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Note

Effect of mesh coverings on retention and growth of blacklip pearl oyster (*Pinctada margaritifera*) spat during early nursery culture

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Abstract – Hatchery produced pearl oyster spat are generally transferred to the ocean on spat collectors which may, or may not, be covered with fine mesh to prevent spat loss. This study examined the effectiveness of mesh bag covers on culture units for spat of the blacklip pearl oyster, *Pinctada margaritifera*. It assessed spat retention and growth in culture units without mesh bags (REMOVE) and in those where mesh bags were replaced weekly (REPLACE), washed weekly (WASH) or left unwashed (NO WASH) for the duration of the six-week experiment. Culture units in the REPLACE treatment had the greatest number of spat at the end of the experiment and 41% more spat than in the REMOVE treatment. Grading of the spat from each treatment at the end of the experiment showed that the REPLACE treatment had the highest number of spat in large and medium size classes with mean (± SE) dorso-ventral height of 23.9 ± 0.5 mm and 17.4 ± 0.5 mm, respectively. Mean wet weight of the mesh bags at the end of the experiment differed between treatments with bags from the WASH and NO WASH treatments having significantly greater mean wet weights than those from the REPLACE treatment. The results show that the presence of a mesh bag around spat culture units may improve retention of spat by between 18–41%. However, protective mesh bags may foul quickly and, to maximise retention and spat growth, weekly replacement of mesh bags should be undertaken.

Key words: Pearl oyster / Spat collection / Growth / Survival / Nursery culture / *Pinctada margaritifera*

1 Introduction

Development of hatchery production techniques for pearl oysters (e.g. Rose and Baker 1994; Southgate and Beer 1997) has provided pearl culture operations independence from inherent variability associated with collection of wild spat or adults. To capitalize on these developments however, appropriate nursery culture and grow out protocols are required. Culture of the blacklip pearl oyster, *Pinctada margaritifera* has developed rapidly in the Indo-Pacific region over recent years (Fassler 2002), and use of hatchery produced spat in pearl culture operations has assumed increasing importance (Gervis and Sims 1992; Southgate and Beer 2000). Despite this development, relatively little information is available on appropriate early nursery culture protocol for this species.

Transfer of pearl oyster spat from the hatchery to the sea is a critical period and methods employed at this time affect subsequent growth, survival and retention of spat within culture units (Southgate and Beer 1997; Taylor et al. 1998; Pit and Southgate 2000). In general, pearl oyster larvae are settled onto spat collectors which are then transferred to the sea without further protection (Southgate and Beer 1997) or they may be covered with mesh to protect spat from predators and natural elements (Taylor et al. 1998). Spat culture units without protective mesh covering may lose large numbers of spat during transport from the hatchery and when placed into the sea, with spat presumably detaching due to environmental stress (e.g. Taylor et al. 1997a). However, while fine mesh bags surrounding culture units can retain these spat (Taylor et al. 1998), they also have potential disadvantages. The small pore size may encourage a film of sediment and other fouling matter that may reduce water exchange and removal of metabolic wastes (Holliday et al. 1991) and require frequent cleaning. This can be alleviated by either increasing the pore size of the mesh, altering the frequency or method of cleaning, or removing the mesh after a "settling-in" period.

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While a number of recent studies have reported on aspects of nursery culture of *P. margaritifera* (Southgate and Beer 1997; Pit and Southgate 2000; Southgate and Beer 2000; Friedman and Southgate 1999a,b; Pit and Southgate 2003a,b), the effects of mesh covers on culture units containing *P. margaritifera* spat have not been determined. This study examined the effects of mesh bag covers on retention and growth of *P. margaritifera* spat held in plastic trays in suspended culture in northern Australia.

2 Materials and methods

Larvae were cultured according to an established larval rearing protocol (Southgate and Beer 1997). On day 25, larvae large enough to be caught on a 150 μm pore size nylon mesh were placed into 2 × 500 L vigorously aerated settlement tanks containing 1 μm filtered seawater at a density of 0.3 larvae mL^{-1}. Seventy-five spat collectors constructed of 0.5 m^2 of woven shade mesh (50% area) within a 45 cm polyethylene mesh bag (collector bag) were placed in each tank. On day 41, more than two weeks after stocking the settlement tanks, spat collectors were removed and placed into plastic mesh trays with lids (55 × 30 × 10 cm) with 15 × 5 mm pore size (Southgate and Beer 1997). Three collectors were placed into each tray and a closely fitting 1 mm pore size mesh bag (tray bag) was placed over each tray. Trays were suspended at a depth of 5 m from a surface long line in Pioneer Bay, Orpheus Island, north Queensland, Australia (18° 35'S, 146° 29' E), approximately 50 m from a fringing reef flat with a depth of 10–13 m under the longline. When transferred to the long line on day 41, mean (± SE, n = 30) dorso-ventral height (DVH) of spat was 1.41 (± 0.05) mm. Trays were retained under these conditions for 4 weeks before the start of the experiment and washed weekly with seawater from a low pressure, high volume 12-volt bilge pump (88 L min^{-1}).

