

Using concentrated microalgae to condition winged pearl oyster (*Pteria penguin*) broodstock

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Introduction

Pearl production from *Pteria penguin* is often limited by the availability of oysters, particularly in places where it is an exotic species and does not recruit reliably in the wild (Finau 2005; Southgate 2008). Hatchery propagation of *P. penguin* has now become a necessity in areas such as Tonga, where the collection of oysters from the wild can no longer sustain commercial pearl production (Teitelbaum and Ngaluafé 2008).

Hatchery production is reliant on the controlled spawning of mature broodstock during the natural spawning season, which for *P. penguin*, may only span a few months of each year (Milione and Southgate in press). Annual hatchery production of juvenile oysters is, therefore, limited by the short period during which broodstock possess ripe gonads. Due to the sensitivity of embryos and larvae to slight variations in water quality and environmental variables, hatchery rearing of pearl oysters is characterised by high mortality rates (Alagarswami et al. 1989; Rose and Baker 1994; Southgate et al. 2008). Hatchery facilities are, therefore, required to operate for the longest annual period possible in order to maximise the supply of juvenile oysters to the pearl industry.

Gamete production outside of the natural spawning season has been successfully achieved for species of scallop (e.g. Monsalvo-Spencer et al. 1997) and clam (e.g. Ojea et al. 2008) via exposure to adequate water temperature and appropriate feeding regimes of live microalgae. In contrast, pearl oysters are notoriously difficult to artificially condition. Hayashi and Seko (1986) were the first to observe maturation of a pearl oyster species (*Pinctada fucata*) in response to cultured microalgae, although the monospecific diet used did not yield fully mature broodstock. Saucedo et al. (2001) used aquarium culture methods to successfully stimulate the development of ripe oocytes and active spermatozoa *Pinctada mazatlanica*, however, once again gonad development did not reach spawning condition. A recent study by Wassnig (2011) found that *P. penguin* increased filtration and digestion efficiency in response to rapid changes in food availability and elevated water temperature, highlighting the potential use of

these parameters to induce year-round production of gametes from broodstock.

Pearl oysters typically have a far greater filtering capacity than other commercial bivalve species, resulting in a high demand for cultured phytoplankton (Yukihira et al. 1998a). Suspension feeding of bivalves is optimised by maintaining a constant supply of food (Winter 1978), therefore systems for broodstock conditioning require large volumes of marine microalgae, which few pearl oyster hatcheries have the facilities and technical capacity to produce (Southgate 2008). The advent of commercially available concentrated microalgal products has meant that land-based culture of bivalves can be undertaken in areas where the infrastructure for live microalgae production is not available. This study assessed the viability of using concentrated microalgae to condition *P. penguin* broodstock in a flow-through aquarium system.

Materials and methods

The aquarium system used in this study consisted of 5 identical 30-L “flow-through” aquaria capable of holding 3 oysters each. Filtered seawater (FSW) (to 1 µm) was pumped from a temperature controlled 1,200-L tank to a heavily aerated header tank where it was channelled into flow-through aquaria at a rate of 60 ± 2 L aquarium⁻¹ h⁻¹ (Fig. 1), providing a 100% water exchange every 30 min. A diaphragm pump was used to continuously dose seawater with concentrated algae as it flowed from the temperature control tank to the header tank (Fig. 1). FSW entered each flow-through aquarium at a point near to the bottom and moved upwards past the oyster before flowing out at the top. Gentle aeration within individual aquaria was used to ensure that oxygen levels remained above 5.0 mg L⁻¹ and 75% saturation. Aquaria were emptied and cleaned once a day to remove faecal matter.

This study was undertaken at James Cook University’s Marine and Aquaculture Research Facilities Unit (MARFU) in northern Queensland, Australia, and used *P. penguin* collected from a wild population at nearby Orpheus Island (18°36’24 S and 146°29’10 E). The study was conducted for a period of 40 days from late August to early October, to

