statistical analysis and classification trees

Shell APM and DVM were compared amongst the grow-out sites within each spawning cohort using ANOVA (Zar, 1999), as implemented in the SPSS 16.0 software program (Coakes, et al. 2008). When ANOVA indicated significant differences ($P<0.05$) between sites, Tukey's HSD post-hoc multiple comparison test was used as the means separation procedure (Zar, 1999). Homogeneity of variances was confirmed using Levene's Test (Zar, 1999). A bivariate Pearson product-moment correlation was used to look at the relationship between APM and nacre deposited (Zar, 1999). To estimate the importance of site selection for categorical pearl quality traits (ie colour, lustre, shape, complexion), a classification tree analysis was undertaken using Treesplus (Breiman, et al., 1984). Classification tree analysis was chosen as it can be used for interactive exploration of categorical datasets and for description and prediction of variance patterns (De'ath & Fabricius, 2000).

2.3 Results

2.3.1 Effect of site selection on oyster shell growth traits

Site was found to significantly influence pearl oyster shell growth traits (Table 2.2). Cohort 1a oysters exhibited significant differences among sites in APM ($F_{3, 715} = 36.25$, $P <0.001$) and DVM ($F_{3, 715} = 25.61$, $P <0.001$), with the Sasanaflapo site producing the oysters with the largest overall shell growth. The Sasanaflapo site also produced oysters with the largest DVM for cohorts 1b and 2, as well as APM in cohort 2. As the superior growth differences at the Sasanaflapo site were observed across all three cohorts, including two distinct genetic spawnings, these results suggest that the environmental conditions at this site were the most conducive for fast

oyster shell growth. Conversely, the Maratlap site was found to produce the slowest growing oysters for all three cohorts.

Table 2.2. Mean growth (±SE) for shell characters measured in two cohorts evaluated between four Indonesian grow-out sites (Duyef, Maratlap, Sasanaflapo and Wulu). APM = Shell anterior-posterior measurement (mm), DVM = shell dorsal-ventral measurement (mm).

	Traits	*Duyef*	*Maratlap*	*Sasanaflapo*	*Wulu*
Cohort 1a	APM	135.6±0.78^{a}	132.9±0.79^{b}	143.7±0.79^{c}	136.2±0.72^{ab}
	DVM	146.6±0.78^{a}	144.2±0.77^{b}	152.7±0.68^{c}	148.5±0.60^{a}
Cohort 1b	APM	141.7±0.81^{a}	140.5±0.83^{a}	138.8±0.83^{a}	141.4±0.82^{a}
	DVM	151.2±0.74^{ab}	150.2±0.84^{ab}	153.2±0.82^{a}	150.1±0.81^{b}
Cohort 2	APM	133.7±0.67^{a}	132.5±0.61^{a}	136.2±0.61^{b}	136.4±0.65^{b}
	DVM	143.6±0.67^{a}	143.1±0.65^{a}	148.6±0.64^{b}	145.1±0.61^{a}

Means with the same superscript do not differ significantly within cohorts ($P < 0.05$)

2.3.2 Effect of site selection on pearl traits

2.3.2.1 Pearls harvested

Significant differences were observed among cohorts in the percentage of pearls harvested ($F_{2, 6} = 33.8$; $P < 0.05$), with oysters in cohort 2 producing the highest percentage of pearls (Cohort 1a 75.4%, Cohort 1b 61.9% and Cohort 2 82.5%) (Table 2.3). Differences were also evident between the percentage of pearls harvested from Cohorts 1a and 1b, despite the fact that these oysters originated from the same genetic stock. Within each cohort, there was minimal effect of site on the proportion of oysters that produced a pearl. However, among the pearls produced site had a significant impact on the percentage considered as being of low quality, or rejected for sale ($F_{3, 6} = 7.9$, $P < 0.05$). The Sasanaflapo site had the highest percentage of reject pearls (mean = 9.9%), while the Wulu site had the least (mean = 5.7%). Differences were also observed in proportion of reject pearls due to cohort ($F_{2, 6} = 40.6$, $P < 0.001$),

with cohorts 1b and 2 exhibiting a significantly higher number of reject pearls than cohort 1a.

Table 2.3. Total oysters seeded from each cohort at each evaluation grow-out site, the number of pearls produced per site, percentage pearls harvested as a proportion of total seeded per site, and percentage of pearls harvested which were reject quality per site.

Cohort		*Duyef*	*Maratlap*	*Sasanaflapo*	*Wulu*
1a	Total animals seeded	261	240	229	229
	Pearls	173	176	188	183
	% harvested	66.3%	73.3%	82.1%	79.9%
	% rejects	1.7%	2.3%	4.3%	2.7%
1b	Total animals seeded	259	234	259	264
	Pearl	152	143	163	171
	% harvested	58.7%	61.1%	62.9%	64.8%
	% rejects	10.5%	7.0%	14.1%	7.6%
2	Total animals seeded	275	285	249	267
	Pearl	228	220	210	228
	% harvested	82.9%	77.2%	84.3%	85.4%
	% rejects	8.3%	9.5%	11.4%	7.0%

2.3.3 Pearl quality traits

2.3.3.1 Pearl size and weight

Table 2.4 summarizes the mean pearl size and weight between the grow-out sites for all cohorts evaluated. Site selection significantly affected pearl size ($F_{3, 766} = 4.1$, $P < 0.05$) and weight ($F_{3, 766} = 3.3$, $P < 0.05$) for cohort 1a, and pearl weight for cohort 1b ($F_{3, 690} = 2.8$, $P < 0.05$), but not pearl size in cohort 1b ($F_{3, 690} = 1.8$, $P > 0.05$). For cohort 2 no significant differences in pearl weight and pearl size between grow-out sites were established. Although, pearl size across sites followed a similar trend to that of shell growth of the host oysters, with the largest pearls harvested at the Sasanaflapo site coming from the oysters that exhibited overall fastest growth. Likewise, pearls from Maratlap were significantly lower in size and weight in cohort 1a, however, in

cohort 1b Duyef was significantly lower in pearl weight compared to the other sites evaluated.

Table 2.4 Mean (±SE) pearl size (mm) and pearl weight (g) measured in three cohorts evaluated at four grow-out sites within a large Indonesian farm.

	Trait	*Duyef*	*Maratlap*	*Sasanaflapo*	*Wulu*
Cohort 1a	Pearl size	10.15 ± 0.09^{ab}	9.93 ± 0.08^{a}	10.30 ± 0.08^{b}	10.18 ± 0.08^{ab}
	Pearl weight	1.64 ± 0.04^{ab}	1.54 ± 0.04^{a}	1.68 ± 0.04^{b}	1.64 ± 0.04^{ab}
Cohort 1b	Pearl size	10.33 ± 0.06^{a}	10.43 ± 0.07^{a}	10.55 ± 0.06^{a}	10.40 ± 0.06^{a}
	Pearl weight	1.69 ± 0.03^{a}	1.74 ± 0.03^{ab}	1.81 ± 0.03^{b}	1.74 ± 0.03^{ab}
Cohort 2	Pearl size	10.13 ± 0.05^{a}	10.12 ± 0.05^{a}	10.05 ± 0.06^{a}	10.05 ± 0.06^{a}
	Pearl weight	1.58 ± 0.03^{a}	1.60 ± 0.02^{a}	1.57 ± 0.03^{a}	1.56 ± 0.03^{a}

Means with the same superscript do not differ significantly within cohorts ($P>0.05$)

Cohort effects in pearl size and weight as determined by nacre deposition (mm) were also observed (Table 2.5). Nacre deposition was observed to be higher in Cohort 1a, than 1b at all sites, despite oysters from these two cohorts being of the same genetic stock. Cohort 1a oysters were seeded at a younger age than those from Cohort 1b, and given the same genetic background of these two cohorts, the differences observed are likely to be due to differences in nacre deposition rate as a factor of oyster seeding age, or possibly environmental temporal differences between seeding dates due to earlier seeding of Cohort 1a. The Sasanaflapo site produced the heaviest nacre deposition for both cohort 1a and 1b, indicating that site performance did not change over time with oysters comprising the same genetic background.

Table 2.5.Mean nacre deposited (g) between cohorts within the culture sites. Mean nacre was measured as the final weight of pearls harvested minus the corresponding initial weight of the seed nucleus.

	Nacre deposited		
	Cohort 1a	Cohort 1b	Cohort 2
Duyef	1.20 ± 0.04^{a}	0.94 ± 0.02^{b}	0.93 ± 0.03^{b}
Maratlap	1.09 ± 0.04^{a}	0.98 ± 0.03^{ab}	0.95 ± 0.02^{b}
Sasanaflapo	1.24 ± 0.04^{a}	1.07 ± 0.03^{b}	0.91 ± 0.03^{c}
Wulu	1.20 ± 0.04^{a}	0.98 ± 0.03^{b}	0.90 ± 0.03^{b}

Means with the same superscript do not differ significantly ($P<0.05$)

2.3.3.2 – Pearl colour, lustre, shape and complexion

Classification tree analyses for the categorical pearl quality traits suggest that except for Cohort 1a, site location had little influence on pearl colour, lustre, shape or complexion, with no significant splits due to site effects being evident within the trees. However, for cohort 1a, analyses indicated that substantial amounts of the variance observed in both pearl colour and lustre could be partitioned by site, with the Duyef and Wulu sites producing on average more white pearls with pink overtones (n = 380), while Maratlap and Sasanaflapo produced higher numbers of silver pearls with pink overtones (n = 388). Likewise for lustre a split was formed in Cohort 1a whereby the Wulu site produced more pearls exhibiting the highest lustre grade (Grade 1, n = 257) (Figure 2.2).

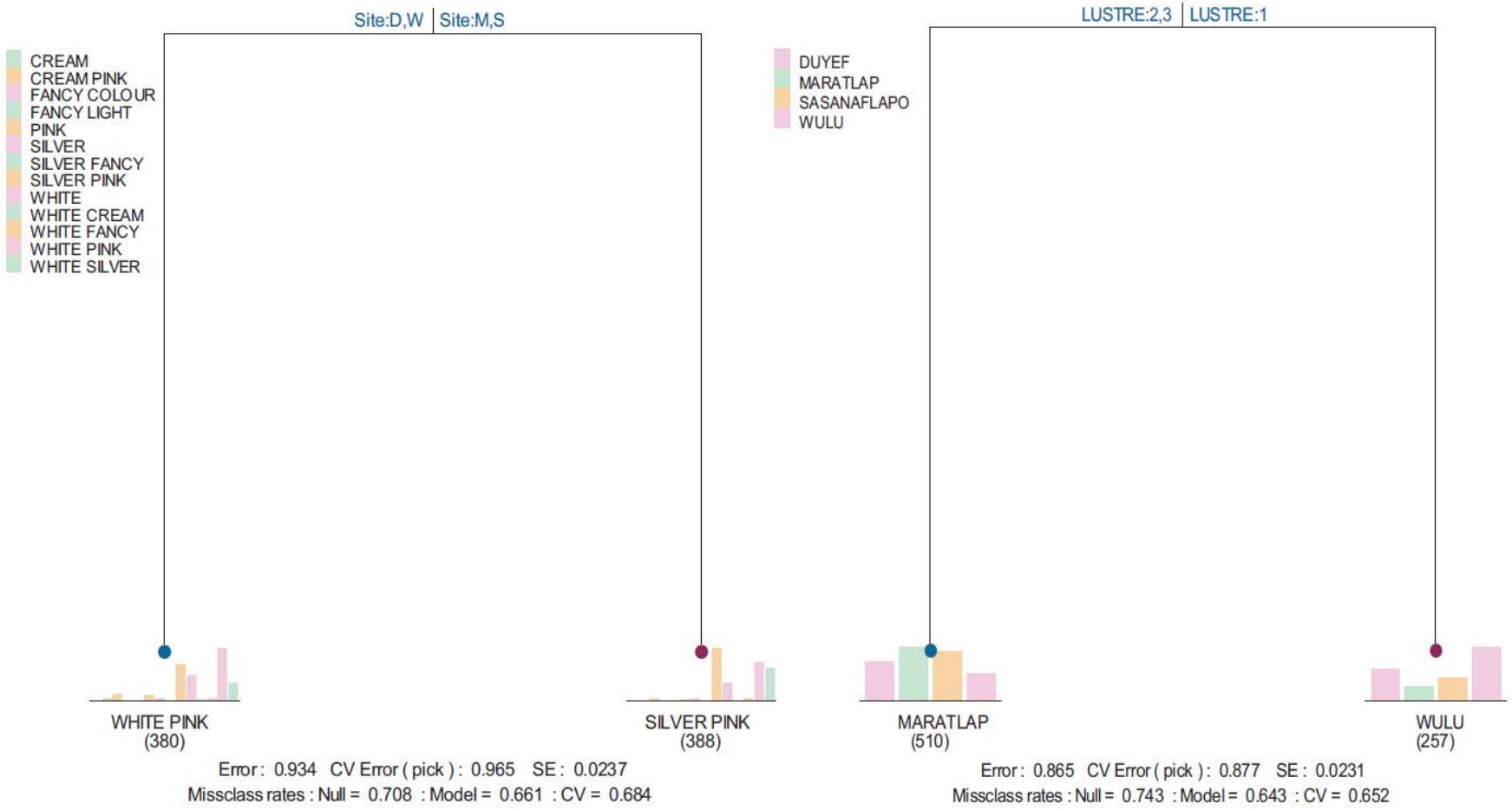


Figure 2.2. Classification tree for cohort 1a with the effect of site selection on pearl colour (a) and lustre (b). Note D=Duyef, M=Maratlap, S=Sasanaflapo and W=Wulu

2.3.4 Effect of site selection on pearl economic value

In cohort 1a, pearls significantly differed in their average value (US$) between grow-out sites ($F_{3, 696} = 2.9$, $P < 0.05$)(Table 2.6), with the Maratlap site producing pearls of average lower value than the other three sites. Overall, pearls from Duyef were of the highest value for cohorts 1a and 2, but in cohort 1b the trend was reversed with pearls produced at the Duyef site being of the lowest value. It is not known why this is the case as the oysters in cohort 1b were the same genetic stock as in cohort 1a; however one hypothesis might be that older animals were seeded as host oysters in cohort 1b and age impacted on the quality of the pearl. For cohort 1b, Sasanaflapo produced pearls with the highest average value, however, these differences were not significant across sites ($F_{3, 628} = 2.1$, $P > 0.05$) despite pearls being on average $30 higher in value between Sasanaflapo and Duyef. Cohort 2 followed the same trend as cohort 1a, with Duyef producing pearls with the highest value; again across all sites these differences were not statistically significant ($F_{3, 768} = 3.5$, $P > 0.05$) (Table 2.6). These results indicate that site selection within our trial farm did not have a major impact on the overall value of pearls produced, however, it seems the genetic composition and age of the host oysters may be of higher importance.

Table 2.6 Average pearl price (US$) ± SE measured within each cohort from the four sites evaluated. Means with the same superscript do not differ significantly within cohorts ($P < 0.05$)

Economic value	*Duyef*	*Maratlap*	*Sasanaflapo*	*Wulu*
Cohort 1a	149.81 ± 12.72^{a}	99.35 ± 5.38^{b}	124.50 ± 8.05^{ab}	137.33 ± 9.76^{a}
Cohort 1b	99.96 ± 6.16^{a}	123.63 ± 9.17^{a}	129.62 ± 9.27^{a}	126.32 ± 10.87^{a}
Cohort 2	117.77 ± 7.95^{a}	107.75 ± 7.35^{a}	106.05 ± 7.64^{a}	105.10 ± 6.48^{a}

Means with the same superscript do not differ significantly within cohorts ($P<0.05$)

2.3.5 Other analyses

To further explore the effect of host oyster growth on nacre deposition a bivariate correlation was performed. Here a weak positive correlation was found whereby oysters with larger APM produced pearls with thicker nacre ($r = 0.262$, $P < 0.05$; Figure 2.3).

