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The Effect of Egg Density on Hatch Rate of Pearl Oyster (*Pinctada maxima* and *P. margaritifera*) Larvae

P.C. SOUTHGATE[1], J.J. TAYLOR[1,2] AND M. ITO[1]

[1]*Aquaculture Department, James Cook University of North Queensland, Townsville, Queensland 4811, Australia.*

[2] *Pearl Oyster Propagators P/L, 4, Daniels Street, Ludmilla, Northern Territory, Australia.*

Abstract - This paper reports on two experiments in which the effect of egg density on hatch rate of pearl oyster *Pinctada maxima* (Jameson) and *P. margaritifera* (L.) larvae was determined. Fertilized eggs were stocked at densities of 10, 20, 30, 40, 50 and 100 ml^{-1} for *P. maxima* and 10, 20, 30, 50, 100 and 150 ml^{-1} for *P. margaritifera*. After 24 h, the number of D-stage veliger larvae was assessed and survival was calculated. Highest survival was shown at an egg density of 10 ml^{-1} for both *P. maxima* (82.5%) and *P. margaritifera* (93.2%). Lowest survival was shown at a density of 100 ml^{-1} for both *P. maxima* (74.1%) and *P. margaritifera* (79.4%). However, for both species, there was no significant difference in survival between densities of 20 and 50 eggs ml^{-1} or between 30 and 100 eggs ml^{-1}.

Hatchery-produced pearl oyster spat are becoming an increasingly impor-tant source of stock for pearl culture industries in the Asia-Pacific region (Gervis and Sims 1992) and hatchery techniques have now been described for the major commercial species of *Pinctada maxima* (Rose 1990; Rose and Baker 1994), *P. margaritifera* (Alagarswami et al. 1989), and *P. fucata* (Alagarswami et al. 1987). Once spawning has occurred, fertilized eggs are usually stocked into incubation tanks where embryological and early larval development take place. Approximately 20-24 h after fertilization, the resulting D-stage veliger larvae are removed from incubation tanks and stocked in larval rearing tanks.

Rose (1990) recommended that egg density during incubation should not exceed 30 ml^{-1} for *P. maxima*. However, the majority of studies on pearl oyster larvae make no reference to the density at which fertilized eggs were incubated (Alagarswami et al. 1987; Alagarswami et al. 1989) and, despite recent research efforts into larval culture techniques for pearl oysters, there is as yet no published data on the effect of egg density on survival to D-stage. This study was undertaken to determine the effect of egg density on survival to D-stage for *P. margaritifera* and *P. maxima*.

Fertilized *P. maxima* and *P. margaritifera* eggs were obtained from routine spawnings in commercial-scale hatcheries. Fertilized *P. maxima* eggs were placed in 50-ml plastic vials containing 1-µm filtered seawater at densities of

10, 20, 30, 40, 50 and 100 ml^{-1} without aeration. The same batch of fertilized eggs, composed of a mixture of eggs from a number of females, was used for each treatment. Each treatment was conducted with 5 replicates. Fertilized *P. margaritifera* eggs were placed in 5-l plastic aquaria containing 1-µm filtered seawater at densities of 10, 20, 30, 50, 100 and 150 ml^{-1}. Again, the same batch of fertilized eggs, composed of a mixture of eggs from a number of females, was used for each treatment. Aquaria were provided with gentle aeration (air filtered to 0.45-mm) from a 4 mm glass rod. Each treatment was conducted in triplicate.

Both experiments were terminated after 24 h. D-stage *P. maxima* larvae were removed onto a 20-µm mesh screen and all larvae were counted microscopically. *P. margaritifera* larvae were removed from each aquarium onto a 25-µm mesh screen and concentrated into a glass beaker. The number of larvae in each was estimated from triplicate counts of 1-ml sub-samples removed from it. Percentage of survival was calculated for each aquarium and data were arcsine-transformed prior to statistical analysis by one-way ANOVA. Significant differences between treatment means were determined using the Least Significant Difference test (Zar 1984). Differences between treatment means were considered significant at the 0.05 level.