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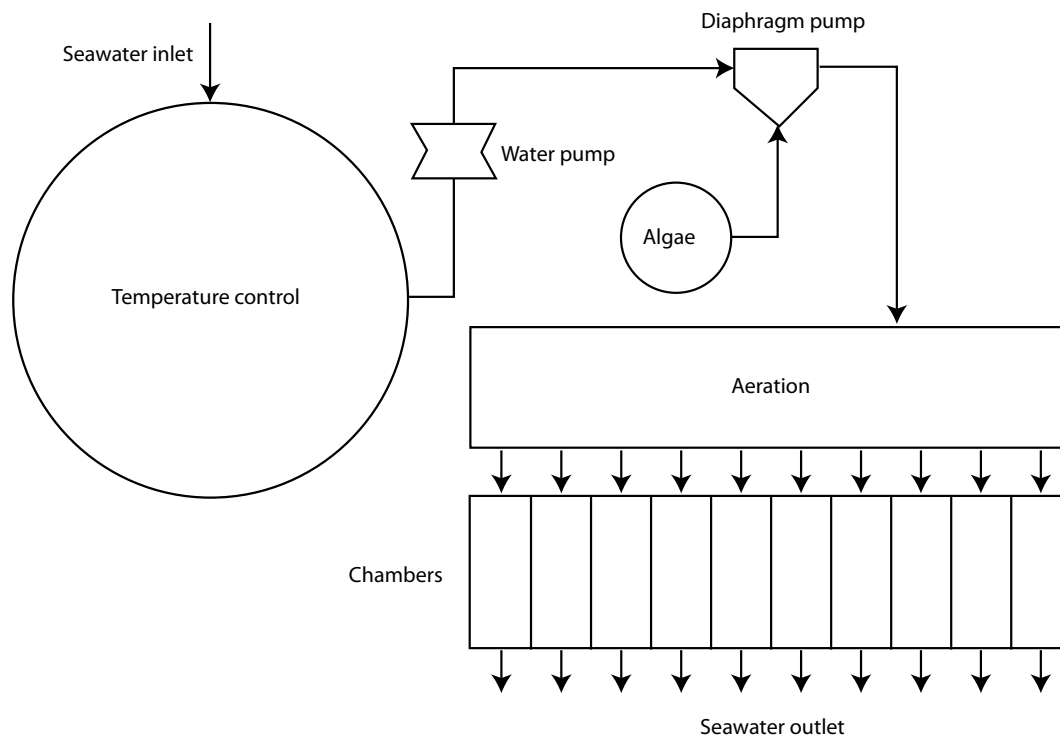


Figure 1. Diagram of the aquarium system containing *Pteria penguin* broodstock. Arrows indicate the flow of filtered seawater from inlet to outlet.

assess if aquarium culture techniques could be used to promote gamete production prior to the natural spawning season of December to March (Milione and Southgate in press). Fifteen adult *P. penguin* were placed among the 5 chambers (3 oysters per chamber), with no significant difference ($\alpha = 0.05$) in mean shell height (SH) between each chamber (overall mean SH = 204 mm).

A mixed diet of concentrated microalgae from the Instant Algae® range (Instant Algae®, Reed Mariculture Incorporated, Campbell, CA, USA 95008) was supplied to broodstock continuously throughout the study period. The diet consisted of, in terms of the number of cells mL^{-1} , 50% *Isochrysis* sp. (Haptophyceae), 25% *Pavlova* sp. (Haptophyceae), 15% *Thalassiosira weissflogii* sp. (Bacillariophyceae) and 10% *Tetraselmis* sp. (Chlorophycophyceae); 1 mg L^{-1} dry weight of the mixed diet was equal to approximately 10,000 cells mL^{-1} .

Broodstock were continuously provided with suspended concentrated algae, which at the beginning of the study period was supplied at a density of 10×10^3 cells mL^{-1} ; twice the average phytoplankton density experienced by pearl oysters in their natural habitat on the Great Barrier Reef (Yukihira et al. 1998a). Food density was increased by 10×10^3 cells mL^{-1} at 10-day intervals beginning on day 11. Water temperature was maintained at an ambient 23.5°C for the initial 10 days and was increased by 1.5°C at 10-day intervals beginning on day 11. This regime

was based on the results of Wassnig (2011), which showed an increase in energy absorption by *P. penguin* with food density and water temperature up to 40×10^3 cells mL^{-1} and 28°C , respectively.

The clearance rate (CR) (L h^{-1}), the rate at which ambient water is cleared of algae cells, was measured for each aquarium every 3 days beginning on day 4. CR was estimated using the equation by Hildreth and Crisp (1976): $\text{CR} (\text{L h}^{-1}) = F (C_1 - C_2)$, whereby:

F = the flow rate of water entering each experimental chamber (L h^{-1}),

C0 = the density of algae surrounding each oyster (cells mL^{-1}),

C1 = the density of algae at the inflow, and

C2 = the density of algae at the outflow of each aquarium.