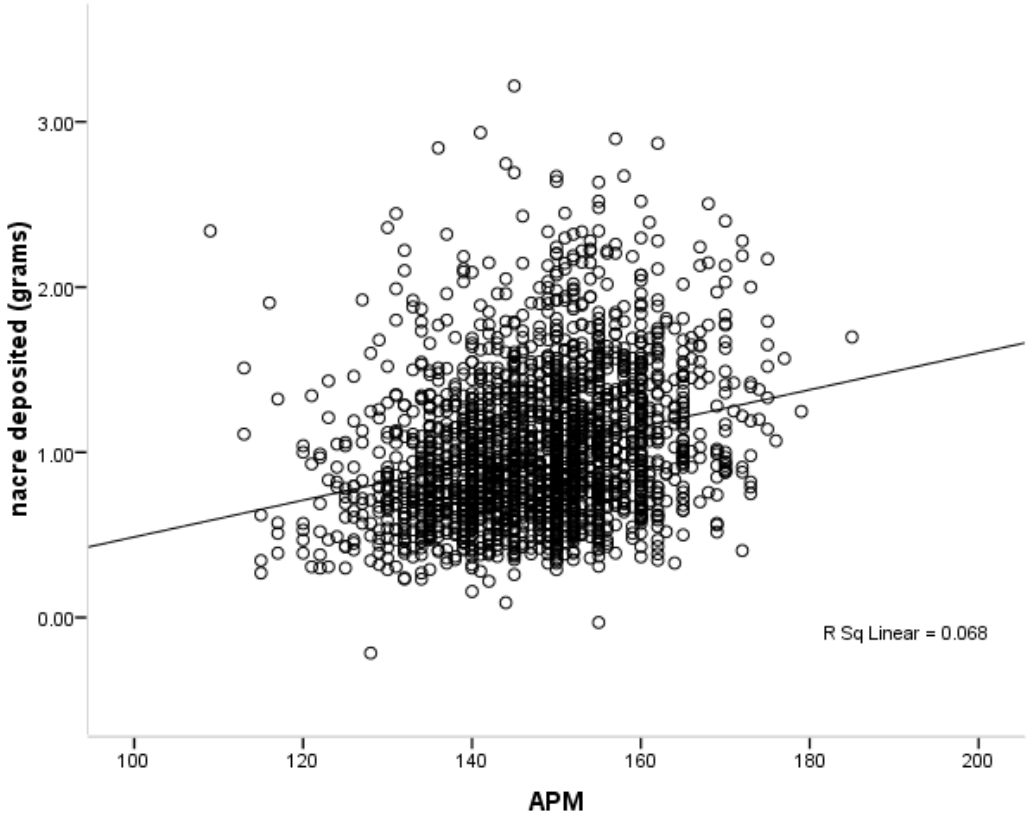


Figure 2.3. Scatterplot showing correlations between host oyster APM (mm) and pearl nacre deposited (grams).

Finally, as nacre colour of the host oysters used in this study could be classified into two distinct *P. maxima* phenotypes (gold-lipped and Silver-lipped) we examined if host nacre colour was associated with pearl nacre deposition. When pearls were grouped within nucleus sizes implanted, gold nacre coloured host oysters produced on average larger pearls for the same nucleus size than silver nacred hosts (e.g. nucleus

size 6.7 mm ($F_{1, 359}$ = 8.2; P <0.05), 7.3 mm ($F_{1, 434}$ = 13.8; P<0.001), 7.6 mm ($F_{1, 433}$ = 12.2; P<0.001) and 7.9 mm ($F_{1, 574}$ = 4.2; P <0.05) (Table 2.7). However, size differences were not significantly different for the smallest and largest nucleus sizes used (e.g. 6.1 mm ($F_{1, 213}$ = 1.0; P>0.0.05) and 8.2 mm ($F_{1, 324}$ = 1.8; P>0.05), although gold-nacred hosts were observed to still produce slightly larger pearls for these nucleus sizes.

Table 2.7 Mean pearl size (mm) between silver and gold nacre colour host oysters, for each pearl seed nucleus size implanted

Nucleus size	Pearl size	
	Silver Nacre	Gold Nacre
6.1 mm	9.45 ± 0.11[a]	9.57 ± 0.11[a]
6.7 mm	10.01 ± 0.09[a]	10.35 ± 0.07[b]
7.3 mm	9.88 ± 0.07[a]	10.27 ± 0.06[b]
7.6 mm	9.86 ± 0.05[a]	10.14 ± 0.05[b]
7.9 mm	10.29 ± 0.06[a]	10.45± 0.05[b]
8.2 mm	10.59 ± 0.06[a]	10.71 ± 0.08[a]

Means with the same superscript do not differ significantly (P<0.05)

2.4 Discussion

2.4.1 Effect of site selection within a farm on important pearl oyster growth traits

Growth of oysters destined to be hosts is an important trait in pearl farming. Generally, the faster the growth the less time needed for oysters to be cultured to a shell size where they can be seeded. As this phase of culture takes approximately 2 years in *P. maxima,* substantial cost savings could be achieved if site location was shown to significantly influence shell growth. In this case, sites could be chosen specifically for the purpose of culturing fast growing host oysters, expediting the total time taken to produce a pearl. Accordingly, our results indicate that site location does have a significant impact on host oyster shell growth. We found that oysters at one

site in particular, that of Sasanaflapo, grew significantly faster than those at the other sites. These growth differences manifested regardless of the age or genetic composition of the cohort evaluated, and therefore, will primarily be due to environmental influences within the sites, rather than individual genetic or temporal effects. Extensive environmental parameters were not collected in this study and identification of the factors responsible for faster growth at Sasanoflapo was not the purpose of this study. However, the Sasanaflapo site is exposed to the fastest water current velocities out of all sites evaluated (Atlas South Sea Pearl, unpublished data), and as *P. maxima* is a sedentary filter feeder, these faster moving currents may have brought a more constant supply of suspended food particles and dissolved oxygen to oysters. Fast current velocities have been shown to positively influence growth in another pearl oyster species (i.e., *P. margaritifera* (Mavuti, et al., 2005), as well as other mollusks (Lenihan, et al., 1996), and identification of site locations with faster water currents may be one parameter that farmers can use to identify superior locations to grow host oysters. The observation that the Sasanoflapo site produced faster growing oysters in two genetic cohorts, as well as across two independent evaluation time periods, also suggests that these site effects are reasonably consistent in their effect on growth over time and across genetic cohorts.

In addition to reduced time to seeding, a potential added benefit of identifying sites leading to superior oyster growth is that host oyster growth in *P. maxima* is correlated with nacre deposition rate (albeit weakly)(but see discussion below). Here we found that host oysters from the Sasanoflapo site, or more generally hosts with larger APM, produced bigger and heavier pearls (after standardization for the implanted nucleus size/weight). A similar correlation between shell growth and pearl weight was also

shown for the Japanese pearl oyster, *P. fucata martensii* (Wada & Komaru, 1996), indicating that the host oyster has an influential role in determining pearl size and weight. Exactly how the host genetically contributes to the formation of a cultured pearl is not understood, but the correlation observed with shell growth rate may be related to a more general capacity of host oysters to allocate energy for cellular growth and nacre deposition processes (Wada and Jerry, 2008). As highlighted above, access to more food resources may provide hosts with an overall greater energy budget to put into pearl sac tissue growth and biomineralization activity.

2.4.2 Effects of site selection within a farm on pearl quality traits and economic outcome

2.4.2.1 Economic value

In two of the three cohorts, site effects on the average value of pearls harvested were not statistically different among sites. This suggests that within our trial farm site differences did not strongly affect overall pearl value. However, in the case of Cohort 1b in particular, it has to be noted that while differences were non-significant, Duyef oysters produced pearls on average US$24-30 less valuable than at other sites and from a harvest of 100,000's pearls this would represent a significant financial loss to the farm. Economic value of pearls was also inconsistent due to site, as overall the Duyef site produced the most valuable pearls for cohort 1a and 2. These results demonstrate the complexity of predicting patterns in relation to pearl production.

Pearl size is one of the primary traits determining price. The larger that pearls are for a similar quality grade the more valuable. Pearls can be larger due either to a bigger seed nucleus inserted into a host, or from increased nacre deposition rates as observed

in our study. However, our results indicate that speeding up nacre deposition rate whilst leading to larger pearls, may not increase their economic value. For instance, Sasanaflapo produced the fastest growing oysters and correspondingly also produced the largest and heaviest pearls. However, when overall economic value of the pearl was calculated accounting for the other pearl traits lustre, shape, colour and complexion, the Sasanaflapo site did not always produce the most valuable pearls and in fact had the highest rate of pearls classified as rejects (i.e. being of such poor quality they have no economic value). Kafuku & Ikenoue, (1983) similarly found in *P. fucata* that sites producing the largest and heaviest pearls also did not yield pearls with the highest value. Kafuku & Ikenoue, (1983) hypothesized that fast growth possibly associated with strong currents led to an increase in the rate of nacre secreted, however, overall pearl quality was lower as a consequence (Kafuku and Ikenoue, 1983). Commercially, fast deposition of nacre is considered to manifest as pearls with reduced lustre and higher surface blemishes and accordingly it is common practice to lower oysters deeper into non-food rich sections of the water column a few months before harvest to produce a smoother, more lustrous, pearl. Therefore, placing host oysters in fast-growth inducing sites after nucleus seeding should be avoided as there is accumulating evidence that while fast growing hosts will produce larger pearls, there are negative correlated impacts on other traits determining pearl value such as lustre and surface complexion.

2.4.2.2 Pearl colour

Recent experiments using xenografted saibo tissue from two pearl oyster species have shown that base colour of pearls (ie gold, black, silver, etc) is primarily determined by the saibo donor (McGinty et al. 2010) and is caused by the inference of light within

the binding regions of the aragonite tiles (Snow, et al., 2004). While base colour is strongly dependent on the saibo used and is an important determinant in pearl price, pearls also exhibit "overtone" variations from that of the base colour. Overtones also influence pearl price. The primary determinant of these colour overtones is uncertain and may result from variations in light interference due to tile patterning (Snow et al. 2004), polyene organic pigment molecules (Soldati, et al., 2008), or as suggested by Yang et al. (2004) for freshwater pearls, may reflect micro-elements bound up in the organic matrix (Yang, et al., 2004). Our data for cohort 1a indicates that local site environmental conditions have some influence on colour overtones, as classification tree analyses were able to identify a distinct partitioning of pearls with white/pink and silver/pink overtones between the Duyef/Wulu and Sasanaflapo/Maraflap sites (Figure 2.2). This split, however, was not observed among Cohort 1b and 2 oysters, suggesting that the influence of site effects on colour overtones may be small overall and their effects too inconsistent to predict in a commercial setting (as evident in temporal differences between cohort 1a and 1b oysters).

2.5 Conclusion

This chapter investigated the importance of site selection within a farm on pearl oyster shell and pearl quality traits. The results indicate that site selection within a farm affected shell growth, the size and weight of the pearls produced and to some extent pearl colour and lustre. However, once all pearl quality traits are combined to determine the overall economic value of pearls harvested, local site impacts within the trial farm were observed to be inconsistent and of minor importance for two of the

three cohorts evaluated. Unfortunately, water quality parameter data for this trial was unable to be collected and as a result it is uncertain how really different the environment over the 3-7 kms between longline sites really was. Therefore, results from this chapter need to be considered with caution in the case of farms where there are significant variation in water chemistry and flow rates between longline sites.

Chapter 3. Growth and genotype by environment interactions in Silver-lipped pearl oyster (*Pinctada maxima*) spat reared under disparate environments.

3.1 Introduction

Historically, pearl production in the silver or gold lip pearl oyster, *Pinctada maxima*, was based on the harvest of spat from the wild and the subsequent rearing of oysters on commercial long-lines (Gervis and Sims, 1992). The development of hatchery techniques over the last fifteen years has seen a shift to the point where the life-cycle could be considered closed and reliable hatchery production of seed stock has occurred. Closure of the life-cycle has allowed the possibility for selective breeding programs to be instigated (Gjedrem, 2005) and recently there has been increased interest from pearling companies in the application of quantitative genetic breeding methodologies to the improvement of traits of economic interest, such as survival and growth rate (Knauer et al., 2007). The benefits of selective breeding have been amply demonstrated in livestock and several species of fish (Gjedrem, 2000); however, directional selection has rarely been applied to pearl oysters. Applying modern breeding methodologies to the pearling industry could have dramatic impacts on productivity and profitability through improvements in growth characteristics of oysters, as well as increases in the uniformity and quality of pearls (Knauer et al., 2007; Rose and Baker, 1994). In order to exploit the full potential from selective breeding, there needs to be an understanding of the genetic basis of traits under selection and what influence the environment has on the overall realization of the phenotype. Of particular interest to pearling companies is whether selection decisions under one set of environmental conditions will be correlated with similar genetic

gains when progeny are reared under disparate environments (so-termed genotype by environment (G x E) interactions).

G x E interactions are pervasive in natural and culture biological systems (Baker, 1987) and occur when levels of gene expression regulating a trait changes between environments, most commonly as a consequence of differing selective pressures. An understanding of their potential impact is essential to ensure maximum genetic gains are achieved before targeted breeding begins. For example, genotype by environment interactions were shown to influence growth rates in selectively bred Pacific oysters (*Crassostrea gigas*), where selection responses were lower than predicted when progeny were reared at locations other than that in which their parents were selected (Langdon, et al., 2003). This demonstrates, in this species at least, that the genetic potential of selectively bred animals is not always realized when reared in environments different from where selection actually took place. Significant occurrences of G x E interactions have also been identified in other bivalves, including the eastern oyster (*C. virginica*) (Newkirk, 1978, 1980) and hard clam (Rawson and Hilbish, 1991). When G x E interactions are considerable like in the examples above, a breeding program will need to be tailored for each of the different environments in which the animals are to be commercially produced (Gjedrem, 2005).

This study examined four shell morphology traits in silver-lipped pearl oyster spat when reared under several environmental conditions to an age of 43 days, to firstly determine how early rearing environment influences spat growth, and secondly, to estimate the effect G x E interactions have on phenotypic growth expression in this species. Measures of spat shell growth after 43-days rearing were chosen as

morphological indicators in the study, as shell size is commonly used by farmers to grade individuals into grow-out panels before transfer to ocean long-lines.

3.2 Materials and methods

3.2.1 Experimental animals

Five full-sib families were produced in a commercial hatchery (Atlas South Sea Pearls/Cendana Indopearls, Bali, Indonesia) by spawning five "non-selected" female and five male broodstock. Before spawning, all broodstock were individually tagged using Dymo labels with an identification number and the sex of the animal. To encourage spawning, the broodstock were initially placed out of water in the sun for ~20 min, then positioned upright in racks situated in a 600-l spawning tank. This tank was then filled and aerated for 30 min for acclimation, alternately drained, and then refilled. This technique was used to stress the oyster into spawning. A potential source of error using this methodology is that sperm from spawning males in the tank might fertilize some eggs before the manipulated full-sib crosses were made. To minimize this risk of contamination the eggs were thoroughly rinsed out of the female mantle cavity once she started to spawn. The female was then placed into an individual spawning tray whereby she recommenced releasing eggs which had not come into contact with sperm. The eggs were then passed through a 200 μm screen to filter debris and collected in 10-l buckets. Sperm from individual spawning males were collected in a similar way. Fertilization took place in 10-l buckets by combining eggs and sperm from an individual male and female, and each full-sib family was stocked into separate 400-l larval tanks for rearing.

After 48 hours, tanks were drained down, larvae collected onto 45 µm screens, and each family transferred into separate 3000-l rearing tanks where they were reared under standard commercial conditions until settlement. Once the larvae approached plantigrade metamorphosis, polypropylene ropes were placed into tanks to provide a substrate for settlement. Spat were allowed to grow in the larval tanks for five days before the initiation of experimental trials (25 days post-spawning age).

3.2.2 Experimental setup

To obtain appropriate spat numbers for the different trials family ropes were cut into sections containing approximately 50 spat and transferred into 20-l experimental tanks at ambient temperatures (~ 28 °C) and with aeration. 100% water exchanges were conducted every 2nd day. Water quality parameters, including pH (8.0-8.3), ammonia (NH_3) (~ 0 mg/l), dissolved oxygen (> 6 mg/l), and salinity (34-35 ppt) were monitored daily and maintained within accepted ranges.

Except for the feeding quality trial, spat were reared according to commercial feeding protocols using four different algae species (*Chaetoceros calcitrans, C. gracilis, Isochrysis sp., Pavlova lutheri*) (Table 3.1).

Table 3.1. The four different algae species used to rear spat in the experimental trials. Note: stocking rates were adjusted to compensate for differences in biomass among algal species to achieve a 1:1:1:1 biomass ratio

ALGAE SPECIES	% OF TOTAL DIET COUNTED AS CELLS/ML	MULTIPLICATION RATIO
Chaetoceros calcitrans	35%	1.20
Chaetoceros gracilis	15%	1.25
Isochrysis sp.	35%	1.00
Pavlova lutheri	15%	1.00

The feeding rate at day 1 of the experiment was 20, 000 algal cells/ml/day which was increased by 2,500 cells/ml each subsequent day.