Prior to the start of the experiment, three trays were randomly removed from the longline for an initial census to determine the mean number of spat per collector and their mean DVH. For each tray, the number of spat within each of the three collectors and those attached to the tray and the tray bag were recorded. Mean (± SE) DVH was 3.9 (± 0.2) mm. Four treatments were tested in triplicate using remaining trays in a six-week experiment. The treatments were:

1. the 4 week-old tray bag was removed from the trays but was not replaced (REMOVE);
2. the 4 week-old tray bag was replaced, then replaced on a weekly basis for the duration of the experiment (REPLACE);
3. the 4 week-old tray bag was replaced, and the tray was washed thoroughly each week (WASH); and
4. the 4 week-old tray bag was replaced, then not cleaned for the remainder of the experiment (NO WASH).

During the washing procedure, low pressure water was flushed over the tray from the top, bottom and sides until no further sediment was washed from the tray. Spat attached to tray bags removed from trays in the REPLACE treatment were counted and included in final spat counts. The experiment was terminated after 6 weeks (10 weeks in nursery culture and 111 days post fertilisation). Weekly samples of >30 spat from replicate trays not included in this experiment but treated according to the REMOVE treatment (controls), were taken to determine changes in mean DVH. This sampling began when trays were transferred to the long line and were continued until the end of the experiment. Water temperature at a depth of 5 m on the longline was recorded continuously during the study using a YSI 6600 Multiparameter Water Quality Monitor.

When trays were brought ashore for final census at the end of the experiment, the tray bag (where applicable) was removed, drained to remove excess water and weighed. Spat collectors were then removed from the trays and spat were removed from the collector bags, trays and tray bags (where appropriate) of each spat collector and counted. Spat were then graded through two mesh sieves (10 mm and 5 mm pore size) to generate three size classes; large (>10 mm), medium (10–5 mm) and small (<5 mm) (Southgate and Beer 1997), and the proportion in each size class was calculated.

Spat counts and tray bag weight data were examined for homogeneity of variance and analysed using one-way ANOVA (Zar 1996). Homogenous subsets of means were determined using Tukey test (Zar 1996). DVH data were analysed using one-way ANOVA and Tukey test.

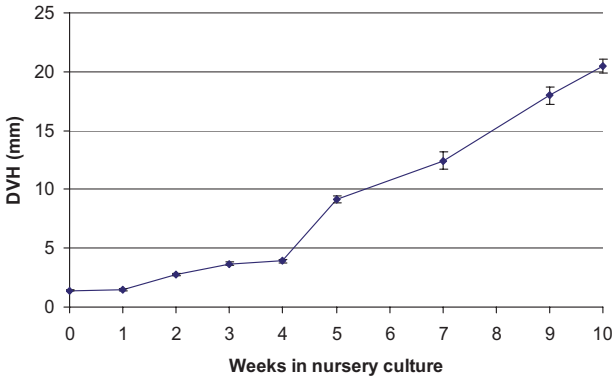

Fig. 1. Mean (± SE) dorso-ventral shell height (DVH) of *P. margaritifera* spat (controls) over a 10 week period following transfer from settlement tanks to nursery culture at sea. Spat were cultured according to the "REMOVE" treatment with the mesh bag removed from the trays at the start of the experiment (4 weeks).

3 Results

Growth of spat (controls) was rapid (Fig. 1) with mean (± SE) DVH increasing from 1.4 ± 0.1 mm at transfer from the hatchery, to 3.9 ± 0.2 mm four weeks after transfer and to 20.5 ± 0.6 mm 10 weeks after transfer. A rapid increase in growth rate occurred after 4 weeks, following removal of the tray bag (Fig. 1). Sea water temperature ranged from 28 °C when the spat were transferred to the sea (March) to 24 °C in May at the end of the experiment.

At the initial census (after four weeks in nursery culture) there was considerable variation in the number of spat within collector bags and attached to trays. The majority of the spat recorded in each unit were found within the collector bags

Table 1. Mean (± SE) number of *P. margaritifera* spat per spat collector, tray and tray bag at the initial census four weeks after transfer to the sea. [1]Each tray contained three spat collectors which contributed to the total.

Substrate	Mean	Minimum	Maximum
Spat collector	384.6 ± 34.5	260	576
Tray	139.0 ± 30.8	80	184
Tray bag	93.3 ± 24.4	67	142
TOTAL[1]	1386.0 ± 87.2	1228	1529

(mean ± SE spat per bag 384 ± 34) with only 139 ± 31 (10%) and 93 ± 24 (<7%) recorded in the tray and tray bag, respectively (Table 1). The initial census determined a mean (± SE) spat abundance of 1386 ± 87 spat per replicate tray (Table 1).