Due to the large volume and high water flow in aquaria holding multiple broodstock, it was not possible to accurately measure CR per oyster; however, the pattern of feeding behaviour over time was monitored by plotting relative CR. Relative CR was calculated by having the uppermost CR measurement equal 100% and scaling all other measurements accordingly.

The faeces remaining in each aquarium were collected every 3 days to monitor absorption efficiency

(AE), the proportion of available organic material absorbed during digestion. AE was calculated using the equation by Conover (1966):

$$AE (\%) = 100 \times (a - f) / (1 - f), \text{ whereby:}$$

f = the fraction of dry faeces lost on ashing, and

a = the fraction of dry algae lost on ashing.

Energy absorption (EA) ($J h^{-1}$) per aquarium was estimated as the product of the energy content of the food source ($J mg^{-1}$), algal density ($mg L^{-1}$), relative clearance rate ($L h^{-1}$) and absorption efficiency (%) (following Widdows 1985). Relative EA was calculated by having the upper most measurement equal 100% and scaling all other measurements accordingly.

Assessing reproductive condition

The 15 *P. penguin* were dissected at the conclusion of the 40-day study so that their gonad tissue could be preserved within FAAC, a formaldehyde-based fixative solution (formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%). Histological analysis was performed on samples of gonad tissue from each oyster according to the methods described by Milione and Southgate (in press). Small sections of tissue were removed from the concentrated gonads, embedded in wax and thinly sliced to provide cross-sections that could be mounted on glass slides and stained for observation under a high-power compound microscope. Images taken at 100X magnification were used to categorise each oyster's condition into one of the five stages of pearl oyster gonad development described by Tranter (1958): 1) inactive (gametes absent), 2) developing (partially filled follicles), 3) ripe (mature filled follicles), 4) spawning (partially emptied follicles), and 5) spent (empty follicles). To enable comparison with the reproductive condition of oysters from the same population remaining in the wild, histological analysis was also conducted on gonad samples taken from 15 similar sized *P. penguin* (mean SH = 201 mm) collected from the Orpheus Island population 3 days after the experiment was concluded.

Results

Regression analyses ($\alpha = 0.05$) revealed a significant difference in clearance rate (CR) over time ($F = 18.041$, $df = 1, 94$, $p < 0.001$). CR dropped roughly 10% with the first increase in food density and water temperature, before increasing a further 20% and staying relatively stable at food densities greater than 30×10^3 cells mL^{-1} and water temperatures greater than $26.5^\circ C$ (Fig. 2A).

AE decreased significantly with time ($F = 58.586$, $df = 1, 58$, $p < 0.001$) in an approximate linear fashion according to the equation $y = -2.61x + 93.98$ ($R^2 = 0.92$), dropping from 89% on day 4 to 62% on day 37. Despite decreased AE over time, an increase in CR and higher food availability resulted in a significant ($F = 481.119$, $df = 1, 94$, $p < 0.001$) increase in EA ($J h^{-1}$) of over 350% from day 4 to day 37. The most dramatic increase in EA occurred when increasing food density from 20×10^3 cells mL^{-1} to 30×10^3 cells mL^{-1} on day 21 (Fig. 2B).

The gonad condition of oysters ranged from being inactive (gametes absent) to ripe (mature follicles) (Table 1). Three experimental oysters had inactive gonads, indicating that not all animals obtained enough energy to develop gametes; however, of the remaining oysters, all were either in the later stages of gonad development or already ripe. The *P. penguin* collected from the wild at the conclusion of the experiment were also classified as either developing or ripe, although compared with experimental oysters, fewer had reached the ripe stage (Table 1). Example images of histological sections of developing and ripe gonad stages in both sexes are shown in Figure 3.

Table 1. Stages in the reproductive cycle of male (M) and female (F) *Ptereria penguin* from experimental and wild groups, as determined via histological examination of gonad samples (see Fig. 3).