For each trial, five replicates each of approximately 50 spat per family were used to evaluate the effect of the relevant environmental variable on performance of growth traits. Experimental treatments ran for 18 days before spat were sacrificed, placed in 70% ethanol for preservation, and measured for differences in growth and shell morphology traits. All experimental animals were measured according to the protocol outlined in section 3.2.8.

3.2.3 Effects of food availability on family growth traits

To examine the effects of food availability on family growth performances, three treatments consisting of a high, medium or low feeding rate were conducted. The commercial feeding rate was considered as the control (medium) feeding rate for this trial. Here spat were fed at the rates outlined above. The lower food availability trial was fed as half of the commercial feeding rate (10,000 algal cells/ml/day), and subsequently the daily increase was only 1,250 cells/ml/day. The high food availability trial was fed as double the commercial feeding rate (40,000 algal cells/ml/day) and was increased by 5,000 cells/ml/day.

3.2.4 Effects of food quality on family growth traits

To examine the effects of food quality on family growth performance, three treatments consisting of high, medium or low food quality were evaluated. The high quality diet consisted of the algae *C. gracilis,* with the low quality diet consisting of

the algae *Nannochloropsis oculata* (Gervis and Sims, 1992). The commercial feeding protocol (Table 1) was used as the medium feed quality, consisting of two high nutritional algae species (*C. calcitrans*, *C. gracilis*) and two low nutritional algae species (*Isochrysis sp.*, *P. lutheri*).

3.2.5 Effects of salinity on family growth traits

To examine the effects of salinity on family growth performance, three treatments consisting of 29, 34 and 40 ppt salinity were tested. Here, animals were acclimated from 34 ppt using gradual salinity changes to lessen any stress caused by the new environment by conducting fifty percent water exchanges at two hour intervals until the seawater was at the required salinity. All salinity treatment animals were fed according to the commercial feeding protocol (Table 3.1).

3.2.6 Effects of hatchery vs. ocean rearing on family growth traits

To examine the effects of two uncontrolled disparate environments, spat were evaluated when reared in an oceanic environment and a commercial hatchery environment. Two replicates of 50 spat from each of the five families were allocated to either an ocean or hatchery rearing environment. The ocean group were reared randomly in the ocean at Penyabangan, Bali, Indonesia, and left untouched until the experiment ended. The hatchery treatment was reared at the same Indonesian site using ambient seawater and the feeding protocols from Table 1, with feeding ratio of 20,000 cells/ml/day at day one, then an increase of 2,500 cells per day for the duration of the experiment. All other water quality conditions were allowed to change according to natural variations.

3.2.8 Measurements and statistical analyses

Due to the small size and fragility of spat when they first settled it was not possible to standardize the initial family mean size and variances for this experiment. An assumption was therefore made that if there were significant family x environment effects influencing shell size then early family growth differences would be overcome and we would still see differences in the relative growth performance of families regardless of initial size. This proved to be correct (see results section).

All spat were photographed using a Leica DC300 digital camera connected to a microscope. The Leica Image Manager software was used to collect measurements on between 20-50 spat per replicate. Shell anterior posterior measurements (APM - the greatest horizontal distance between the anterior and posterior margins of the shell taken parallel to the hinge line), dorsal ventral measurements (DVM - greatest distance from the umbo, or original point of growth, to the furthest margin - used by several studies as the best dimension for measurement of comparative growth (Gervis and Sims, 1992), hinge length (HL - the distance between the tips of the anterior and posterior ears along the hinge line) and total shell area (tracing landmark points of the spat shell surface with the software) were compared among families, treatments, and treatment by family using ANOVA (Zar, 1999). Evidence for genotype by environment interactions on trait expression were also evaluated using a MANOVA. A Tukey HSD post-hoc multiple comparison test was also used when significant differences were identified (Zar, 1999). Homogeneity of variances was confirmed using Levene's Test (Zar, 1999). All statistical analyses were performed using the SPSS 13.0 software program.

3.3 Results

3.3.1 Effects of food availability on family growth traits

Phenotypic variances in shell traits among families for the experimental treatments are presented in Table 3.2 and Figure 3.1 (note: DVM, hinge length and shell area responded in a similar manner to APM and for brevity only the results for APM are presented).

Table 3.2. Mean (+/- SE) growth for shell characters measured in five *Pinctada maxima* families evaluated under the different parameters of food availability, food quality, salinity, and ocean vs hatchery rearing environment. DVM = shell dorso-ventral measurement (mm), APM = shell anterior-posterior measurement (mm), area = total shell area, tracing landmark point of shell (mm^2). Subscripts indicate significant differences between families at $P < 0.05$).

Parameter	Treatment	Trait	Family				
			1	2	3	4	5
Food availability	2x	DVM	1.2 (+/- 0.0)	1.2 (+/- 0.1)	1.2 (+/- 0.0)	1.2 (+/- 0.1)	1.3 (+/- 0.1)
		APM	1.7 (+/- 0.1)	1.5 (+/- 0.2)	1.5 (+/- 0.1)	1.6 (+/-0.1)	1.7 (+/-0.1)
		Hinge Length	1.6 (+/-0.1)	1.3 (+/-0.2)	1.5 (+/- 0.1)	1.5 (+/- 0.1)	1.6 (+/-0.1)
		Area	1.6 (+/-0.2)	1.3 (+/-0.3)	1.4 (+/-0.1)	1.5 (+/- 0.1)	1.7 (+/- 0.2)
	0.5x	DVM	1.4 (+/-0.0)	1.3 (+/-0.1)	1.3 (+/-0.0)	1.3 (+/- 0.0)	1.3 (+/-0.0)
		APM	1.8 (+/- 0.1)	1.7 (+/-0.1)	1.6 (+/- 0.1)	1.7 (+/- 0.1)	1.7 (+/- 0.1)
		Hinge Length	1.7 (+/-0.1)	1.6 (+/-0.1)	1.5 (+/- 0.1)	1.6 (+/-0.1)	1.6 (+/-0.1)
		Area	1.8 (+/- 0.1)	1.6 (+/- 0.2)	1.6 (+/-0.1)	1.6 (+/- 0.1)	1.8 (+/-0.1)
Food quality	High	DVM	1.4 (+/- 0.0)^{a}	1.6 (+/-0.1)^{ab}	1.4 (+/-0.0)^{ab}	1.5 (+/-0.0)^{ab}	1.5 (+/-0.0)^{b}
		APM	1.7 (+/-0.1)^{a}	2.1 (+/-0.2)^{ab}	1.8 (+/- 0.1)^{a}	1.9 (+/-0.1)^{ab}	2.0 (+/-0.1)^{b}
		Hinge Length	1.6 (+/-0.1)^{a}	2.0 (+/-0.2)^{ab}	1.6 (+/-0.1)^{ab}	1.7 (+/-0.1)^{ab}	1.9 (+/-0.1)^{b}
		Area	1.8 (+/-0.1)^{a}	2.6 (+/-0.4)^{ab}	1.8 (+/-0.1)^{a}	2.1 (+/-0.1)^{ab}	2.4 (+/-0.1)^{b}
	Low	DVM	1.2 (+/-0.0)^{a}	1.3 (+/-0.1)^{ab}	1.3 (+/-0.0)^{ab}	1.4 (+/-0.0)^{b}	1.4 (+/-0.0)^{b}
		APM	1.5 (+/-0.0)^{a}	1.7 (+/-0.1)^{ab}	1.7 (+/-0.0)^{ab}	1.8 (+/- 0.1)^{b}	1.7 (+/-0.1)^{b}
		Hinge Length	1.3 (+/-0.0)^{a}	1.6 (+/-0.1)^{ab}	1.5 (+/-0.0)^{ab}	1.6 (+/- 0.1)^{b}	1.6 (+/- 0.1)^{b}
		Area	1.4 (+/- 0.1)^{a}	1.7 (+/-0.2)^{ab}	1.7 (+/-0.1)^{ab}	1.9 (+/-0.1)^{b}	1.8 (+/-0.1)^{b}
Salinity	29ppt	DVM	1.6 (+/-0.1)	1.5 (+/- 0.1)	1.5 (+/-0.1)	1.5 (+/-0.1)	1.6 (+/-0.0)
		APM	2.1 (+/-0.1)	2.1 (+/-0.1)	2.0 (+/-0.1)	2.0 (+/-0.1)	2.2 (+/-0.1)
		Hinge Length	2.0 (+/- 0.1)	2.0 (+/- 0.1)	1.9 (+/- 0.1)	1.9 (+/-0.1)	2.1 (+/-0.1)
		Area	2.4 (+/-0.2)	2.4 (+/- 0.2)	2.3 (+/-0.2)	2.3 (+/-0.2)	2.7 (+/-0.1)
	40ppt	DVM	1.3 (+/- 0.0)	1.4 (+/-0.1)	1.3 (+/- 0.0)	1.3 (+/-0.1)	1.3 (+/-0.0)
		APM	1.7 (+/- 0.0)	1.8 (+/-0.2)	1.7 (+/-0.0)	1.7 (+/- 0.1)	1.7 (+/-0.1)
		Hinge Length	1.6 (+/-0.0)	1.8 (+/-0.2)	1.6 (+/-0.0)	1.6 (+/-0.1)	1.6 (+/-0.1)
		Area	1.6 (+/-0.1)	2.0 (+/-0.3)	1.7 (+/- 0.1)	1.7 (+/-0.1)	1.7 (+/-0.1)
Ocean vs. hatchery	Ocean	DVM	2.1 (+/-0.1)^{a}	3.0 (+/-0.1)^{b}	2.0 (+/-0.1)^{a}	2.0 (+/-0.1)^{a}	1.8 (+/-0.1)^{c}
		APM	2.5 (+/-0.1)^{a}	3.7 (+/-0.1)^{b}	2.4 (+/-0.1)^{a}	2.4 (+/- 0.1)^{a}	2.2 (+/- 0.1)^{a}
		Hinge Length	2.4 (+/-0.1)^{a}	3.5 (+/-0.1)^{b}	2.3 (+/-0.1)^{a}	2.3 (+/-0.1)^{a}	2.1 (+/-0.1)^{a}
		Area	3.7 (+/-0.2)^{a}	7.9 (+/-0.4)^{b}	3.5 (+/-0.2)^{a}	3.6 (+/-0.2)^{a}	2.9 (+/-0.2)^{a}
	Hatchery	DVM	1.2 (+/-0.1)	1.4 (+/- 0.1)	1.3(+/-0.0)	1.4 (+/-0.1)	1.4 (+/-0.0)
		APM	1.6 (+/- 0.1)	1.8 (+/-0.2)	1.7(+/- 0.1)	1.6 (+/-0.1)	1.8 (+/-0.0)
		Hinge Length	1.5 (+/-0.1)	1.7 (+/-0.2)	1.5(+/-0.1)	1.5 (+/- 0.1)	1.6 (+/- 0.0)
		Area	1.4 (+/- 0.1)	1.8 (+/- 0.3)	1.6(+/-0.1)	1.4 (+/-0.2)	1.7 (+/-0.1)

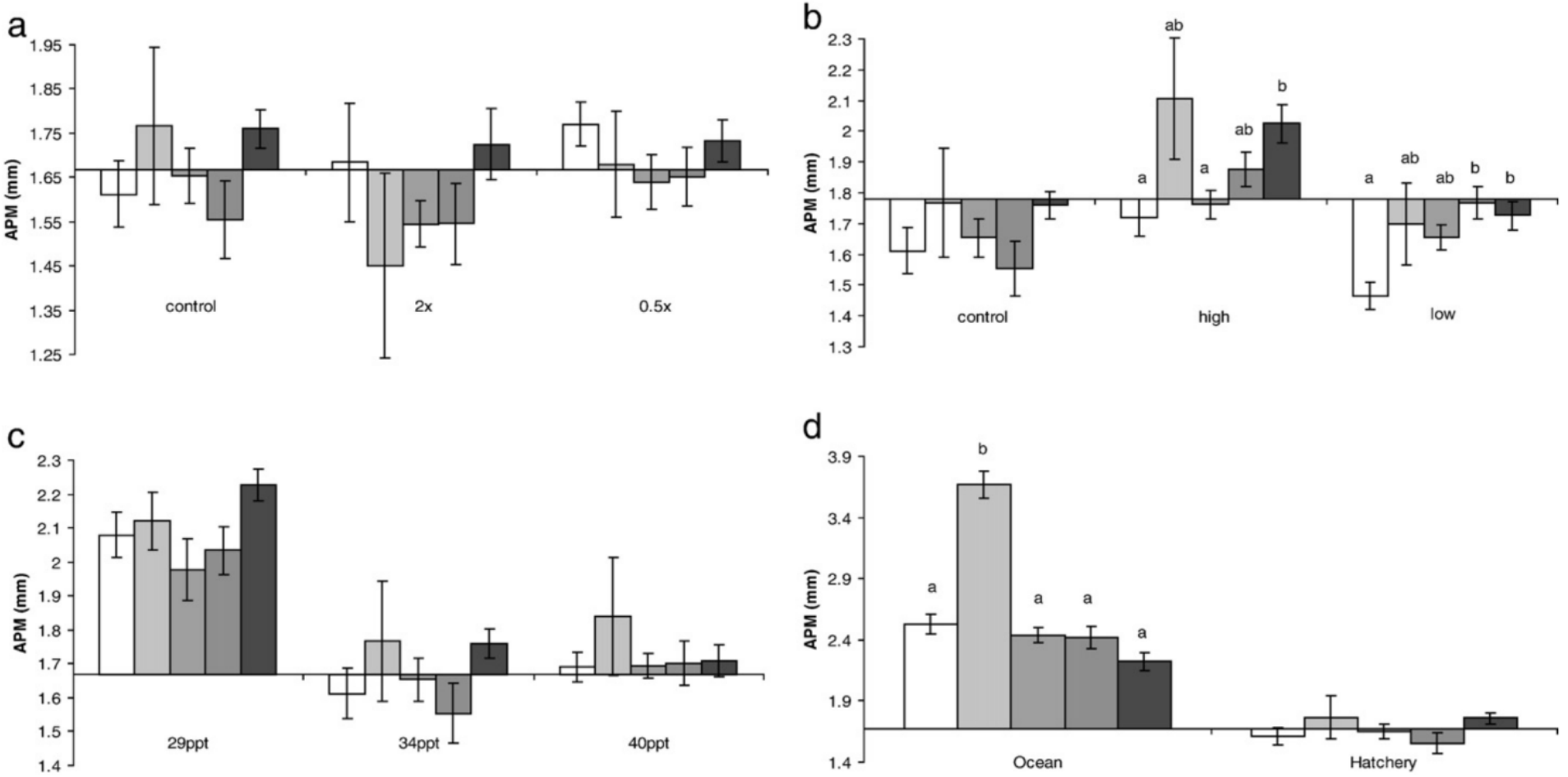


Figure 3.1. Variation in average anterior posterior measurements (APM) (mm ± SE) for five families reared in a) three different food availability treatments, b) three disparate food quality treatments, c) three different salinity treatments and d) the hatchery vs. ocean reared treatments. Notes: graph shows deviation from the mean of the control (commercial feeding or hatchery conditions) treatment. Means with the same superscript do not differ significantly (P>0.05). Bar represents SE.

When all morphological parameters were analyzed simultaneously by MANOVA significant effects due to food availability were found between experimental treatments ($P<0.05$), families ($P<0.01$) and treatment x family ($P<0.001$) (Table 3.3). However, these significant differences as indicated by the multivariate analysis appeared to be influenced largely by the inter-family difference in APM, as univariate ANOVA tests showed that on an individual trait basis the amount of food availability did not significantly change growth (Tables 3.2, 3.3).

Table 3.3 Univariate (ANOVA) and multivariate (MANOVA) analyses on the effect of food availability, food quality, salinity, and ocean vs hatchery rearing environment on the expression of four *Pinctada maxima* shell growth traits; dorsal ventral shell length (DVM), anterior posterior shell length (APM), shell hinge length and shell area.