The mean number of spat per tray (spat collectors, tray and tray bag where applicable) at the end of the experiment for each of the four treatments were: WASH 1380 ± 161; NO WASH 1355 ± 347; REMOVE 1147 ± 151; and REPLACE 1614 ± 99 (Table 2). The REPLACE treatment retained the greatest number of spat per tray and 41% more than the REMOVE treatment; however, spat abundance did not differ significantly between treatments ($p > 0.05$). Weekly replacement of the tray bags also resulted in improved growth rates of spat. When graded at the end of the experiment, the four treatments showed significant differences in the number of spat recorded in the large and medium size classes (Fig. 2). Significantly more large spat were recorded in the REPLACE treatment than in all other treatments. The REPLACE treatment also held significantly greater numbers of medium size class spat (421 ± 35 spat per unit) than the REMOVE (205 ± 75) and WASH (164 ± 30) treatments ($p < 0.05$). The mean DVH (± SE, $n = 30$) of spat from the REPLACE treatment in the large and medium size classes were 23.9 ± 0.5 mm and 17.4 ± 0.5 mm, respectively. There were no significant differences in the number of small (<5 mm) spat between treatments.

Tray bags from the REPLACE treatment, which were immersed for one week only, contrasted with the heavily fouled bags from the WASH and NO WASH treatments. Mean wet weight of the tray bags differed significantly ($p < 0.001$) between the REPLACE (74.9 ± 1.8 g) and WASH (337 ± 9 g) and NO WASH (382 ± 8 g) treatments.

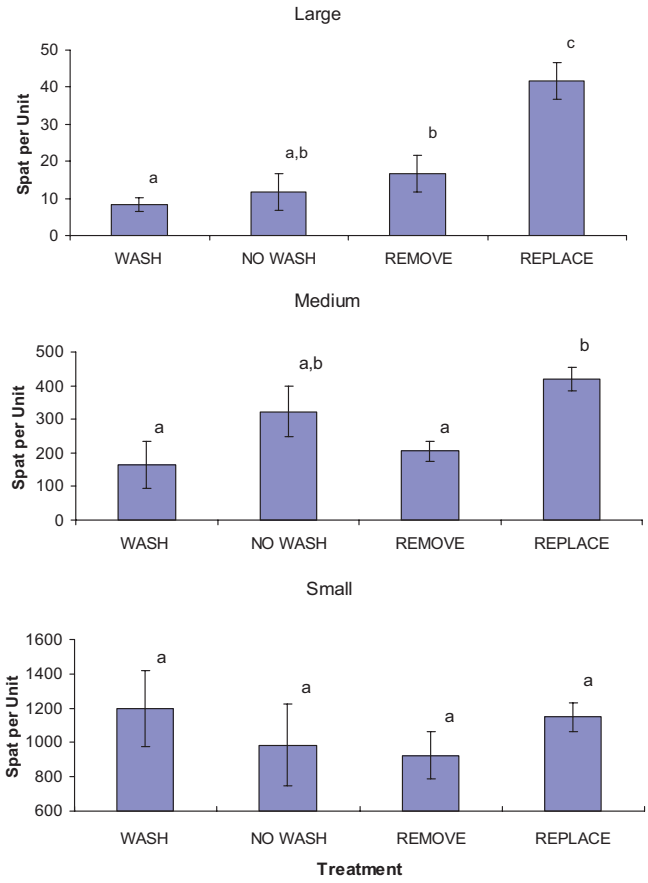

Fig. 2. Mean (± SE) number of *P. margaritifera* spat per tray in Large, Medium and Small size classes after 10 weeks in nursery culture and 6 weeks in one of four treatments (WASH, NO WASH, REMOVE and REPLACE). Means within each size class with the same superscript are not significantly different ($p > 0.05$).

4 Discussion

Use of mesh bags to cover trays for the period between transfer from the hatchery and the first four weeks of nursery culture provided considerable benefit in retaining detached spat. Although a significant proportion (17%) of the spat had detached from spat collectors during or after transfer and were attached to the tray or the tray bag, most of these (10%) were attached to the tray. The tray bag may have prevented the loss of many of these spat; however, the data suggest that *P. margaritifera* spat preferentially reattached to the more rigid plastic tray.