Group	No.	Inactive	Developing	Ripe	Spawning	Spent
Experimental	15	3	2 (M), 3 (F)	5 (M), 2 (F)	0	0
Wild	15	0	6 (M), 5 (F)	3 (M), 1 (F)	0	0

Discussion

CR escalated when food density exceeded 30×10^3 cells mL^{-1} and water temperature reached $26.5^\circ C$, which is likely to be a response to both variables. The results of Wassnig (2011) showed that *P. penguin* can increase CR in response to elevated food density, a response that has also been reported for other bivalve species and is thought to be a consequence of the ability to detect and capitalise on greater nutrient availability (e.g. Willows 1992). A positive influence of increased water temperature on ingestion is a phenomenon common to pearl oysters (Numaguchi 1994; Yukihiro et al. 2000). Higher water temperatures are assumed to facilitate greater ingestion by improving respiration and, thus, metabolic rate (Kobayashi and Tobata 1949; Lucas 2008).

Oysters did not eject excess food as pseudofaeces at any point during this study, including at the highest

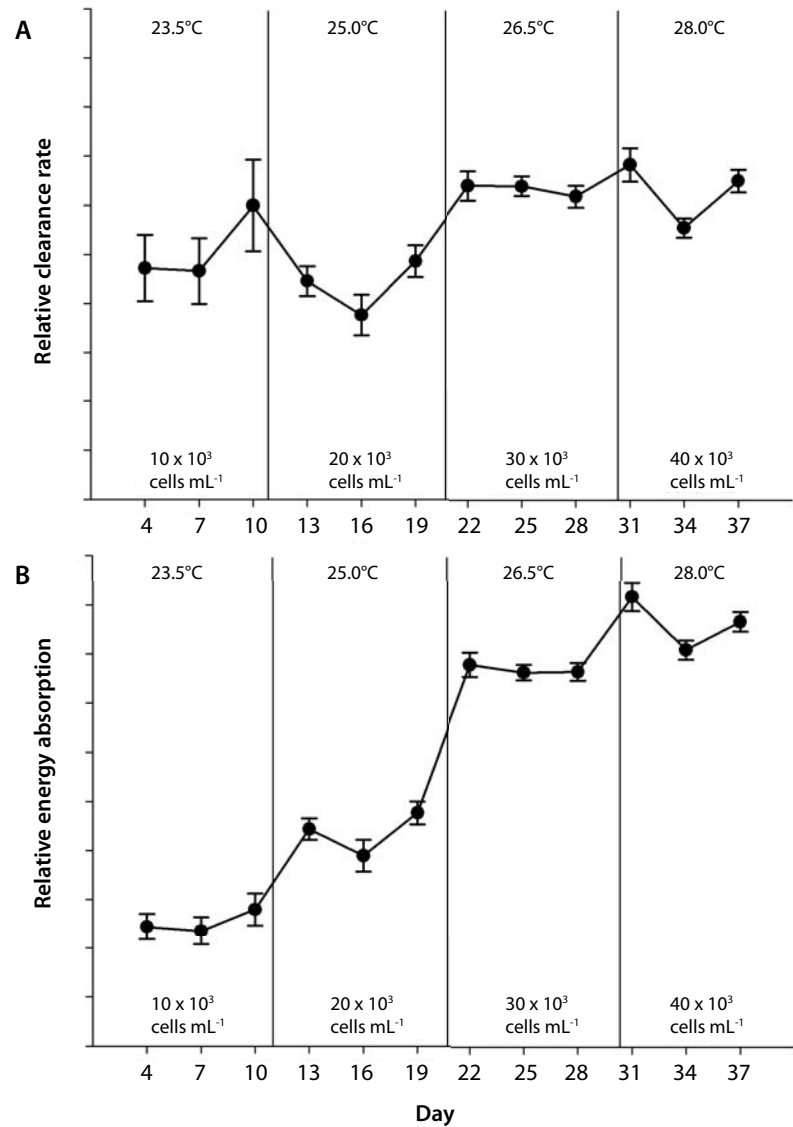


Figure 2.

Feeding behaviour of *Pteria penguin* over time when feeding on a mixed diet of concentrated algae.

- A) Mean (\pm SE) relative clearance rate;
 B) mean (\pm SE) relative energy absorption. Food density and water temperature were increased at 10-day intervals beginning on day 11.