Experiment	Trait	F	DF	SIG.
Food availability				
Treatment	DVM	2.181	2, 868	0.114
	APM	1.685	2, 868	0.186
	Hinge Length	1.858	2, 868	0.157
	Area	0.93	2, 868	0.395
	MANOVA	2.385	8, 1730	0.015
Family	DVM	1.229	4, 868	0.297
	APM	2.603	4, 868	0.035
	Hinge Length	1.863	4, 868	0.115
	Area	1.885	4, 868	0.111
	MANOVA	2.263	16, 2643	0.003
Treatment * Family	DVM	1.363	8, 868	0.209
	APM	0.848	8, 868	0.560
	Hinge Length	0.959	8, 868	0.467
	Area	0.767	8, 868	0.632
	MANOVA	2.174	32, 3191	0.000
Food quality				
Treatment	DVM	8.632	2, 1139	0.000
	APM	9.846	2, 1139	0.000
	Hinge Length	7.814	2, 1139	0.000
	Area	12.981	2, 1139	0.000
	MANOVA	11.721	8, 2272	0.000
Family	DVM	6.492	4, 1139	0.000
	APM	8.2	4, 1139	0.000
	Hinge Length	7.99	4, 1139	0.000

	Area	7.487	4, 1139	0.000
	MANOVA	3.206	16, 3471	0.000
Treatment * Family	DVM	0.678	8, 1139	0.711
	APM	1.057	8, 1139	0.391
	Hinge Length	0.901	8, 1139	0.515
	Area	1.209	8, 1139	0.290
	MANOVA	2.274	32, 4191	0.000
Salinity				
Treatment	DVM	27.795	2, 1036	0.000
	APM	36.584	2, 1036	0.000
	Hinge Length	36.092	2, 1036	0.000
	Area	33.446	2, 1036	0.000
	MANOVA	10.444	8, 2066	0.000
Family	DVM	2.737	4, 1036	0.028
	APM	3.176	4, 1036	0.013
	Hinge Length	3.413	4, 1036	0.009
	Area	2.012	4, 1036	0.091
	MANOVA	2.286	16, 3157	0.003
Treatment * Family	DVM	0.956	8, 1036	0.469
	APM	0.928	8, 1036	0.492
	Hinge Length	1.057	8, 1036	0.391
	Area	0.818	8, 1036	0.586
	MANOVA	1.231	32, 3811	0.174
Ocean vs. hatchery reared spat				
Treatment	DVM	448.976	1, 525	0.000
	APM	268.851	1, 525	0.000
	Hinge Length	256.786	1, 525	0.000
	Area	415.808	1, 525	0.000
	MANOVA	164.627	4, 525	0.000
Family	DVM	19.825	4, 525	0.000
	APM	15.097	4, 525	0.000
	Hinge Length	15.254	4, 525	0.000
	Area	29.729	4, 525	0.000
	MANOVA	11.919	16, 1595	0.000
Treatment * Family	DVM	14.664	4, 525	0.000
	APM	9.415	4, 525	0.000
	Hinge Length	8.305	4, 525	0.000
	Area	23.394	4, 525	0.000
	MANOVA	12.733	16, 1595	0.000

Despite the results of the ANOVA, however, there was some evidence for relative family growth ranking or magnitude growth changes for some morphological traits (Fig. 3.1). For example, both relative ranking and magnitude differences of APM and DVM were observed for Family 1, suggesting that if the experiment was run for longer, food availability may have led to disparate family growth among treatments for these shell traits. However, at the time the experiment concluded these effects were not large enough to translate into significant treatment by family interaction effects based on univariate ANOVA tests (Table 3.3).

3.3.2 Effects of food quality on family growth traits

Food quality significantly affected the growth performance of pearl oyster spat, with spat reared in an environment where they had access to algae of a higher quality, growing faster than those where food quality was lower or more variable. MANOVA and univariate ANOVA showed that experimental treatment (P<0.001; Table 3.3), family (P<0.001; Table 3.3) and treatment x family (MANOVA only) (P<0.001) effects significantly influenced growth variance. Therefore, the quality of food not only influenced overall spat growth as would be expected, but that within treatments families demonstrated different capacities to utilize the available nutrition source and translate this into growth. This can be observed in the high food quality treatments with family 5 showing significantly increased growth rate compared to families 1 and 3. Also in the low food quality treatment there is a significant difference in the growth rate between the families, with families 4 and 5 growing faster than family 1 (Table 3.2).

Although the MANOVA showed a significant interaction between experimental treatment and family growth response, breakdown of the analysis into individual morphological response variables failed to demonstrate differential expression of traits due to confounding

environment (Table 3.3). This once again suggests that when growth variance due to all morphological traits is pooled there are differences in the way the oyster families are growing in the disparate nutritional environments, however, these differences are not as obvious on a trait by trait basis.

3.3.3 Effects of salinity on family growth traits

Salinity was found to significantly influence the overall growth performance of pearl oyster spat (Tables 3.2, 3.3), with all families growing appreciably faster when reared at 29 ppt, compared to the higher salinities of 34 ppt (seawater control) and 40 ppt. Mean family growth expression within salinity treatments were also observed to vary, with family 5 being significantly different than family 3 in the 29 ppt treatment for hinge length. There was an obvious effect of both salinity treatment (MANOVA , $P< 0.001$; Table 3.3) and family ($P<0.01$) on the realization of spat growth. However, there was no evidence of a genotype by salinity effect on the realization of growth, with both MANOVA ($P> 0.05$) and univariate ANOVA ($P> 0.05$) treatment x family interaction terms being non-significant (Table 3.3).

3.3.4 Effects of hatchery vs. ocean rearing environment on family growth traits

Unlike the preceding experiments where only single environmental parameters were manipulated, rearing spat in an ocean versus hatchery environment produced the most dramatic differences in growth responses. Those spat from the families which were reared in the ocean grew significantly faster than those in the hatchery (MANOVA, $P<0.05$. ANOVA $P<0.001$; Tables 3.2, 3.3). There were also appreciable differences between overall family growth responses. In particular, the ocean environment favored growth in family 2, which

grew approximately twice as fast as spat in other families. As a result, significant family effects in growth response were present ($P<0.01$) (Table 3.3).

Compared to the experiments involving manipulation of single environmental parameters, evidence for a genotype by environment interaction under the uncontrolled, complex, environmental conditions were stronger, with both MANOVA ($P<0.001$) and all univariate treatment x family interactions being significant (Table 3.3).

3.4 Discussion

It is important before commencing a selective breeding program to have an understanding of how the environment might affect the realization of genetically determined growth, particularly where progeny may be reared under different environmental conditions from which their parents were selected. Different genotypes might respond in differing ways to environmental challenges and often those genotypes selected that perform well under one environment may perform poorly in another (Gjedrem, 2005). This phenomenon has been termed a genotype by environment effect and these interactions can constitute a significant proportion of the overall growth variance observed in a population.

This current study examined in *P. maxima* spat whether different environmental parameters influenced family-based phenotypic expression of early-growth related shell morphology traits, and more importantly, whether there was evidence for plasticity or modification in growth expression due to family (genotype) by environment interactions. The results from this study demonstrated that when the differences between rearing environments were large and complex (i.e. ocean vs. hatchery environments) family by environment treatment effects were evident, whereby relative family ranking performance was observed to change.

However, except for the ocean vs. hatchery rearing trial, the overall interaction effect was moderately small and was not usually present when single growth traits were evaluated.

At the environmental treatment level, a significant difference in expression of growth traits between ocean versus hatchery reared animals ($P<0.001$) was found, with superior growth observed in the spat reared in the ocean treatment. This increased growth is consistent with that observed for the closely related pearl oyster *P. margaritifera* where early spat transfer (3 weeks after settlement) to the ocean resulted in higher growth rates than for spat transferred at 6 and 9 weeks after settlement, likely as a consequence of access to a more complete nutritional diet (Pit and Southgate, 2000). Although there were no significant differences observed in growth rate between families when grown under the three food availability treatments, there was a decrease in overall growth when the level of food availability was two-fold that of standard commercial feeding practices. Several studies have likewise found that hatchery-reared oyster larvae fed high food rations exhibit lower growth (Doroudi and Southgate, 2000; Doroudi et al., 1999a; Riisgard, 1991); possibly as a result of an increase in overall microbial activity leading to a deterioration in culture water quality. However, it is uncertain if this was the cause of the slower growth observed in *P. maxima* spat, as critical water quality parameters such as ammonia did not differ between food availability treatments. Another explanation of the slower growth observed when food availability was high may be related to the ability of the pearl oyster to process high densities of algae. It is known that gut clearance rates decrease as oysters near satiation leading to valve closure and overall reduced metabolism (Riisgard, 1991) and this may have been one factor leading to the slower growth in *P. maxima* spat when reared at high algal densities.

A more important factor than food availability on overall growth performance of *P. maxima* spat was the nutritional value of the microalgae used. In this trial there was a significant difference in growth when spat from the five full-sib families were reared under the different levels of food quality. As could be predicted, animals fed the most nutritional algal diet had the highest growth rate among the treatments. Significant interactions due to nutritional diet influenced relative family growth performance within both the low and high food quality treatments, indicating the inability of some of the families to grow consistently when the diet was manipulated.

Salinity is one of the major factors affecting growth rate and survival of bivalve larvae, and previous research has found that development of bivalve embryos occurs only in conditions close to those at which spawning was conducted (Doroudi et al., 1999b). The results in our study showed that overall family growth rate was highest when spat were raised at the lowest salinities tested (i.e. 29 ppt; $P<0.001$). This result agrees with Taylor et al., (2004), who found pearl oyster spat reared at 30 ppt for 20 days exhibited significantly faster growth when compared to spat reared at 25, 34, 40 and 45 ppt. Interestingly, although our study and that of Taylor et al., (2004) demonstrates that salinity has a strong selective influence on pearl oyster growth, no evidence of a disparate interaction between salinity and the genetic realization of family growth was observed among the five families we tested. This may have purely been a consequence of the relatively few families tested and it should not be taken that all family genotypes will respond consistently if salinity varies during spat grow-out.

3.5 Summary

It is well established that productivity of aquaculture systems is largely dependent on environmental factors like water temperature, water quality, salinity and day length, which

usually cannot be controlled. As a consequence more attention should be given to quantifying potential genotype by environment effects in aquatic organisms than in other farmed animal species such as cattle or poultry, where environmental conditions are much easier to standardize (Wild, et al., 1994). Several studies have found significant genotype by environment interactions in commercially farmed aquatic animals (Gjedrem, 2005), and this study also found evidence in *P. maxima* spat for modification of growth due to genotype by environment interactions within the first 43 days after oysters settle as spat. The finding that G x E is detectable at such an early period of growth suggests that these effects may be further magnified over the two years it takes to grow an oyster to nucleus seeding size. If this is so future breeding programs aiming to improve host oyster growth rates and rear improved stocks in several locations will need to factor differential growth performance into their selection designs. However, the actual long-term impacts and importance of different environments on the realization of adult oyster growth is still unknown and this was examined in more detail in the next chapter (Chapter 4) of this thesis.

Chapter 4. Population and family growth response to disparate rearing sites and genotype x environment interaction in the Silver-lipped pearl oyster (*Pinctada maxima*)

4.1 Introduction

South-Sea pearl production is based on culture of the silver-lipped pearl oyster *Pinctada maxima*. In this species, pearl production takes four years and comprises two distinct culture phases. The first phase, or the growth phase, typically takes 18-24 months. During the growth phase oysters are reared from spat in the hatchery to a large enough size for a pearl nuclei, along with a piece of mantle tissue from a donor oyster, to be implanted into the gonad (>120 mm anterior-posterior measurement). After implantation, the second phase comprises the actual period of pearl culture and lasts a further 2 years to allow sufficient deposition of nacre to occur.

Whilst the pearling industry has embraced mechanical and technological advancement to improve efficiencies of production, the application of modern breeding methodologies as a tool to further enhance profitability is yet to be routinely applied within this industry (Knauer et al., 2007). This lack of improvement through targeted breeding programs has been due to several reasons, including a past reliance on wild oysters for pearl production, unreliable hatchery production of spat which made breeding programs hard to implement, and most importantly, a poor understanding of the role genetics has in oyster growth, pearl formation, and the realization of specific pearl quality traits such as colour, lustre and surface complexion (Wada and Jerry, 2008). In fact, despite nearly 100 years of implementing cultured pearl seeding techniques in *P. maxima*, and the South Sea pearl industry being worth US$412 million worldwide, there has been limited research into understanding the genetic

basis of oyster growth and pearl quality traits that could be used as a basis in the design of efficient breeding programs for this species (Wada and Jerry, 2008).

Whilst improving pearl quality traits such as colour, shape, lustre, surface complexion and size are considered of primary interest for selection programs, the time taken for the host oyster to complete the first phase of culture also significantly determines profitability. During the 18-24 months required to grow host oysters to a size suitable for implantation the oysters need to be constantly cleaned of bio-fouling organisms and husbandry equipment such as mesh panels and long-lines need ongoing repair and replacement. In Indonesia the cost of undertaking these activities equates to ~US$0.40/month/oyster (Joseph Taylor, unpublished data). Using genetic selection to reduce the time taken for pearl oysters to grow to a size suitable for nuclei implantation by as little as 1 month would result in substantial savings to pearling companies, as a typical farm may carry 300,000+ pre-implantation oysters (ie a cost savings of US$120,000). Consequently, breeding programs aimed at selecting for improved pearl quality would also benefit from the inclusion of improving shell growth as an additional breeding objective.

Before breeding programs for *P. maxima* should commence it is important that the genetic basis of all traits included in the breeding objective are understood. Without sufficient additive genetic variance for the trait under selection, genetic response will be slow and other genetic forms of improvement might be more effectively applied (e.g. crossbreeding, chromosome manipulation). Of particular relevance to South-Sea pearl culture is improvement of shell height and shell width, as these two traits influence the time of seeding and the size of the pearl nucleus that can be implanted. To date there have been no studies on the heritability and genetic correlation of these shell growth traits in *P. maxima*, with the only

estimates available for any pearl oyster species being based on selection response for shell convexity ([shell width]/[shell height + hinge line length + shell width]) ($h^2 = 0.35$) and width ($h^2 = 0.47$) in the related Akoya pearl oyster, *P. fucata* (Wada 1986, Velayudhan et al.,1996). Estimates of shell growth traits in other bivalves including edible oysters, however, show similar heritabilities to those seen in *P. fucata* (Sheridan, 1997) and based on these studies it is conceivable that shell growth traits in bivalves in general should be responsive to selection.

As well as the need to estimate accurate genetic parameters, an additional consideration for future breeding programs for *P. maxima* will be whether oysters realize their genetic potential when cultured at multiple grow-out locations (so called genotype by environment (G x E) interactions). Understanding genetic by environment effects in pearl culture is particularly important, as many pearling companies produce spat in hatcheries which service multiple farms, many of which may be geographically distant and subject to disparate environmental regimes. As a result, the likely influence G x E effects will have on the realization of genetic gains, both for pearl quality and oyster growth traits, may need to be factored into future breeding programs servicing multiple grow-out locations. Indeed, preliminary data on juvenile *P. maxima* (Chapter 3) and from other bivalves suggests that G x E modifications on growth patterns may be common in this group of molluscs. For instance, in Chapter 3 (and the resulting publication of Kvingedal et al., 2008) it was found that families of 43 day old *P. maxima* spat when reared under different nursery culture conditions exhibited environment dependent growth patterns. In another bivalve, the Pacific oyster *Crassostrea gigas*, significant G x E interactions were found for meat yield and survival in families when reared at different sites (Evans and Langdon, 2006), while in juvenile hard clams *Mercernaria mercenaria*, full-sib and half-sibs reared at five locations significantly varied in relative growth (Rawson and Hilbish, 1991). Although the magnitude of these G x E interactions

varied between species, these bivalve studies caution that G x E interactions may need to be factored into pearl oyster breeding program design, with estimates of their impact quantified before such programs begin. Of particular relevance is the long-time it takes to grow pearl oysters to sizes appropriate for seeding and it is possible that the initial differences in spat growth due to environment that were observed in Chapter 3 will be manifested in a larger way by the time oysters are large enough for seeding.

In response to the desire of the South Sea pearling industry to improve growth rate thereby lowering time to pearl nucleus implantation, this chapter determined for the first time in *P. maxima* the additive genetic basis and the importance of genotype by environment deviations in the realization of adult host oyster shell growth.

