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The effects of stocking density and ration on survival and growth of winged pearl oyster (*Pteria penguin*) larvae fed commercially available micro-algae concentrates

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ABSTRACT

Commercially available micro-algae concentrates have been successfully used as an alternative to live micro-algae as a food source during routine larval culture of the winged pearl oyster, *Pteria penguin*. This supports the development of simplified hatchery facilities and larval rearing protocols that are more appropriate to Pacific island nations. An optimal feeding regime based on these products that also accounts for larval stocking density is yet to be developed. Two experiments were conducted at a commercial pearl oyster hatchery facility in the Kingdom of Tonga to examine the combined effects of stocking density and ration on survival and growth of both D-stage (from 1 to 8 days post-fertilsation) and umbo-stage (from 8 to 17 days) *P. penguin* larvae. Both experiments used a factorial design combining three larval stocking densities (D-stage: 2, 6 & 10 larvae mL^{-1}; umbo-stage: 1, 3 & 5 larvae mL^{-1}) and three rations (D-stage: 5, 10 & 15 cells mL^{-1}; umbo-stage: 10, 15 & 20 cells mL^{-1}). Survival during D-stage was significantly improved in aquaria stocked below 10 larvae mL^{-1}, whereby a density of 6 mL^{-1} maximised larval production. An intermediate ration of 10×10^3 cells mL^{-1} maximised both survival and growth during D-stage. Increasing the initial stocking density of umbo-stage larvae from 1 to 3 mL^{-1} resulted in significant reductions in both survival and growth. Growth of umbo-stage larvae stocked at a density of 1 mL^{-1} increased significantly when ration remained below 20×10^3 cells mL^{-1}. The results of this study provide a basis for optimised hatchery culture protocols for *P. penguin* that are more appropriate to Pacific island nations.

1. Introduction

Hatchery propagation has become increasingly important as a source of oysters for the cultured pearl industry and is now a necessity in regions where the collection of wild spat no longer provides adequate numbers of oysters to sustain half-pearl (mabè) production on a commercial scale (Southgate, 2008). The near exhaustion of *Pteria penguin* populations in Japan and China has meant that commercial half-pearl production from this species is now dependent on hatchery propagation (Liang et al., 2001; Yu et al., 2004; Southgate et al., 2008), which potentially ensures a consistent supply of oysters and allows selection for commercially desirable genetic traits such as increased growth rate, disease tolerance and improved pearl quality (Evans et al., 1995).

Despite the advantages of hatchery production, it is often beyond the capability of many developing countries in the Pacific, where the technical resources and skilled personnel required for successful hatchery operation are often lacking. Production of appropriate quantities of high quality live micro-algae as a larval food source is a common bottleneck due to the requirement for dedicated facilities, specialised equipment and technical oversight (Couteau and Sorgeloos, 1992; Helm et al., 2004). A large proportion of the infrastructure and costs associated with establishing a pearl oyster hatchery in the Pacific are attributed to micro-algae culture (Ito, 1999).

Recent years have seen increased availability of commercially available 'off-the-shelf' food products that are designed to replace or supplement live aquaculture foods including micro-algae. A

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number of these products, such as dried and concentrated micro-algae, have been developed specifically for bivalves, and prior studies have assessed the potential of such products for replacing live micro-algae for pearl oyster larvae (e.g. Southgate et al., 1998; Teitelbaum and Ngaluafe, 2008). Various species of phototrophically grown, highly concentrated marine micro-algae are now available commercially and have potential in many aspects of bivalve production, including hatchery culture (Reed and Henry, 2014). These products are now used routinely as the sole food source during hatchery production of the winged pearl oyster, *Pteria penguin*, in Tonga, and have greatly simplified hatchery procedures (Southgate et al., 2016).

Micro-algal concentrates are composed of intact but non-viable, non-motile cells (Reed and Henry, 2014) that are negatively buoyant. As such, micro-algae concentrates behave differently to live micro-algae when introduced to larval rearing tanks (Heasman et al., 2000) and optimised feeding regimes may be different even if the nutritional content of the food source is similar. Ration must allow access to an appropriate level of nutrition to support larval development, without causing reduced water quality resulting from the decomposition of unconsumed feed (Loosanoff and Davis, 1963; Doroudi et al., 1999). The efficacy of ration varies according to larval density (Doroudi and Southgate, 2000; Liu et al., 2010), therefore an understanding of the specific interactions between these two factors is required to achieve efficient larval production and to minimise food waste. This study therefore, investigated the effects of stocking density and ration on survival and growth of both D-stage and umbo-stage *P. penguin* larvae fed commercially available micro-algae concentrates.