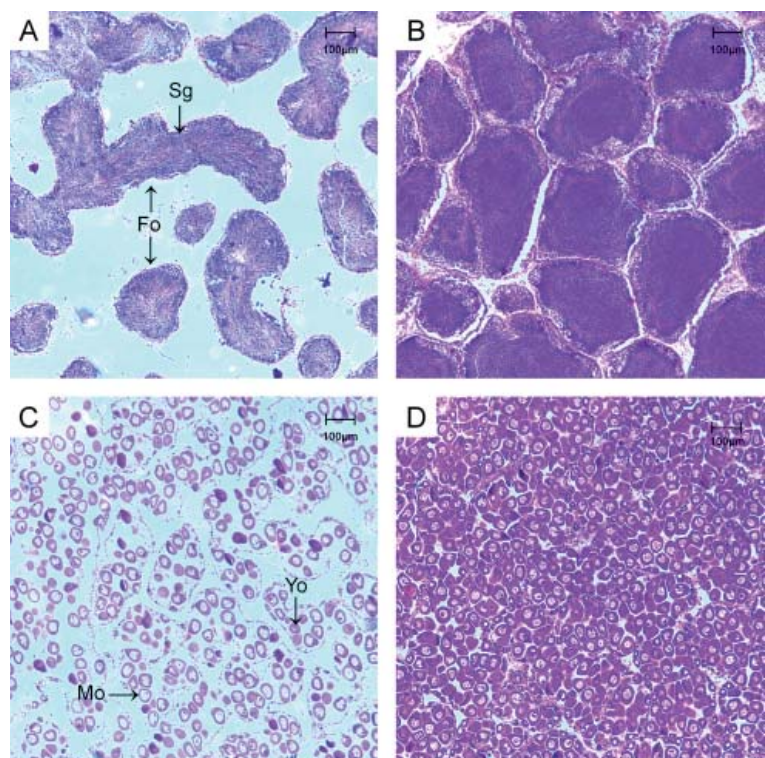


Figure 3.

Images of developing and ripe gonad stages for *Pteria penguin*. A) Developing male with expanding follicles (Fo) and spermatogonia (Sg) lining the follicle walls; B) ripe male with densely packed spermatozoa; C) developing female with both young oocytes (Yo) and mature oocytes (Mo); D) ripe female with densely packed mature oocytes.

food density of 40×10^3 cells mL^{-1} . This suggests that *P. penguin* has a superior ability to maintain ingestion efficiency at high food densities when compared with pearl oyster species belonging to the genus *Pinctada*, which typically begin producing pseudofaeces at algal densities between 10×10^3 cells mL^{-1} and 30×10^3 cells mL^{-1} (Yukihira et al. 1998b). CR of *P. penguin* stabilised at food densities greater than 30×10^3 cells mL^{-1} and water temperatures greater than 26.5°C . A plateau in feeding behaviour at high food concentrations has been seen previously in other bivalve species and is thought to be a mechanism for avoiding energy wastage associated with the production of pseudofaeces at food concentrations surpassing maximum ingestion capacity (Kobayashi and Tobata 1949; Lucas 2008). The short-term absorption efficiency of microalgae by *P. penguin* decreased with increasing ingestion rate, most probably due to decreased gut retention time and corresponding enzymatic digestion (e.g. Iglesias et al. 1992).

The reproductive condition of oysters after the 40-day study period suggested that male *P. penguin* were able to access the energy required to produce spermatozoa at a rate exceeding that observed in the wild environment over the same time period. The production of mature oocytes in females was less reliable. Milione and Southgate (in press) found that approximately 20% of adult *P. penguin* from the same Orpheus Island population used in this study, possess inactive gonads during the month of August. Therefore, it is possible that the oysters used in this study that were inactive at the end of the study actually entered the aquarium system in that condition. Ripe gametes are typically produced earlier in male than females oysters (Loosanoff and Davis 1952); thus the period of optimal energy absorption provided in this study (10–20 days) may not have been long enough to allow for the reliable production of energetically expensive oocytes. This notion is supported by the results of Saucedo et al. (2001) who found a positive relationship between the proportion of female *Pinctada mazatlanica* producing ripe oocytes and the period of exposure to high densities of microalgae (75×10^3 cells mL^{-1}), with periods of 45–60 days yielding the greatest oocyte production.

This study provides preliminary observations regarding the use of concentrated microalgae to prompt gametogenic activity in *P. penguin*, although more research is required to determine the nutrition and time period required to reliably bring female broodstock to spawning condition. Further research should also be conducted to assess whether the techniques used in this study can be used to extend the annual period of hatchery production by maintaining broodstock in a ripe condition in the months following cessation of the natural spawning season.

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