4.2 Materials and Methods

4.2.1. Experimental animals

Pearl oyster families were produced in a commercial hatchery (Atlas South Sea Pearl, Bali, Indonesia) by selecting broodstock from three Indonesian populations -Aru (6°43'S, 134°63'E), Bali (8°23'S, 115°14'E) and West Papua (1°13'N, 130°54'E). Spawnings were conducted solely between oysters from the same population (ie no inter-population crosses were undertaken). Before spawning, all broodstock were individually tagged using Dymo™ labels with an identification number and the sex of the animal. As per standard commercial practices, the broodstock were initially placed in the sun for ~20 min, then positioned upright in racks situated in a 600 L spawning tank. This tank was then filled with 10 µm filtered sea water and aerated for 30 min before it was alternately drained and refilled to encourage oysters to spawn. Once an oyster began to release gametes it was removed from this shared environment and its cavity rinsed with filtered seawater to remove gametes originating from

other oysters that might have been circulating in the communal tank. Spawning oysters were then moved onto a tray with individual partitioned raceways designed to allow the collection of unfertilized eggs and sperm. Female and male gametes were passed through a 200 µm screen and collected in individual 10 L buckets, where thereafter 32 maternal full-sib family groups were created by mixing the sperm from a single male with eggs from a single female. Although the object of the experiment was to initially create a number of full-sib families from each population, DNA parentage analyses subsequently indicated numerous half-sib families, most likely as a result of sperm retention from the original spawning tank in the mantle cavity of females after they were flushed clean. After ~20 minutes post fertilization, developing embryos were filtered onto a 20 µm screen and stocked into separate 400 L larval rearing tanks. After 48 hours, the 400 L tanks were drained down, larvae collected onto 45 µm screens and communally stocked as pooled cohort groups of 6 to 7 maternal full-sib families into 5000 L tanks where they were reared under standard commercial conditions until settlement. Once the larvae approached plantigrade metamorphosis, polypropylene ropes were placed into tanks to provide a substrate for settlement. When spat had settled they were transferred to long-lines at Penyabangan, Bali, Indonesia for a common grow-out period of 65 days. After the 65 days growth, oysters from each cohort were split and transferred to two commercial grow-out sites, Penyabangan, Bali (8°11'S, 114°50'E) and Malaka, Lombok (8°30'S, 116°40'E). Oysters were allowed to grow for a further 8 months before being uniquely numerically tagged for long-term identification using superglued Dymo™ labels which were replaced after each episode of biofouling treatment. Net panel location was also recorded in case of tag loss. Temperature measurements were taken from both grow-out environments using a TinyTagTM automated temperature logger every 4 hr. Temperature fluctuations at the two sites followed a similar pattern; however, temperature at the Lombok site was on average lower throughout the year (Figure 4.1). Oyster shell growth

measurements were collected at both 14 and 18 months of age. At the same time as the 18 month measurements were collected, a tissue biopsy of the foot was also taken and preserved in 70% ethanol for subsequent DNA parentage analyses.

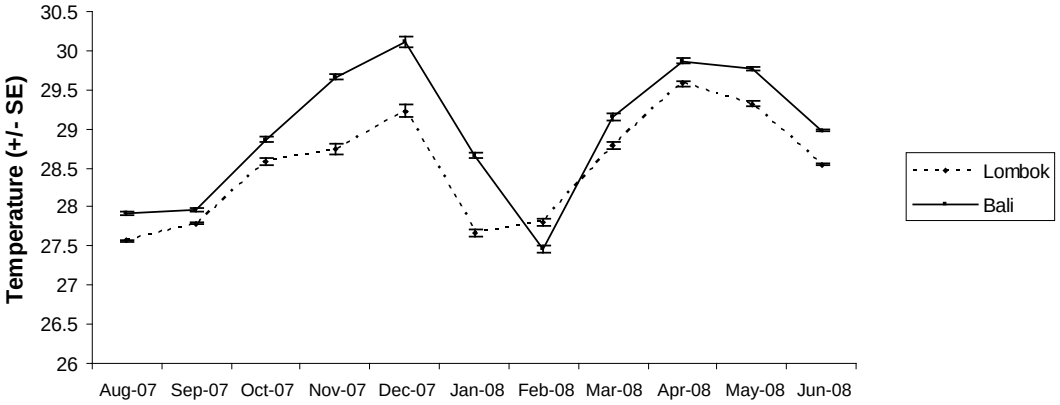


Figure 4.1. Mean monthly seawater temperature at 5 m depth at the two Indonesian grow-out locations.

4.2.2. Markers and genotyping

To assign individual oysters to their family of origin, DNA parentage analyses based on six microsatellite DNA markers were used (Pmx+022, Pmx16_23, Pmx16_41, Pmx18_21 (Smith et al., 2003), JCUPm_1g8 (Evans et al., 2006), JCUPm_26h5 (Lind et al., 2009). Here, DNA was extracted from pearl oysters by digesting ~1 mm^2 of foot tissue at 55°C for 3-4 hours in 100 µl of buffer containing 670 mM Tris-HCl pH 8.0, 166 mM NH4SO4, 0.2% Tween-20 ® (Sigma-Aldrich), 0.2% IGEPAL CA-630 ® (Sigma-Aldrich) and 1 µg µl-1 Proteinase K (Lind et al., 2009). Immediately after digestion was completed samples were incubated at 95°C for 5 min to deactivate the Proteinase K and frozen at -20°C until use. Prior to PCR, the genomic DNA (gDNA) was thawed, vortexed briefly and centrifuged for 1 min at 1000 g to remove cellular debris from the supernatant. Microsatellite markers were PCR amplified using the following conditions; 0.5 µL of crude gDNA digest was added to 1x PCR buffer (BIOLINE, no MgCl2), 2x Q-solution (QIAGEN), 1.7 mM MgCl2, 0.2 mM dNTPs, 0.33 µM

of both forward and reverse primers, 0.3 U of Taq DNA polymerase (BIOLINE) for markers Pmx+022, Pmx16_23, Pmx16_41, Pmx18_21. Total PCR reaction volumes were 15 µL. For primer sets JCUm_1g8 and JCUPm_26h5 PCR conditions were slightly modified as follows: 1x buffer (BIOLINE), 3 mM MgCl2, 0.3 mM dNTPs, 0.2 µM of both forward and reverse primers, 0.3 U of Taq DNA polymerase (BIOLINE) and 0.5 µL of DNA digest in a 15 µL total volume. PCR thermocycling conditions for Pmx16_23, Pmx16_41, Pmx18_21 and Pmx+022 were: 94 °C for 2 min; 34 cycles of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 1 min; and a final extension step of 72 °C for 5 min. For markers JCUPm1_g8 and JCUPm_26h5 the PCR conditions were: 94 °C for 3 min; 16 cycles of 94 °C for 30 sec, 58 °C for 40 sec (with a decrease by 0.5 °C for every cycle), 72 °C for 1 min; 15 cycles of 94 °C for 30 sec, 50 °C for 40 sec, 72 °C for 1 min; and a final extension of 72 °C for 5 min. To remove residual salts prior to genotyping, PCR products were purified using an ammonium acetate-ethanol precipitation protocol (Sambrook et al., 1989). PCR products together with a Tamra-400 size standard were subject to capillary electrophoresis on a MegaBACE 1000 DNA analyser (Amersham Biosciences) and allele sizes were then calculated using the MegaBACE fragment profiler version 1.2 software (Amersham Biosciences).

4.2.3 Parentage analyses

FSTAT version 2.9.3.2 (Goudet, 1995) was used to estimate basic genotypic statistics such as number of alleles, heterozygosity and polymorphic information content (PIC) of each marker. All broodstock and progeny genotypes were used as input for parental determination using the program FAP 3.5 (Taggart, 2007). To increase power of parentage assignment, analyses were first conducted based on the 6-7 parental pairs that were known to have been pooled to produce each cohort. For those oysters that could not be assigned unambiguously based on the 6-7 known parent pairs (ie possibly as a result of sperm contamination during spawning,

or their cohort identity being lost during the grow-out phase) all possible broodstock pairings were evaluated in parentage analyses. Any progeny that could not be assigned with high confidence to a parental pair were removed from further analyses.

4.2.4 Measurements and statistical analysis

In total phenotypic data from 8347 pearl oysters were collected. Shell traits recorded were anterior posterior measurements (APM -the greatest horizontal distance between the anterior and posterior margins of the shell taken parallel to the hinge line), dorsal ventral measurements or shell height (DVM -greatest distance from the umbo to the furthest margin), shell width (SW – maximum distance between external surfaces of the two valves when they are closed) and wet weight (WW). The data collected was analyzed in two ways. Firstly, because oysters could be identified to their population of origin by net panel location phenotypic data among populations and sites for the whole 8347 oysters measured at 18 months of age were compared using a general linear model. Secondly, to determine the family level effect 2400 oysters from the original 8347 oysters with phenotypic records were randomly genotyped and assigned parentage (see 4.2.3). These data were then used to estimate variance components, heritability, genetic correlations and G x E interactions for both the 14 and 18 month age datasets using an animal model in ASREML (Gilmour et al., 2002). In the model site and cohort group were treated as fixed effects and animal and dam as random effects. Evidence for genotype by environment interactions on growth trait expression was evaluated by separately analysing each growth trait in both environments as if they were different traits and then examining their genetic correlation (Falconer, 1952). The G x E interaction is the difference between 1 and the estimated genetic correlation, with the

closer the estimated genetic correlation being to unity the smaller the overall G x E interaction. Similarly to Ponzoni et al., (2008) a genetic correlation of 0.5, 0.7 and 0.9 was considered as being severe, moderate and insignificant, respectively.

4.3 Results

4.3.1 Parentage assignment

A total of 2400 oyster genotypes were analyzed of which 1900 individuals were able to be assigned to one of the known 32 male-female full-sib pairings with high confidence. Parentage analyses also identified up to 80 half-sib families, most of which contained less than 10 oysters, or were not present at both sample locations. The higher than expected number of half-sib families may have been caused by residual sperm remaining in the female oyster mantle cavity, after the removal from the communal spawning induction tank. Consequently, to decrease statistical noise from including families with low numbers of trait records, all analyses were restricted to the 47 half-sib families with more than 5 oysters per family. Genetic statistics for the six microsatellite markers are provided in Table 4.1.

Table 4.1. Number of alleles (k), polymorphic information content (PIC), expected heterozygosity (H), average non-exclusion probability for a candidate parent pair (NE-PP) and estimates of null alleles (Null) of *P. maxima* progeny from the Aru, Bali and Raja Empat populations.

Locus	K			PIC			H			NE-PP			Null		
Population	Aru	Bali	Raja Empat	Aru	Bali	Raja Empat	Aru	Bali	Raja Empat	Aru	Bali	Raja Empat	Aru	Bali	Raja Empat
022	13	14	16	0.84	0.83	0.90	0.86	0.84	0.90	0.12	0.13	0.10	-0.03	-0.03	-0.05
16_23	12	17	12	0.86	0.85	0.81	0.88	0.86	0.81	0.10	0.12	0.18	-0.01	0.04	-0.04
16_41	10	10	10	0.85	0.83	0.85	0.87	0.83	0.85	0.11	0.15	0.13	0.09	-0.03	-0.05
18_21	11	14	11	0.82	0.78	0.70	0.84	0.80	0.71	0.14	0.17	0.30	-0.01	0.01	0.00
1_g8	12	14	13	0.75	0.83	0.80	0.77	0.85	0.83	0.20	0.12	0.16	-0.05	-0.00	-0.00
26h5	4	5	4	0.30	0.79	0.52	0.32	0.63	0.60	0.71	0.49	0.53	0.10	-0.01	0.01
Mean	11	10.3	12.3	0.75	0.74	0.78	0.77	0.76	0.80	0.00	0.00	0.00	-	-	-

4.3.2 Shell growth trait analyses

Differences in shell growth traits were analyzed separately by population of origin (section 4.3.2.1) and family (section 4.3.2.2).

4.3.2.1. Population differences

Significant differences were observed between pearl oysters originating from each of the three Indonesian populations for all three shell growth traits measured (DVM -$F_{2, 8347}$= 453.2; P<0.001, APM -$F_{2, 8347}$ = 531.9; P<0.001, SW -$F_{2, 8341}$ = 524.0; P<0.001, WW -$F_{2, 8341}$ = 483.2; P<0.001) (Table 4.2). For all traits, Aru pearl oysters performed the poorest, with oysters from this population being the slowest growing at both locations evaluated. Pearl oysters from Bali and West Papua exhibited similar size metrics; however, West Papuan oysters although not always larger as measured by shell size, were significantly heavier in weight. Overall, oysters reared at the Lombok site grew significantly faster than those reared in Bali indicating site effects on growth (DVM -$F_{1, 8339}$ = 173.1; P<0.001, APM -$F_{1, 8341}$ = 251.8; P<0.001, SW -$F_{1, 8341}$ = 435.3; P<0.001, WW -$F_{1, 8341}$ = 257.8; P<0.001 (Table 4.2).

Table 4.2. Sample size and population of origin means (± standard deviation) for the four growth traits measured in *P. maxima* when grown at two sites (Bali and Lombok). *n* = number of oysters analyzed from each population.

Treatment	*Trait*	*n*	*Aru*	*N*	*Bali*	*n*	*West Papua*
Bali	DVM (mm)		85.4 ± 1.4^{a}		102.8±0.8^{b}		100.0±0.6^{b}
	APM (mm)	66	87.1 ± 1.4^{a}	323	101.8±0.7^{b}	431	100.2±0.6^{c}
	SW (mm)		17.0 ± 0.3^{a}		17.5±0.1^{b}		18.3±0.1^{c}
	WW (g)		81.5 ± 3.3^{a}		117.0±1.9^{b}		114.4±1.7^{b}
Lombok	DVM (mm)		88.5 ± 1.6^{a}		103.7±0.9^{b}		101.0±0.6^{b}
	APM (mm)	65	89.6± 1.6^{a}	254	102.1±0.9^{b}	417	101.0±0.7^{c}
	SW (mm)		17.1± 0.3^{a}		18.3±0.1^{b}		18.7±0.1^{c}
	WW (g)		90.0±3.6^{a}		118.9±2.3^{b}		118.7±1.7^{b}

APM = Shell anterior-posterior measurement (mm), DVM = shell dorsal-ventral measurement (mm), SW = shell width (mm) and WW = wet weight (g). Means with the same superscript do not differ significantly among populations in the relevant grow-out location for the trait of interest (P>0.05).

4.3.2.2 Family differences

Pearl oysters genotyped and assigned to families were observed to differ in mean familial expression for all shell growth traits (DVM – $F_{46, 1890} = 9.5$; $P<0.001$, APM -$F_{46, 1890} = 11.3$; $P<0.001$, SW -$F_{46, 1890} = 5.5$; $P<0.001$, WW -$F_{46, 1890} = 8.5$; $P<0.001$), as well as between Bali and Lombok grow-out sites for APM ($F_{1, 1890} = 4.5$; $P<0.05$) and SW ($F_{1, 1890} = 7.5$; $P<0.05$), but not for DVM ($F_{1, 1890} = 0.9$; $P>0.05$) and WW ($F_{1, 1890} = 2.6$; $P>0.05$) (Figure 4.2). Overall, those families which grew fastest at one site location also grew fastest at the other location (Figure 4.2), with genetic correlation (rg) analyses finding no evidence for family specific G X E interactions for any of the traits measured at the two locations (rg = 0.89 – 0.99, Table 4.3). This trend was consistent for both 14 and 18 month datasets indicating that genetic growth superiority is established at least as early as 14 months into production and is subsequently maintained until time of nucleus implantation.

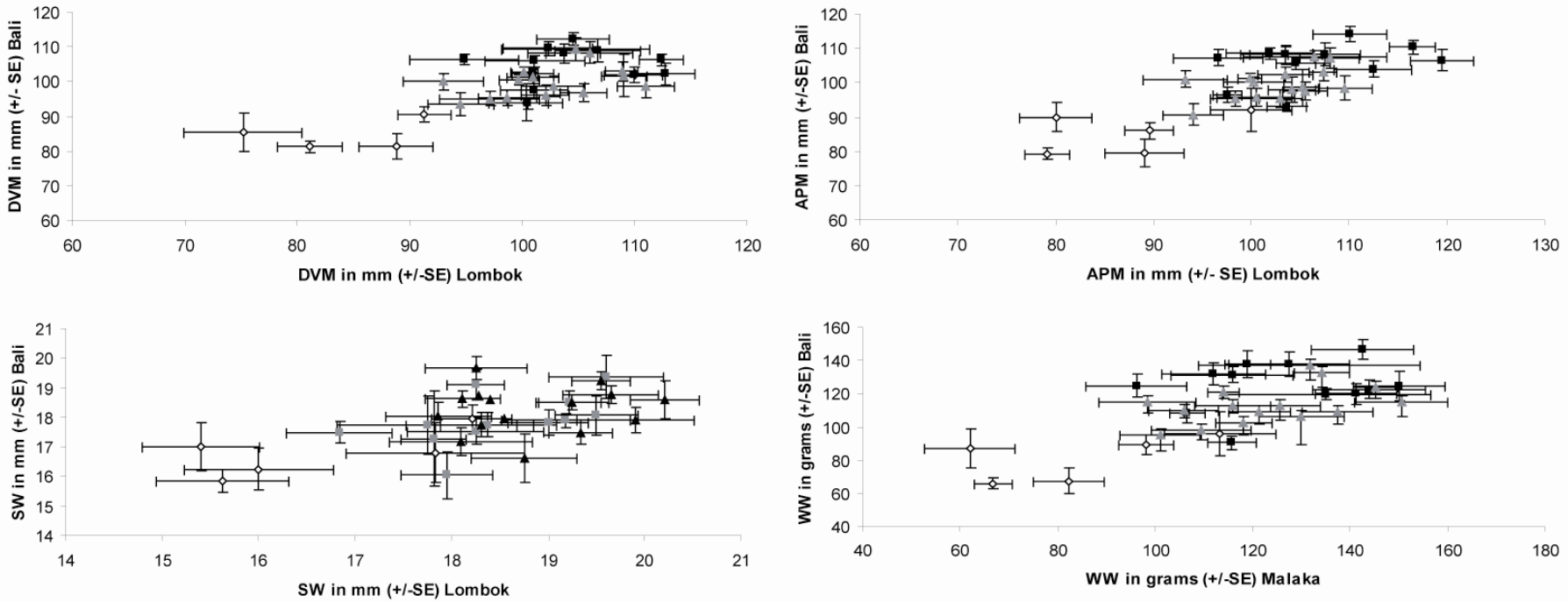


Figure 2. Comparison for a) Dorsal-Ventral Measurements, b) Anteriro-Posterior Measurements, c) Shell width and d) wet weight for *Pinctada maxima* families at 18 months when reared at two disparate locations. Note ◇ Aru families ■ Bali families ▲ West Papua families

4.3.3. Genetic parameters

Estimates of trait heritability and genetic correlations are provided in Tables 4.3 and 4.4. Except for SW at 18 months, all shell traits were moderately heritable and should be responsive to selection, with heritability estimates ranging between 0.15 ±0.003 (DVM) – 0.23 ±0.030 (APM).