Overall growth of spat between transfer to the sea and the initial census was approximately 1 mm per week. However, growth rate improved after the start of the experiment probably in response to improved water exchange following removal of the original tray bags. Fouling has a negative effect on the growth of pearl oysters (Taylor et al. 1997b; Pit and Southgate 2003a) and this was confirmed in our study. Development of fouling on the tray bags of the WASH and NO WASH treatments was rapid. After 2 weeks, filamentous algae began to grow and, by the end of the experiment, major differences in the degree of fouling on culture units were observed between the four treatments. The nature of the fouling community differed between trays and tray bags. Epizoonts (bryozoans, sponges and tunicates) made up the majority of the fouling on trays in the REMOVE treatment whereas macroalgal fouling dominated on tray bags in the WASH and NO WASH treatments. The tray bags in the REPLACE treatment were free of major fouling with superficial siltation only. Wet weights of the mesh bags from each of the treatments showed that weekly washing reduced the weight of fouling material on the bags, which presumably improved water flow thought the trays.

Use of tray bags improved retention of spat in culture units with mean spat totals approximately 41%, 20% and 18% higher in the REPLACE, WASH and NO WASH treatments,

Table 2. Mean (± SE) number of *P. margaritifera* spat per culture unit at the end of the experiment. Data are presented for each part of the spat culture unit (spat collector, the sum of three spat collectors, tray and tray bag). *Means with the same superscript were not significantly different ($P > 0.05$).

	Treatment			
Source	WASH	NO WASH	REMOVE	REPLACE
Spat collector	282 ± 27	313 ± 82	344 ± 43	375 ± 6
Total spat collectors	846 ± 82	939 ± 245	1031 ± 129	1124 ± 18
Tray	27 ± 91	409 ± 133	116 ± 23	487 ± 116
Tray bag	7 ± 1	8 ± 3	–	3 ± 1
TOTAL*	1380 ± 161[a]	1355 ± 347[a]	1147 ± 151[a]	1614 ± 99[a]

respectively, than in the REMOVE treatment. However, the number of spat attached to the tray bags at the end of the experiment was very low. In the WASH treatment, less than 2% of total spat were attached to the tray at the end of the experiment which is low compared to the other treatments (NO WASH, 30%; REPLACE, 30% and REMOVE, 10%). Removing the tray bag from trays did reduce the labour requirement insofar as labour associated with washing the culture units was reduced; however, any benefit was possibly at the expense of lost spat and the more vigorous cleaning required to remove epizoonts. Trays in the WASH and NO WASH treatment differed little with regard to spat abundance which suggests that washing provided no benefits in terms of spat abundance in treatments where the tray bag was retained and not replaced.

Use of mesh bags to cover early nursery culture units had a positive affect on survival and growth of *P. margaritifera* spat resulting in higher retention and improved growth rates when bags were replaced weekly. Improved growth of spat in the REMOVE and REPLACE treatments probably reflected improved water flow and a concomitant increase in food availability. Although water flow in the REMOVE treatment would be expected to be higher than in other treatments, fewer large spat were present in this treatment. This may result from loss by detachment or predation. Fouling of culture units in the WASH and NO WASH treatments probably restricted water flow in the trays, resulting in reduced growth rates. Taylor et al. (1998) reported that mesh bags improved survival of silver- or gold-lip pearl oyster (*P. maxima*) spat held on PVC slats. While pore size of the mesh (0.75, 1.5 and 3 mm) had no effect on oyster survival, controls without mesh bag covers suffered near 100% mortality due to predation (Taylor et al. 1998). Mesh size did, however, affect growth with spat held in the mesh with the largest pore size recording the highest mean DVH (Taylor et al. 1998). Similarly, the present study recorded the lowest numbers of spat in the REMOVE treatment, which did not have a protective mesh bag.

Friedman and Bell (1996) reported that spat collectors inside protective mesh bags (2 × 5 mm pore size) did not yielded significantly greater numbers of pearl oyster (*P. margaritifera* and *P. maculata*) spat than unprotected spat collectors. Spat collectors were, however, deployed for a period of 6 months which was long enough for predators (e.g. *Cymatium* spp. and portunid crabs) to become established within the collectors and influence spat numbers. Protective mesh bags became heavily fouled during the 6 month immersion period and this was also thought to influence growth and survival of spat (Friedman and Bell 1996). The current study, like that of Taylor et al. (1998), clearly shows that there are benefits in using mesh bags as a means of protecting small pearl oysters spat. However, the results of Friedman and Bell (1996) showed that if protective bags are left too long without washing or replacement, fouling and predation may result in significant loss of spat. While regular washing or replacement of protective mesh bags will help reduce fouling, inspection of culture units for predators could only be achieved if protective bags were removed and replaced.

In summary, this study has shown that the use of mesh bags to cover culture units containing *P. margaritifera* spat can improve retention and growth of spat when replaced weekly. Weekly replacement of mesh bags can yield 2–3 times more large and medium sized spat than other treatments at first grading (10 weeks after transfer), and may improve spat retention by between 18–41% when compared to culture units without mesh bags.

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