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Family by environment interactions in shell size of 43-day old silver-lip pearl oyster (*Pinctada maxima*), five families reared under different nursery conditions

Renate Kvingedal [a,*], Brad S. Evans [a], Joseph J.U. Taylor [b], Jens Knauer [b], Dean R. Jerry [a]

[a] *Aquaculture Genetics Research Group, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia*
[b] *Atlas South Sea Pearl, 43 York St. Subiaco, WA 6008, Australia*

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ABSTRACT

To understand the influence the environment and associated genotype by environment interactions will exert on future silver-lip pearl oyster (*Pinctada maxima*) selective breeding programs, this study assessed the relative performance in four shell growth traits 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.

Rearing environment was found to influence growth expression, with significant differences evident when oysters were grown at different salinities in the ocean instead of hatchery, or when fed algae of differing nutritional quality. As indicated by MANOVA, family comparative growth performances were also altered when the environment changed, with significant environment by family interactions apparent in the food quality, food availability and hatchery vs. ocean rearing treatments. Changes in salinity, however, did not affect relevant family performances.

These results indicate that growth and relative family performance in *P. maxima* may change dependent on local environmental conditions and that genotype by environment effects may need to be considered in breeding programs for this species.

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 10 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 (GxE) interactions).

GxE interactions are pervasive in natural and cultured 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 GxE 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 GxE interactions are considerable like in the examples

* Corresponding author. Tel.: +61 7 47815547; fax: +61 7 47814585.
E-mail address: renate.kvingedal@jcu.edu.au (R. Kvingedal).

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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-lip 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 GxE 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.

2. Materials and methods

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 h, 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).

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 1).

The feeding rate at day 1 of the experiment was 20,000 algal cells/ml/day which was increased by 2500 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 2.7.

Table 1
The four different algae species used to rear spat in the experimental trials

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

Note: stocking rates were adjusted to compensate for differences in biomass among algal species to achieve a 1:1:1:1 biomass ratio.

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 1250 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 5000 cells/ml/day.

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*).

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 50% water exchanges at 2 h intervals until the seawater was at the required salinity. All salinity treatment animals were fed according to the commercial feeding protocol (Table 1).

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 2500 cells per day for the duration of the experiment. All other water quality conditions were allowed to change according to natural variations.

2.7. 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