Table 4.3. Heritability (h_2) and genotype by environment (r_g) estimates for *P.maxima* shell growth traits measured at two sites (Bali and Lombok) at 14 and 18 months of age. DVM = shell dorsal-ventral measurement, APM = shell anterior-posterior measurement, SW = shell width and WW = wet weight.

Trait	Parameter	14 months	18 months
DVM	h^2	0.15±0.020	0.15±0.003
	r_g	0.92±0.017	0.98±0.11
APM	h^2	0.23±0.030	0.17±0.004
	r_g	0.99±0.00	0.97±0.11
SW	h^2	0.11±0.009	0.02±0.000
	r_g	0.99±0.00	0.99±0.32
WW	h^2	0.21±0.020	0.16±0.004
	r_g	0.99±0.17	0.89±0.15

However, heritabilities were observed to slightly decrease with age. This may have been due to the variation observed among phenotypes being decreasingly influenced by additive genetic effects, or simply as a result of increased environmental determined variances modifying the traits over time. As expected, all shell traits were highly phenotypically and genetically correlated, with genetic correlations ranging between 0.74 – 0.98 for SW-APM, and DVM-WW, respectively (Table 4.4).

Table 4.4. Genetic correlations (phenotypic correlations in parentheses) between shell growth traits at 14 months (below diagonal) and 18 months (above diagonal) in the Silver-lipped pearl oyster *P. maxima*. DVM = shell dorsal-ventral measurement, APM = shell anterior-posterior measurement, SW = shell width and WW = wet weight.

Trait	DVM	APM	SW	WW
DVM	-	0.93 (0.94)	0.88 (0.73)	0.98 (0.92)
APM	0.93 (0.90)	-	0.74 (0.74)	0.92 (0.93)
SW	0.86 (0.80)	0.82 (0.81)	-	0.94 (0.79)
WW	0.97 (0.89)	0.94 (0.91)	0.94 (0.85)	-

4.4 Discussion

Growth rate is one of the primary determinants of aquaculture productivity and is the most common trait to be included as a breeding objective in selection programs. In bivalves growth primarily occurs through mantle tissue deposition of calcite and aragonite at shell margins and in pearl oysters shell dimensions are the most commonly measured traits to establish growth rate (Gervis and Sims, 1992, Rose and Baker, 1994). Shell size characters, in particular DVM, are also used as the criteria in choosing oysters large enough for pearl nucleus implantation (in *P. maxima,* DVM = 120 mm; Gervis and Sims, 1992). Accordingly, in this study four shell traits were measured in families of *P. maxima* originating from three Indonesian populations when reared at two locations and showed; a) that DVM, APM and WW traits are under moderate additive genetic influence and would respond to targeted selection; b) that pearl oyster families and populations exhibit differences in growth, with oysters originating from around the island of Aru growing the slowest under the conditions evaluated; c) that location significantly influences growth with oysters reared in Lombok growing faster than at the Bali location; and d) that phenotypic expression of shell growth traits is stable among families reared at the two locations indicating non-significant genotype-environment influences on shell growth.

4.4.1 Population growth differences

Before commencing a selective breeding program it is prudent to identify populations or strains with superior characteristics for culture as the foundation base. These populations may either be superior because of exceptional genetic merit for commercial traits, or may possess high amounts of genetic diversity (Jerry, et al., 2002). Correct choice of the founder population is important, as the baseline performance of a superior population for the trait under selection at the start of the program may in fact already equal the genetic gains that would be made by several generations of improvement based on breeding more inferior populations (Jerry, et al., 2002; Knibb, 2000). Consequently, in this chapter the performance of populations of *P. maxima* originating from three geographically distant locations within the Indonesian archipelago were evaluated to establish if growth differences in shell traits were evident and whether there may be advantages in choosing one or more populations as founders in a future breeding program.

DVM is the measurement used to determine if oysters are of sufficient size for nucleus seeding. In this study it was found that DVM in pearl oysters originating from the Aru Islands was on average 8-12% smaller depending on rearing location than oysters sourced from Bali and West Papua. These observed differential patterns in growth between the populations are likely to have a strong genetic basis, as the design of the experiment evaluated a large number of communally cultured families from each population at two different culture locations with consistent patterns in growth observed. Given the slower shell growth response in the Aru *P. maxima* population at both culture sites, it suggests that Aru oysters are either genetically predisposed to slower growth, or alternatively that under the culture conditions evaluated, the genetic potential for growth in Aru oysters was not able to be fully realized. Accordingly, if a breeding program for shell growth is going to culture oysters at the two locations evaluated in

this study, formation of the foundation stock using oysters sourced from West Papuan and Balinese populations would appear to provide the best performing oysters for the traits under selection.

The pattern of growth differences among populations observed and the suggestion that these have a substantial genetic basis is also supported by the microsatellite DNA study of Lind et al., (2007). Here Lind et al., (2007) showed that Balinese and West Papuan *P. maxima* were more genetically similar compared to comparisons with those from Aru, which was significantly differentiated from Balinese and West Papuan stocks (Lind, et al., 2007).

4.4.2 Family growth differences and genotype-environment interactions

Family-specific shell growth differences were found in this study indicating the high levels of variability within populations available for selection. As shown by high genetic correlations for shell traits among sites, the pattern of family growth performance was comparatively similar whereby fast-growing families at one location tended to be fast-growing at the other. Therefore it could be concluded that despite early estimates of growth differences observed in spat (Chapter 3) that genotype by environment deviations for family shell growth traits are of minor consequence when oysters are cultured at the Bali or Lombok site. This has important ramifications to the design of pearl oyster breeding programs for growth, as data suggests that the majority of family-determined growth potential of oysters should be realized regardless of which of the two sites oysters are reared. As a result progeny from selection candidates should perform equally well at both sites necessitating the establishment of only a single breeding nucleus servicing these two locations.

4.4.3 Effect of site location

The effect of site on growth has been evaluated in both *P. maxima* and the closely related black-lip pearl oyster *P. margaritifera*, with previous studies suggesting that localized site effects significantly influence growth in black-lip pearl oysters, but surprisingly less so for *P. maxima*. For example, both Sims (1994) and Pouvreau and Prasil (2000) demonstrated significant site-specific growth effects on *P. margaritifera* reared in the Cook Islands and French Polynesia, while Yukihira et al, (2006) also found that growth rates differed greatly between

P. margaritifera reared at two dissimilar sites within the Great Barrier Reef lagoon, Australia (Pouvreau and Prasil, 2000; Sims, 1994; Yukihira, et al., 2006). Surprisingly in the same study, *P. maxima* reared at the same two locations showed no differences in growth. In the present study, however, it was found that *P. maxima* reared in Lombok grew faster than at Bali indicating, at least at the sites evaluated, that site effects are equally important to the realization of growth in *P. maxima*. Whilst the actual cause of these growth differences among sites is unknown, field studies show that water temperature, food availability, salinity and current flow rate all have an influence on growth (reviewed in Saucedo and Southgate, 2005). Given that the Lombok site experiences more thermally stable annual water temperatures (Figure 4.1), higher phytoplankton abundance, and is exposed to slightly higher current velocities than the Bali site (J. Taylor; personal observation), these environmental effects are likely to have contributed to the site-specific differences in growth detected.

4.4.4 Heritability and genetic correlations

This study is the first to report heritability and genetic correlation estimates of shell traits in the silver-lipped pearl oyster, *P. maxima*, and the second in a pearl oyster species to report estimates for shell width (Wada, 1986). Except for width, all shell traits in *P. maxima* exhibit

moderate additive genetic variances and should respond to targeted selection as has been observed in breeding programs to increase shell growth traits in other bivalve species (i.e scallops *Euvola ziczac,* Perez and Alfonsic, 1999; *Argopecten ventricosus,* Ibarra et al., 1999). However, heritability of shell width was substantially lower than that predicted in *P. fucata* (h^2 = 0.47)(Wada, 1986), indicating either small additive genetic effects or the influence of large environmental variance for this trait in *P. maxima*. Whilst shell width is not the primary trait influencing time of nucleus implantation, along with the size of the gonad it does have some impact on the size of the nucleus seed that can be implanted. Therefore it will be important to ensure that as breeding programs progress that selection intensity is maintained on this trait to prevent adverse changes in shell morphometrics. Due to its low heritability, direct selection to improve shell width may be ineffectual. However, given this trait shows positive genetic correlation with DVM (the primary trait likely under selection) indirect improvement of this trait should occur as a result of correlated genetic responses.

Chapter 5. Heritability estimates and the effect of genotype x environment interaction on the production of pearl quality traits in the Silver-lipped pearl oyster, *Pinctada maxima*

5.1 Introduction

For selection programs to be successful traits of economic importance should exhibit both genetic variation within populations and importantly a moderate to strong additive genetic component (i.e. heritability). To limit the impact of unintended phenotypic consequences, or conversely to aid selection of difficult to measure traits, it is also desirable to have a good appreciation of how primary traits under selection are genetically correlated. A sound knowledge of the genetic basis of traits is therefore a prerequisite for the design and conduct of efficient, long-term commercial selection programs (Gjedrem, 2005). In aquaculture, the acquisition of genetic information over the last three decades in several species including Atlantic salmon, rainbow trout, marine shrimp and tilapia has allowed the development of large-scale breeding programs (Gjedrem, 2005). However, for many other aquaculture species targeted selection is still impeded by a fundamental lack of knowledge about the genetic basis of economically important traits. One such aquaculture industry that is interested in applying selection to improve profitability, but for which there are currently gaps in genetic understanding, is that of South Sea pearl farming, an industry based on the culture and harvest of pearls from the Silver-lipped pearl oyster, *Pinctada maxima*.

As a commercial process, the production of cultured pearls is both unique and biologically complex compared to that of other aquaculture industries. Firstly, the primary objective of pearl farming is not to produce food, but to produce a highly valued gemstone that is essentially a by-product of shell biomineralisation. The biomineralisation process leading to pearl formation is still poorly understood and after the point of nuclei implantation into a host

oyster the farmer has limited control over how a pearl develops. The quality of a harvested pearl is a product of how smooth and uniform biomineralisation of the nacreous layer around an artificial nucleus has occurred, with its overall value determined by five traits (size, colour, lustre, surface complexion and shape). Besides size, which can be quantitatively measured on a continuous scale, these traits are classified by categorical grades and determined by the expert eye of professional pearl graders (Table 5.1). It is the complex interplay of variation observed in these five traits that determines whether the pearl is classed as "gem" quality or not. The second complexity, and a complication for breeding programs, is that production of a cultured pearl is a two-step process involving an initial two year oyster grow-out phase and then requiring a further two years after oysters have been "seeded" to grow the actual pearl. The "seeding" process is essentially a surgical operation conducted by a trained technician who inserts a small piece of tissue (saibo) cut from the outer mantle edge of a sacrificed "donor" oyster, along with a small bead nuclei, into the gonad of a second "host" oyster. The host oyster is then returned to the water where hopefully the mantle graft will grow and encapsulate the nuclei and evenly deposit nacre over the following two years it takes to grow the pearl. Thus the pearl culturing process involves two oysters, each of which may genetically contribute to one or more pearl quality traits. If breeding programs are to be successful at producing higher percentages of gem quality pearls it will be essential to not only understand the genetic basis of each trait, but also to elucidate the respective genetic contribution, if any, made by the donor and/or host oyster.

Genetic studies involving pearl oysters to date have primarily focused on determining genetic parameters for shell growth. For example, Wada, (1986) and later Velayudhan et al., (1996) reported realized heritability for shell width and convexity based on selection responses in the Akoya pearl oyster *P. fucata*. Similarly for *P. maxima* moderate heritabilities for shell dorsal-

ventral and anterior-posterior measurements were found (Chapter 4 and published as Kvingedal et al., 2010). These studies demonstrate that there is the potential to increase productivity of pearl farming by selective breeding for oysters with faster shell growth, thereby reducing the time required to grow oysters to a suitable seeding size (Kvingedal, et al., 2010; Velayudhan, et al., 1996; Wada, 1986). In addition, Wada and Komaru (1996) examined the phenotypic correlation between shell valve weight and size of pearls in Akoya oysters and found a small positive relationship whereby oysters with heavier valves produced bigger pearls. Although their findings were based on phenotypic relationships it is possible that an underlying genetic correlation exists between the two traits whereby selection for faster growing oysters might also improve at least one pearl quality trait, that of pearl size (Wada and Komaru, 1996). Overall though, despite a few studies looking at shell growth, there have been no rigorous published studies reporting on the heritability of pearl quality traits and their phenotypic and genetic correlations.

Another major consideration for many pearling companies who may want to start breeding programs is that often hatcheries supply seed (i.e. juvenile oysters) to numerous grow-out sites, many which are geographically distant and subject to vastly different environmental conditions. As a result, future breeding programs also have to account for the impacts genotype by environment (G x E) interactions have on the realization of genetic gains in their design. To date, there have only been two studies in pearl oysters examining family-specific G x E and both of these have been restricted to survival and shell growth traits (Chapters 3 & 4, Kvingedal, et al., 2008; Kvingedal, et al., 2010). Furthermore, there are no reports within the literature where potential G x E impacts have been examined for pearl quality traits (Wada and Jerry, 2008).

The aim of this study therefore was to estimate heritability and genetic correlations for pearl quality traits in the silver-lipped pearl oyster, *P. maxima*; namely pearl size, colour, lustre, shape and complexion. As future breeding programs for pearls are likely to involve oysters that will be reared in geographically disparate locations the impact G x E interactions on the realization of genetic gains for pearl quality traits was also evaluated.

5.2 Materials and Methods

5.2.1 Experimental animals and site selection

Pearl oyster families were produced in a commercial hatchery (Atlas South Sea Pearl, Bali, Indonesia) using broodstock from three Indonesian populations. The population experiment utilized wild caught broodstock from Aru (6°43'S, 134°63'E), Bali (8°23'S, 115°14'E) and West Papua (1°13'N, 130°54'E). Spawnings were conducted solely between oysters originating from the same population (i.e. no inter-population crosses were undertaken). Before spawning, all broodstock were individually tagged using Dymo™ labels with an identification number and the sex of the animal. As per standard industry practices, the broodstock were initially placed in the sun for ~20 min, then positioned upright in racks situated in a 600 L spawning tank. This tank was then filled with 10 µm filtered sea water and aerated for 30 min before it was alternately drained and refilled to encourage oysters to begin spawning. Once an oyster began to spawn it was removed from this shared environment and its cavity rinsed with filtered seawater to remove the majority of gametes that might have been floating in the communal tank. Spawning oysters were moved onto a tray with individual partitioned raceways designed to allow the collection of unfertilized eggs and sperm. Female and male gametes were passed through a 200 µm screen and collected in individual 10 L buckets. Thirty-two full-sib maternal family groups were then created by

mixing the sperm from a single male with eggs from a female. Although the object of the experiment was to create a number of full-sib families from each population under evaluation, DNA parentage analyses subsequently indicated the creation of up to 80 half-sib families, possibly due to retention of sperm from the original spawning tank in the mantle cavity of several females after they were flushed clean. After ~20 minutes post-fertilization, developing embryos were filtered onto a 200 µm screen and stocked into separate 400 L larval rearing tanks.