Survival to D-stage for both species is shown in Table 1. For *P. maxima*, highest mean survival of 82.5% was shown at a density of 10 ml^{-1}; however, survival at this density did not differ significantly from that at 20 ml^{-1} and mean survival did not differ significantly between 20 ml^{-1} and 50 ml^{-1}.

Highest survival of *P. margaritifera* larvae (93.2%) was shown at a density of 10 eggs ml^{-1}; however, this did not differ significantly from survival at 20 eggs ml^{-1} (90.2%). The lowest mean survival (79.4%) was shown at 100 eggs ml^{-1} but this did not differ significantly from that at 30 eggs ml^{-1} (84.6%). Mean survival at the highest density tested (150 eggs ml^{-1}) was 79.6%; however, there was no significant difference between survival at 150 egg ml^{-1} and 30 eggs ml^{-1} (84.6%).

Although survival was greatest at a density of 10 eggs ml^{-1} in both experiments, the results show that *P. maxima* and *P. margaritifera* eggs can be incubated at densities of up to 100-150 ml^{-1} and still achieve high rates of

Table 1. Survival of *Pinctada maxima* and *P. margaritifera* larvae to D-stage at different densities during egg incubation[1].

	Survival (%, Mean ± se)	
Density (eggs ml^{-1})	*P. maxima*	*P. margaritifera*
10	82.48 (+1.39)^{a}	93.20 (+0.55)^{a}
20	79.65 (+1.51)^{a,b}	90.22 (+1.21)^{a,b}
30	77.52 (+0.52)^{b,c}	84.61 (+0.38)^{b,d}
40	77.26 (+0.98)^{b,c}	N/A
50	76.60 (+1.27)^{b,c}	87.80 (+1.40)^{b,d,e}
100	74.10 (+1.20)^{c}	79.36 (+1.96)^{c,d}
150	N/A	79.57 (+5.96)^{c,e}

[1]Means within the same column with the same superscripts are not significantly different (P>0.05)
N/A=Not assessed.

survival to D-stage. These densities are considerably higher than the 30 eggs ml^{-1} recommended by Rose (1990) for *P. maxima*. However, a major factor influencing survival of eggs to D-stage is the quality of the eggs themselves, which can vary considerably between batches. For example, prior to this study, an egg density of 50 ml^{-1} was used routinely for *P. margaritifera* at James Cook University's Orpheus Island hatchery. Although survival to D-stage at this density is usually in the range of 60-80%, survival as low as 6% has also been recorded. Similar variations in hatch rate have been recorded for *P. maxima* during commercial propagation in Australia and Indonesia. Survival of eggs stocked at 20-30 ml^{-1} is usually within the 60-75% range but has been as low as 12%.

The two batches of eggs used in these experiments were obtained from routine pearl oyster spawnings in commercial-scale hatcheries. As such, the quality or condition of the eggs used in the experiments is representative of the quality of eggs used in commercial hatchery production of pearl oysters. The high survival shown in this study indicates that batches of good quality eggs were used in both experiments. However, in batches of eggs which show relatively low survival, the resulting organic load and bacterial population of the culture water would be expected to increase with increasing egg density. As a result, proportionally lower survival may result from eggs stocked at higher densities.

It should be emphasized that egg density is one of a number of factors influencing development, survival and growth of eggs and larvae in bivalve hatcheries. Factors such as aeration, temperature, salinity and the use of antibiotics have considerable influence on the viability of eggs and larvae (Dos Santos and Nascimento 1985; Lemos et al. 1994). However, there is a paucity of information on the effects of such factors on the development of the eggs and larvae of pearl oysters. Egg quality is perhaps the major influence on the viability of early life stages of bivalves. Although egg quality can be improved by appropriate broodstock conditioning (Quayle and Newkirk 1989), information relating to the conditioning of pearl oyster broodstock is very limited (Gervis and Sims 1992) and attempts to condition *P. maxima* have been unsuccessful (Rose et al. 1986). Future advances in the hatchery production of pearl oysters will clearly benefit from research to develop appropriate methods for broodstock conditioning, and to determine optimal conditions for incubation and culture of eggs and larvae.

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