2. Materials and methods

2.1. Larval production

This study was conducted at the Aquaculture Facility of the Ministry of Agriculture and Food, Forests and Fisheries (MAFFF) at Sopu in the Kingdom of Tonga (21°07′21″S; 175°13′36″W). Fifty *Pteria penguin* brood-stock were collected from a long-line located 500 m offshore and cleaned, before being induced to spawn via repeated air exposure (Victor et al., 2001). Broodstock were placed in a shallow spawning tank containing UV-treated 1 μm filtered sea water (FSW) for 1 h, before tanks were completely drained, leaving the oysters exposed to direct sunlight for 10 min. The tank was then refilled with FSW at a temperature of 28° C and the oysters left undisturbed for 30 min. This process was repeated twice before several males began to release sperm, prompting the females to spawn 3–5 min later.

Once spawning began, the procedures for achieving fertilisation followed the standard methods described for pearl oysters (Southgate, 2008). Fertilised eggs were rinsed onto a mesh sieve to remove excess spermatozoa and then placed into static incubation tanks containing gently aerated 1 μm FSW at a stocking density of 50 eggs mL^{-1} (Southgate et al., 1998; Doroudi and Southgate, 2003). Embryonic development was monitored using an optical microscope (200X). After an incubation period of 22 h, the shelled D-stage larvae that had developed were washed and counted, and the required number of larvae were distributed into the aquaria used for Experiment 1. D-stage larvae that were not used in Experiment 1 were cultured for a further 8 days in 1,000 L tanks using typical methods for *P. penguin* larvae (Southgate et al., 2016) and then used in Experiment 2.

Aquaria in both experiments were filled with UV-treated 1 μm FSW and underwent a complete water exchange every two days, in accordance with typical culture methods for *P. penguin* larvae (Southgate et al., 2016). Aquaria were held in a temperature controlled water bath at 28 °C throughout the experiments. Each aquarium had a removable lid and was gently aerated through a glass tube inserted from the top.

2.2. Micro-algae concentrates

Larvae were fed commercially available micro-algae concentrates from the Instant Algae® range (Reed Mariculture Inc., San Jose, CA, USA) in both experiments. The products used in this study were mono cultured *Isochrysis* sp. ("Isochrysis 1800®"), mono-cultured *Pavlova* sp. ("Pavlova 1800®"), and a third product composed of a mixture of *Isochrysis* sp., *Pavlova* sp., *Thalassiosira weissflogii* and *Tetraselmis* sp. ("Shellfish Diet 1800®"). All were obtained from an Australian distributor of the products and were stored in their original bottles in a refrigerator at 4 °C for the duration of the study. Prior to use, a 5 mL aliquot of each concentrate was added to 2 L of FSW in a separate container and gently hand-shaken to disperse the micro-algae cells. The cell densities in resulting suspensions were determined using a haemocytometer and the volume needed to provide the required ration for each aquarium was dispensed through a 20 μm mesh sieve to remove or break up any clumps of micro-algae cells that may have been present.

2.3. Experiment 1

Experiment 1 assessed the combined effects of stocking density and ration on survival and growth of D-stage *P. penguin* larvae from 1 to 8 days post-fertilisation. The mean antero-posterior shell measurement (APM) of 50 randomly selected D-stage larvae at the start of the experiment was 81.0 ± 3.8 μm. Larvae were stocked into 5 L aquaria at densities of 2, 6 and 10 larvae mL^{-1} and fed a 1:1 ratio (based on cell count) of "Isochrysis 1800®" and "Pavlova 1800®" at a ration of either 5, 10 or 15×10^3 cells mL^{-1} once daily. The combined effects of larval density and ration each at 3 levels with 3 replicates required a total of 27 experimental aquaria.

2.4. Experiment 2

Experiment 2 examined the effects of stocking density and ration on survival and growth of umbo-stage *P. penguin* larvae from 8 to 17 days post-fertilisation. Prior to reaching umbo-stage, larvae were raised according to the intermediate conditions applied in Experiment 1 (i.e. density = 6 larvae mL^{-1}, ration = 10×10^3 cells mL^{-1}). The APM of 50 randomly selected umbo-stage larvae at the start of the experiment was 105.9 ± 10.7 μm. Larvae were stocked into 5 L aquaria at densities of 1, 3 and 5 larvae mL^{-1} and fed a 1:1:1 ratio of "Isochrysis 1800®", "Pavlova 1800®" and "Shellfish Diet 1800®" at a ration of either 10, 15 or 20×10^3 cells mL^{-1} once daily. The resulting cell count of the feed used constituted approximately 43% *Isochrysis* sp., 40% *Pavlova* sp., 10% *Thalassiosira weissflogii* and 7% *Tetraselmis* sp. The combined effects of larval density and ration each at 3 levels with 3 replicates required a total of 27 experimental aquaria.

2.5. Sampling and data analysis

At the end of each experiment, the contents of individual aquaria were collected on a 25 μm mesh screen and washed into separate 50 mL vials containing 4% formaldehyde solution in buffered seawater. Survival and growth were estimated by removing triplicate 1 mL sub-samples from each 50 mL vial and examining the contents using an optical microscope (200X). The number of surviving larvae were counted and the shell length (APM) of 20 random individuals was measured. Relative larval production, or yield mL^{-1}, was calculated by multiplying initial density by mean survival. The normality of raw data was assessed using probability plots of residual