After 48 hours, the 400 L tanks were drained, larvae collected onto 45 µm screens and the families then communally stocked in groups of 6 to 7 maternal full-sib families (termed a cohort) into 3000 L rearing tanks where they were reared under standard commercial conditions until settlement. Once the larvae approached plantigrade metamorphosis, polypropylene ropes were placed into tanks to provide a substrate for settlement. The spat were then transferred to long-lines in Penyabangan, Bali, Indonesia for a common grow-out period of 65 days. After 65 days growth, oysters were split into two groups and transferred to two grow-out locations Bali (8°11'S, 114°50'E) and Lombok (8°30'S, 116°40'E) to evaluate site-specific and genotype by environment influences on pearl quality traits.

5.2.2 Initial oyster grow-out and pearl nuclei seeding

Animals were reared under commercial conditions for 18-22 months until they were large enough for pearl nuclei seeding. A total of 585 oysters (average 55 per cohort) were chosen at random to provide saibo mantle tissue for seeding. Saibo mantle tissue from each oyster was cut into a maximum of 16 3x3 mm^2 pieces and implanted into the gonads of a total of 9810 host oysters. Like donor oysters, host oysters destined for implantation were chosen randomly from each cohort. Only oysters originating from the same parental broodstock population as

the host oyster were used as donors. To reduce the incidence of rejection after the pearl nuclei are inserted, the host oysters were conditioned prior to seeding so that they were in an active phase of gametogenesis. Host oysters were conditioned by covering with a mesh-cloth 3 weeks prior to seeding operations. Oysters were seeded with pearl nuclei over a three day period. This was undertaken for all three populations. As the surgical technique and hygiene used by grafting technicians may influence pearl quality, all seeding operations were undertaken using the same six technicians at the Bali culture site.

Once all oysters had been implanted with a nucleus they were transported in panels to a single long-line for 10 weeks to recover from the seeding operation. After this time host oysters were randomly split into two groups and again relocated to their respective grow-out sites in Bali and Lombok.

5.2.3 Measurement of pearl quality

At harvest, anterior posterior (APM), dorsal ventral (DVM) shell growth and oyster weight measurements were taken from the host oyster. Pearls, if present, were harvested and placed into an individually labeled bag for grading. If hosts did not contain a pearl, or if a keshi pearl had formed (a small irregular shaped pearl resulting after nuclei have been rejected), this was also recorded. After cleaning, pearl size (mm), weight (g), shape, lustre, complexion and colour were measured and commercially graded according to the criteria outlined in Table 5.1. The values of individual pearls were calculated from a proprietary industry formula which simultaneously evaluated all pearl quality traits (Atlas South Sea Pearl, unpublished data).

5.2.4 Markers and genotyping

DNA was extracted from all oysters used as broodstock and 2768 host and donor oysters by digesting ~1 mm^2 of sampled foot tissue (preserved in 70% ethanol) at 55°C for 3-4 hours in 100 µl of buffer containing 670 mM Tris-HCl pH 8.0, 166 mM NH_4SO_4, 0.2% Tween-20 ® (Sigma-Aldrich), 0.2% IGEPAL CA-630 ® (Sigma-Aldrich) and 1 µg $µl^{-1}$ Proteinase K. Immediately after digestion was completed samples were incubated at 95°C for 5 min to deactivate the Proteinase K and frozen at -20°C until use. Prior to PCR, the genomic DNA (gDNA) was thawed, vortexed briefly and centrifuged for 1 min at 1000 g to remove cellular debris from the supernatant.

For parentage analyses, six microsatellite DNA markers were amplified (Pmx+022, Pmx 16_23, Pmx 16_41, Pmx 18_21 (Smith, et al., 2003), JCUPm_1g8 (Evans, et al., 2006) and 26h5 (Lind, et al., 2009) using the following conditions; 0.5 µL of crude gDNA digest was added to 1x PCR buffer (BIOLINE, no $MgCl_2$), 2x Q-solution (QIAGEN), 1.7 mM $MgCl_2$, 0.2 mM dNTPs, 0.33 µM of both forward and reverse primers, 0.3 U of *Taq* DNA polymerase (BIOLINE) for markers Pmx+022, Pmx 16_23, Pmx 16_41, Pmx 18_21. Total PCR reaction volumes were 15 ul. For primer sets JCUm_1g8 and 26h5 PCR conditions were slightly modified as follows: 1x buffer (BIOLINE), 3 mM $MgCl_2$, 0.3 mM dNTPs, 0.2 µM of both forward and reverse primers, 0.3 U of *Taq* DNA polymerase (BIOLINE) and 0.5 µL of DNA digest in a 15 µL total volume. PCR thermocycling conditions for Pmx 16_23, Pmx 16_41, Pmx 18_21 and Pmx+022 were: 94 °C for 2 min; 34 cycles of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 1 min; 72 °C for 5 min, and 12 °C hold. For markers JCUPm_1_g8 and 26h5 the PCR conditions were: 94 °C for 3 min; 16 cycles of 94 °C for 30 sec, 58 °C for 40 sec (with a decrease by 0.5 °C for every cycle), 72 °C for 1 min; 15 cycles of 94 °C for 30 sec, 50 °C for 40 sec, 72 °C for 1 min; 72 °C for 5 min, and 12 °C hold. To remove residual

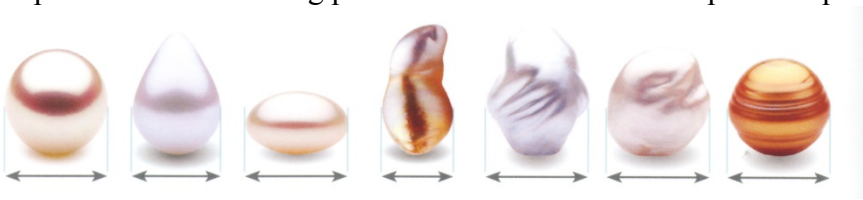
salts prior to genotyping, PCR products were purified using an ammonium acetate-ethanol precipitation protocol (Sambrook, et al., 1989). PCR products together with a Tamra-400 size standard were subject to capillary electrophoresis on a MegaBACE auto sequencer (Amersham Biosciences) and allele sizes were then calculated using the MegaBACE fragment profiler version 1.2 software (Amersham Biosciences).

5.2.4 Parentage analyses

FSTAT version 2.9.3.2 (Goudet, 1995) was used to estimate basic genotypic statistics such as heterozygosity (H), polymorphic information content (PIC) number of alleles (K) and estimates of null alleles (Null) (see Kvingedal et al. 2010). All parent and progeny genotypes were used as input for parental determination using the program FAP 3.5 (Taggart, 2007). Parentage assignment was determined firstly within cohorts based on the 6-7 parental pairs that were known groupings of progeny from a subset of the broodstock. For those oysters that could not be assigned unambiguously due to residual sperm contamination, or being moved accidently during the grow-out phase, all possible parental broodstock pairings were evaluated and examined for assignment. This approach allowed for errors and/or mix-ups in identification at any point throughout production to be identified. Any progeny that could not be assigned to a parental pair with high confidence were removed from further analyses.

5.2.5 Measurements and statistical analysis

Table 5.1. Grading system for *P. maxima* pearls. A summary version from Atlas South Sea Pearls Ltd. (Taylor, 2007)

Characteristics	Grading description
Size: How a pearl is measured is dependent on shape	The position for measuring pearl diameter relative to the pearl shape 
Shape: In order to assist in pearl classification and valuation some broad shape categories are utilized	Round, Near Round, Semi Round, Oval, Drop, Semi Drop, High Button, Flat Button, Semi Button, Semi Baroque, Baroque, Circle
Colour	White, White with silver overtone, White with pink overtone, White with fancy overtone, Silver, Silver with pink overtone, Silver with fancy overtone, Pink, Cream, Cream with pink overtone, Cream with fancy overtone, Yellow, Light Gold, Metallic Gold, Metallic Gold with fancy overtone, Fancy colour; i.e. apricot, purple, chocolate and platinum
Complexion: A blemish is an imperfection in the surface of a pearl, the cleaner the complexion, the higher the grade	A1=no blemishes or 1 small blemish, B1=1-3 very small blemishes, B2=3 or more blemishes, C1=minor blemishes all over the pearls surface, C2=blemished but still demonstrating lustre that is not appropriate for retail sale, D1=blemished low quality pearl
Lustre: a combination of reflection and refraction of light from the surface of a pearl	1=gem/brilliant: pearls appear very bright, light appears to reflect from within the pearl, 2=excellent: pearls are bright and have a good inner reflection, 3=high: pearls are bright on the surface but have minimal inner reflection, 4=modest: reflection is not clear and the pearls appear slightly opaque, 5=poor: opaque to the point of appearing "milky"

Data was analysed with a linear mixed model using ASReml (Gilmour, et al., 2006). Variance components were estimated by restricted maximum likelihood (REML) procedures using an animal model based on donor oyster relationships. The model was;

$$y = Xb + Z_a a + e$$

where y is the vector of observations, b, a and e are the corresponding vectors of fixed, direct genetic, and random residual effects, respectively. Host genetic variation was considered as a common environmental effect. The design matrices, X, and Z_a relate the fixed, and direct genetic effects to observations. The fixed effects in the model included: location (two levels), cohort (five levels) and technician (five levels). Two way interactions were tested. The variance covariance structure for the effects was;

$$v\begin{bmatrix} a \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & 0 & I_n\sigma_e^2 \end{bmatrix}$$

where A is the numerator relationship matrix, I_n is the identity matrices for animals, and σ^2_a and σ^2_e are variance components for direct additive genetic, and residual effects, respectively. Univariate analyses were used to estimate heritabilities, whilst bivariate analyses were used to estimate the genetic and phenotypic correlations between traits.

Genotype by environment effects were quantified for pearl quality traits by bivariate analysis and examination of genetic correlations when the same traits are considered in the two different growout locations (Falconer, 1952). The residual covariance was set to zero in the model because observations for each trait were recorded on separate groups of sibs in different locations. Only those half-sib families with 10 or more progeny records were included in analyses.

5.3 Results

5.3.1 Family assignment

DNA parentage analyses identified 80 half-sib families of pearl oysters. However, only 40 of these half-sib families were found to fit the statistical requirements of heritability and genetic correlation analyses (i.e. families detected at both pearl grow-out sites with > 10 progeny records/site and that produced a sellable gem grade pearl.

5.3.2 Site and population effects on pearl quality

2516 pearl quality records were analysed from the two sites. The effect of fixed factors and their significance in the statistical model are provided in Table 5.2.

Table 5.2. Significance of the fixed effects of nucleus size, grow-out location, cohort and technician on pearl quality traits.

Fixed effect	Weight	Size	Shape	Surface	Colour	Lustre
Nucleus size[1]	**	**	Ns	*	*	**
Location (L)	ns	ns	*	Ns	Ns	**
Cohort (C)	*	*	Ns	**	*	Ns
Technican (T)	ns	ns	**	Ns	Ns	**
L*C	ns	ns	*	Ns	Ns	**
L*T	**	**	Ns	Ns	Ns	*
C*T	**	**	**	Ns	Ns	**

[1] Nucleus size was modelled as cubic polynomial
P<0.01 = **, P<0.05 = *, ns = not significant

Analyses comparing differences in pearl quality showed a significant site effect for pearl size ($F_{1, 2516}$ = 51.06; P<0.001), pearl weight ($F_{1, 2516}$ = 46.46; P<0.001) and pearl value ($F_{1, 2516}$ = 4.69; P<0.05), with pearls harvested from Lombok overall being bigger and more valuable than those harvested from Bali (Table 5.3).

Table 5.3. Mean (± SD) pearl size (mm), weight (g) and value (US$) of pearls harvested from *P. maxima* when reared at two sites (Bali, n = 1435) and Lombok, n = 1081).

Treatment	Bali	Lombok
Pearl size	9.90 ±0.03[a]	10.22 ±0.03[b]
Pearl weight	1.5 ±0.0[a]	1.7 ±0.0[b]
Pearl value	92.9 ±2.9[a]	104.1 ±4.0[b]

Means with different superscript indicate significant differences at P<0.05 for the trait when measured at the two grow-out locations.

This suggests that the Lombok site was a more productive site for pearl grow-out. Oysters from different genetic origins and the quality of pearls they produced were also evaluated at the two sites. Here no significant differences in either pearl size or weight were observed between oysters from different genetic populations in either Bali (pearl size - $F_{2,1326} = 1.553$; P>0.05; pearl weight - $F_{2,1326} = 0.174$; P>0.05) or Lombok (size - $F_{2,874} = 2.447$; P>0.05; pearl weight - $F_{2,874} = 0.308$; P>0.05) (Table 5.4), although pearls harvested from oysters from West Papua were slightly bigger and heavier at both grow-out sites (Table 5.4). Pearl value was found to significantly differ between the Bali and Aru populations at the Bali grow out site (P<0.05).

Table 5.4. Mean (± SD) pearl size (mm), weight (g) and value (US$) of pearls harvested (n) from oysters originating from three *P. maxima* populations when reared at reared at Bali and Lombok.

Treatment	*Trait*	*N*	*Aru*	*N*	*Bali*	*N*	*West Papua*
Bali	Pearl size	101	9.72 ±0.06	423	9.91 ±0.09	802	10.02 ±0.02
	Pearl weight	101	1.4 ±0.0	423	1.5±0.0	802	1.5±0.0
	Pearl value	87	62.33 ±11.2[b]	350	92.93 ±5.6[a]	727	89.99 ±3.9[ab]
Lombok	Pearl size	82	10.11 ±0.07	167	10.25±0.05	625	10.33 ±0.06
	Pearl weight	82	1.5±0.1	167	1.6±0.1	625	1.7±0.5
	Pearl value	68	71.31 ±14.1	152	93.28 ±9.5	584	101.21 ±4.8

Means with the same superscript do not differ significantly among populations in the relevant grow-out location for the trait of interest (P>0.05).

5.3.3 Heritability and genetic correlations

Heritability estimates for donor-derived pearl quality traits and their genetic and phenotypic correlations are provided in Table 5.5. A low to moderate heritability was estimated for size (h^2 = 0.13 ±0.05), weight (h^2 = 0.15 ±0.06), complexion (h^2 = 0.25 ±0.07), colour (h^2 = 0.15 ±0.05) and lustre (h^2 = 0.14 ±0.06). This indicates for the first time an additive genetic contribution by the donor oyster to these quality traits that may be selected upon. Pearl shape, however, did not have a strong heritable basis (h^2 = 0.06 ±0.03) attributable to the donor.

Table 5.5. Heritability (bold diagonal), genetic (below diagonal) and phenotypic (above diagonal) correlations (± standard error) for pearl traits

Trait	Weight	Size	Shape	Complexion	Colour	Lustre
Weight	**0.15 ± 0.06**	0.94 ± 0.01	-0.02 ± 0.03	-0.18 ± 0.03	-0.14 ± 0.03	-0.10 ± 0.03
Size	0.99 ± 0.01	**0.13 ± 0.05**	0.01 ± 0.03	-0.21 ± 0.03	-0.14 ± 0.03	-0.10 ± 0.03
Shape	-0.46 ± 0.32	-0.46 ± 0.33	**0.06 ± 0.03**	0.09 ± 0.03	-0.02 ± 0.03	-0.17 ± 0.03
Complexion	-0.11 ± 0.26	-0.15 ± 0.26	0.65 ± 0.24	**0.25 ± 0.07**	0.12 ± 0.03	0.01 ± 0.03
Colour	-0.03 ± 0.29	0.06 ± 0.30	0.62 ± 0.27	0.09 ± 0.25	**0.15 ± 0.05**	0.13 ± 0.03
Lustre	-0. 15 ± 0.29	-0.13 ± 0.30	-0.07 ± 0.38	-0.76 ± 0.18	0.13 ± 0.31	**0.14 ± 0.06**

High positive genetic correlations were found between pearl weight and size (r_g = 0.99 ±0.01), pearl shape and complexion (r_g = 0.65 ±0.24) and shape and colour (r_g = 0.62 ±0.27), whilst pearl complexion and lustre (-0.76 ± 0.18) and pearl weight and shape (r_g = -0.46 ±0.32) were negatively correlated. Estimation of genetic correlation (r_g) between the same traits at two different locations showed the possibility of severe G x E interactions occurring in oyster families grown in Bali and Lombok for pearl size (r_g = -0.22 ±0.58), colour (r_g = 0.28 ±0.36), weight (r_g = 0.34 ±0.57), shape (r_g = 0.56 ±0.39) and lustre (r_g = 0.59±0.47). However, large standard errors caused by differential representation of family data among the two sites meant that correlations could not be established very accurately. Therefore the magnitude of these G x E estimates need to be viewed with some caution and may be only indicative of the true overall effects (Table 5.6).

Table 5.6. Genetic correlations (± standard errors) between the same pearl quality traits (G x E) when measured at Bali and Lombok.

Trait	Weight	Size	Shape	Complexion	Colour	Lustre
Weight	0.34 ± 0.57					
Size		0.22 ± 0.58				
Shape			0.56 ± 0.39			
Complexion				0.85 ± 0.21		
Colour					0.28 ± 0.36	
Lustre						0.59 ± 0.47

5.4 Discussion

Improvement of pearl quality through the application of selective breeding is reliant on a good understanding of the genetic basis of quality traits. In this study it is reported for the first time in pearl oysters genetic parameters for five traits that underpin pearl value. Results show that pearl weight, size, colour, complexion and lustre are heritable and could therefore be improved by selecting donor oysters with high genetic merit. Analyses also suggest that donor oyster selection will not be effective in improving the shape of pearls (ie reducing variance from round), as this trait exhibited low additive genetic variance. Finally, macro-geographical site effects may influence the realization of some pearl quality traits such as size and weight and thus site selection is likely to have an important contribution to the production of higher quality pearls. G x E effects when farming pearls also may need to be considered when farming similar genetic stocks at multiple locations.

Variation of all pearl traits except shape exhibited an additive genetic component attributable to donor oysters. This finding confirms the significant genetic role the implanted mantle allograft plays in the biomineralisation process of a cultured pearl. Surprisingly though, until recently the fate of the mantle allograft following the grafting process and its overall genetic contribution to pearl formation has been uncertain. For example, the fate of the mantle tissue

cells after implantation and during formation of the pearl sac had not been clearly elucidated and it was unknown if they survived the immunological reaction of the host oyster (Cochennec-Laureau, et al., 2010; Herbaut, et al., 2000; Kawakami, 1952a, b). It was not until DNA was detected from *P. margaritifera* donor mantle tissue in pearl sacs using microsatellite genetic markers that it became clear that the donor cells survived the implantation procedure (Arnaud-Haond, et al., 2007). Later, McGinty et al., (2010, 2011) applied the novel approach of using *P. margaritifera* and *P. maxima* xenografts to confirm that mantle-derived cells in the pearl sac actively express two putative biomineralisation genes and that the base colour of pearls was primarily derived from the nacre colour of donor oysters (McGinty, et al., 2010; McGinty, et al., 2011). These molecular studies are in agreement with the quantitative genetic analyses presented herein and conclusively confirm that the donor oyster mantle tissue has a very active genetic role in pearl formation. Therefore selection programs aimed at improving traits such as pearl colour, lustre, complexion, and size should be achievable through targeted donor oyster selection.

Unfortunately, highly skewed numbers of individuals that produced pearls from each host oyster family prevented estimates of host oyster-derived genetic parameters for pearl traits. It is therefore possible that a proportion of the genetic variance in pearl quality traits is also contributed by the host oyster. This may be particularly true for pearl shape which was found to exhibit very little donor-derived additive variance. Shape of pearls may be influenced by the morphology of the host oyster gonad and whether the nuclei moves during the initial processes of pearl sac formation. The handling of oysters and how they are hung in panel nets after implantation may also influence shape (Taylor and Strack, 2008). How much variance in pearl shape is of environmental and/or host genetic origin is still to be determined, although analyses did find a significant effect of technician on shape and lustre, indicating

that at least for these quality traits there is a non-genetic influence dependent on the skills of the operating technicians (Table 5.2). Therefore one way to reduce variance in shape of harvested pearls is to refine implantation techniques. Pearl size may also have a host additive genetic component, as previous studies by Wada and Komaru (1996) found in *P. fucata martensii* a significant phenotypic correlation between host shell valve weight and harvested pearl size. In *P. maxima* a weak correlation in anterior-posterior measurements (APM) of the host shell valve and the amount of nacre deposited onto pearl nuclei was also observed (Chapter 2, Figure 2.3). Variance in *P. maxima* shell growth traits have heritable components (APM $h^2 = 0.23 \pm 0.030$; DVM $h^2 = 0.15 \pm 0.003$ (Chapter 4, Kvingedal et al., 2010) and consequently correlations between host oyster shell growth and deposition of pearl nacre may be dependent on the capacity of host oysters to allocate energy for cellular growth and nacre deposition processes in general (Wada and Jerry, 2008). Further studies focused on defining host heritability of pearl traits are required before it is known whether breeding programs would significantly benefit from the selection of host oysters over that which can be achieved through selecting donor oysters alone.

Another interesting finding from this study was that the value of pearls produced from pearl oysters sourced from different Indonesian populations differed when reared communally at the Bali site. Here oysters from West Papua and Bali on average were found to produce more valuable pearls. This trend was also present at the Lombok site, although statistical differences due to wider variation in pearl value were not evident. This is the first time *P. maxima* oysters from different populations have been scientifically shown to vary in the quality of pearls they produce. Based on mtDNA sequences, the Bali and West Papuan populations evaluated in the current study are genetically differentiated from those from Aru (Lind, et al., 2007) and these genetic differences also manifest as growth differences (Chapter

4, Kvingedal et al. 2010), with Aru oysters exhibiting slower growth in the environments tested. Therefore, identification of genetically superior foundation stock through conducting strain comparison trials could benefit future breeding programs, as populations like those from West Papua and Bali grow both faster to seeding age and continue on to produce higher quality pearls.

Genotype by environment interactions might also need to be factored into future breeding programs as estimated genetic correlations between two locations were less than 0.8 for all traits, except that of complexion (Falconer and MacKay, 1996). These differences were primarily driven by re-ranking of family performances indicating that the best performing family in one location may not be necessarily the family with the highest genetic merit when reared at the alternate site. Re-ranking of families will confound breeding programs and if G x E effects are found to be equally pervasive when oysters are reared at other sites this may dictate the establishment of site-specific breeding programs. Caution though needs to be exercised on the magnitude of G x E results observed as high variances in estimates were evident as a result of unequal family representation in the pool of pearl producing oysters genotyped at the two sites.

In conclusion, the current study has shown for the first time that many of the important pearl quality traits have a heritable basis and will therefore respond to selection. Whilst further work is still required to refine our understanding on the role of the host oyster to observed pearl phenotypic variance and on the overall magnitude of G x E effects, results can be used as a foundation for the design of future breeding programs aimed at improving pearl quality in pearl aquaculture.

Chapter 6 General Discussion

Pearl oyster aquaculture is unique as a primary production industry in that the farmed marketable product is primarily not the oyster itself, but the pearl it produces. Cultured pearls are the only gemstone produced by an animal, with the process of pearl production reliant on the cellular cooperation of two genetically distinct animals. This makes pearl production inimitable because it requires two distinct genotypes to create the product. It is this conceivable genetic contribution from two sources that has prevented the easy estimation of genetic parameters for pearl traits that are essential for the design of efficient selective breeding programs.

This thesis aimed to provide the fundamental knowledge essential to the development of selective breeding programs for the gold/silver lipped pearl oyster *Pinctada maxima*. As such it focused on elucidating the importance of genetic and environmental factors on pearl oyster growth and pearl quality at all levels of production, from the importance of site selection and genetic effects within a farm, through to genotype by environment influences on traits among farms at geographically distinct locations. It also dramatically shifted the paradigm of our understanding of the genetic basis of important traits for pearl aquaculture by estimating for the first time in *P. maxima* genetic parameters for host oyster growth, as well as in any pearl oyster species the heritability and genetic correlative relationships of pearl quality traits. The accumulation of this new knowledge and recognition that pearl quality traits are under additive genetic control will finally allow well-designed selection programs for this pearl producing species to be achievable.

6.1 Constraints in the South Sea pearl industry in relation to implementing a selective breeding program

Previously the Australian/Indonesian pearling industry was reliant on catching oysters from the wild to produce pearls. Due to the rapid decline of wild pearl oyster banks the fishery became heavily regulated and restricted, pushing the industry towards developing hatchery techniques to supply the demand for oysters for pearl production. Hatchery domestication was successful and by 1990 the lifecycle of the silver-lipped pearl oyster *P. maxima* was considered sufficiently closed to allow efficient seed-stock production. Due to a lack of knowledge of fundamental genetic information, however, the industry continued to collect wild broodstock and merely used hatchery production to produce larvae and spat.

As of the commencement of this thesis, no selective breeding programs had been established for *P. maxima*. There were several reasons for this. Firstly, critical knowledge on the genetic basis and stability of pearl quality traits was non-existent. Without knowing if traits were heritable (and equally the magnitude of additive genetic influence) no efficient breeding programs could be designed. Secondly, the magnitude of genotype by environment (G x E) interactions which may modify realization of genetic gains were unknown. Knowledge on the potential impact of G x E is empirical in selection of broodstock as pearling companies often operate with several farms spread across a wide geographical area and the underlying foundation of any selective breeding program is the stock population.

Understanding the significance of G x E, involves further expanding our knowledge of the environmental influence on pearl production, both at a macro-environmental level, i.e. between geographically distinct sites, and at a micro-environmental level, i.e. the subtle differences within a farm site. As a response to the paucity of information related to culture

site effects on pearl oyster growth and pearl quality, Chapter 2 examined a common grow-out situation where pearl oysters from two genetically differentiated spawning were each reared at four geographically isolated sites within a large Indonesian pearl farm. The results from this chapter indicated that site selection at a micro-scale within a farm did influence shell growth, as well as the size and weight of the pearls produced. This information is of upmost importance to pearlers as this study only investigated geographical distances of < 7 km between sites and often spat are transported greater distances than this for grow-out. So the different growth response of the pearl oysters and pearl quality traits found are likely to become even more evident in commercial farms where the animals are transported greater distances and where environmental parameters are likely to be more disparate.

Chapter 2 established to some extent that pearl oyster shell growth and some pearl quality traits were influenced by grow-out locations within a pearl farm, and to some extent by genetic background of oysters. In order to exploit the full potential from selective breeding there needs to be an understanding of the genetic basis of traits under selection, as well as what influence the environment has on the overall realization of the phenotype. Unfortunately in Chapter 2 due to not being able to collect robust environmental data it was unknown what environmental parameters might be driving changes in growth. Consequently, Chapter 3 looked at shell growth in five families of *P. maxima* spat when reared under several defined environmental conditions including salinity, food availability, food quality and hatchery vs. ocean rearing. Here differential growth between families due to salinity, food quality and hatchery vs. ocean rearing was established, as well as differences in the relative growth performance of families in the hatchery vs. ocean rearing treatment. This showed that under the environmental treatments that spat were exposed to in this experiment that weak genotype by environment interactions could occur in commercial culture if conditions were disparate

enough. However, a limitation of this experiment was that oysters were only reared for a short period of time and it was uncertain if the early family growth differences observed might manifest themselves under long-term commercial rearing conditions. Consequently, the thesis then went on in Chapters 4 and 5 to quantify the effects of G x E on shell growth and pearl quality traits among three commercially farmed pearl oyster populations when reared at two sites (Bali and Lombok), as well as estimated genetic parameters such as heritability and genetic correlations.

6.2 Understanding of heritability of important shell growth and pearl traits and the importance of determining G x E interactions.

Estimating the heritability of shell growth traits for the pearl oyster is important because of the added costs of having to rear and maintain oysters until they reach a suitable time for nuclei implantation. Selecting for faster growing oysters will improve the profitability of pearl farming by overall lowering the time it takes to produce a quality pearl (currently 4 years in *P. maxima*). Similarly, selecting for pearl quality traits that will respond to selection will result in an overall larger percentage of gem quality pearls that are harvested from each crop.

Chapter 4 determined that shell growth traits DVM, APM and WW are under moderate additive genetic influence with heritability estimates for DVM $h^2 = 0.15$, APM $h^2 = 0.23$ and WW $h^2 = 0.21$, indicating that these traits should respond to targeted selection. Chapter 5 similarly showed that five of the six pearl quality traits (pearl weight $h^2 = 0.15$, size $h^2 = 0.13$, colour $h^2 = 0.15$, complexion $h^2 = 0.25$ and lustre $h^2 = 0.14$) exhibit moderate levels of heritability based on the contribution from the donor-oyster. Shape, however, showed low

heritability. These heritability results indicating that the donor oyster genetically contributes to pearl quality are supported by McGinty et al (2010, 2011) who used xenografts between *P. margaritifera* and *P. maxima* to confirm that mantle-derived cells in the pearl sac actively express two putative biomineralisation genes and that the base colour of pearls was primarily derived from the nacre colour of donor oysters. All together these results have advanced our knowledge on the intricate relationship between host and donor oyster and although more research is needed into understanding the genetic contribution of the host oyster in regards to pearl quality traits, it is clear the donor oyster determines many of the important pearl quality traits and as such the industry should respond with creating two separate lines; one line with the breeding objective of producing faster growing host oysters, and the other line selecting donor oysters for improved pearl quality traits such as colour, lustre, complexion and size.

As well as the need to estimate accurate genetic parameters, an additional consideration for future breeding programs for *P. maxima* will be whether oysters realize their genetic potential when cultured at multiple grow-out locations. If there is a strong G x E effect on *P. maxima* pearl quality traits, there may be a need to produce several lines of oyster for the geographically different grow out sites commercial pearlers commonly farm. Chapter 3 showed that families of 43 day old *P. maxima* spat, when reared under different nursery culture conditions, exhibited environment dependent growth patterns. However, when three populations and numerous families were reared under commercial grow-out conditions for two years the results from this larger scale experiment suggested that G x E growth interactions were negligible, indicating that superior oyster families tend to be superior at the two commercial sites evaluated in this thesis. In contrast, the results from the pearl quality data indicated some family re-ranking occurring for five of six pearl quality traits indicating

that the best performing family in one of the farm sites was not necessarily the family with the highest genetic merit when reared at the alternative commercial site.

Together the results indicate that G x E effects when farming pearls (donor oysters) will need to be considered and independently evaluated when farming similar genetic stocks at multiple locations, whilst for the host oyster the pattern for family growth performance was comparatively similar whereby fast-growing families at one location tended to be fast-growing at the other necessitating the establishment of only a single-breeding nucleus for host oysters.

6.3 Future direction and concluding remarks

The information collated in this thesis provides a foundation for the establishment of *P. maxima* selective breeding programs. Based on calculated heritability estimates for both shell growth traits and pearl quality *P. maxima* is responsive towards selective breeding, and commercial gains can be therefore be made through targeted selection approaches. This research has also identified that at a minimum, two separate selection lines needs to be incorporated into selective breeding programs; one for host oysters for shell growth traits, and another for pearl quality based on donor oysters.

In addition to the knowledge generated by this thesis there are still several gaps in our understanding of the genetics behind pearl production which should be addressed in the future to further exploit the commercial benefits that a selective breeding program may achieve. Firstly, auxiliary examination is required on the heritability of pearl quality traits.

Commercial practicalities meant that this study was unable to collect enough data to accurately estimate host oyster-derived genetic parameters for pearl traits measured. Therefore the additive genetic contribution by the host oyster to pearl quality is still presently unquantified. It is conceivably possible that although pearl shape did not have a donor-oyster genetic variance component that the host oyster may influence this trait and that shape may be improved through selecting host oysters. Secondly, this thesis did not quantify what the actual environmental drivers were for the differences seen in G x E expression. Elucidation of what parameters are important in driving differences may allow the future determination of sites which will minimize possible G x E perturbations in genetic gain. Also this thesis only examined the possibility of G x E at two Indonesian island sites. To further collaborate the generality of the results found in this thesis in regard to the potential impacts G x E may have in pearl production additional growth and pearl production studies should be conducted in other regions where pearl farming is conducted (i.e. Australia).

Now that essential quantitative genetic information has been gathered the next step is to determine the breeding objective of selection programs and to conduct simulations of the different breeding schemes that can be undertaken to identify the breeding design most suited to the pearling industry that will achieve genetic gains for traits such as pearl colour, luster and size.

Throughout the course of this research it has been apparent that this industry has significant scope for improvement, and the establishment and development of future selective breeding programs will undoubtedly provide quantifiable commercial gains with an increased proportion of gem quality pearls harvested from a typical production cycle. The industry now has the essential genetic information required to make this reality finally possible.

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