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**Stocking density and shelter type for the optimal growth and survival of western rock lobster Panulirus cygnus (George).**

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## Abstract

The growth and survival of three size classes of wild caught western rock lobster, Panulirus cygnus (post-pueruli, mean  $2.14 \pm 0.07$  g,  $13.2 \pm 0.1$  mm CL; year one post-settlement juveniles,  $57.1 \pm 1.1$  g,  $38.7 \pm 0.28$  mm CL; and year two post settlement juveniles, mean  $138.2 \pm 2.26$  g,  $51.9 \pm 0.25$  mm CL) were examined at combinations of two stocking densities (post-pueruli: 50 and 100 m<sup>-2</sup>; year one: 11 and 23 m<sup>-2</sup>; year two: 10 and 19 m<sup>-2</sup>) and two shelter types (a novel rigid plastic mesh shelter or bricks) over a period of 6 months. Survival of lobsters held at the lower densities (90% – 95%) was significantly greater than for lobsters held at higher densities (post-pueruli 78%, year one 86%, year two 88%). Post-pueruli survival was significantly higher in tanks with mesh shelters (91.7%) than brick shelters (75.8%) with a similar trend exhibited by year 1 and 2 lobsters. Densities tested did not significantly affect lobster growth for any size class. Growth of post-pueruli was considerably higher in tanks with mesh shelters (641.7% weight gain; specific growth rate 1.07 BW day<sup>-1</sup>) (p<0.05) but there was no difference in the growth of year 1 and 2 lobsters between mesh and brick shelters. Feed intake (g pellet dry matter lobster<sup>-1</sup> day<sup>-1</sup>) was not significantly different between densities. However, food conversion ratios (FCR) were significantly better for post-pueruli and year 2 lobsters held at lower densities (FCR of 1.9 and 2.3 respectively) and marginally better for year 1 lobsters held at lower densities (FCR 1.4). FCR was also significantly better for all size classes held in tanks with mesh shelters than brick shelters (p<0.05). This study has shown that P. cygnus is well suited for aquaculture based on the collection and on-growing of wild caught pueruli, as this species exhibits good survival at high densities (up to 100 m<sup>-2</sup>) without adverse effects on growth, and shows no captivity-related health problems. We recommend mesh shelters, with stocking densities of 50 m<sup>-2</sup> for post-pueruli and between 20 and 25 m<sup>-2</sup> for year 1 and 2 juveniles, to maximise survival and production.

**Keywords:** rock lobster, density, shelter, survival, growth, aquaculture

## Introduction

Increasing global demand, a high market value and concern for the sustainability of wild stocks have created significant interest in the development of spiny lobster aquaculture (Jeffs and Hooker, 2000; Kittaka and Booth, 2000; Cox and Johnston, 2003; Phillips and Liddy, 2003). Experimental and commercial scale aquaculture production has commenced in a number of countries including New Zealand, Japan, Vietnam, Australia, Phillipines, India, Singapore and Taiwan (Jeffs and Davis, 2003). There are three options for spiny lobster aquaculture; closed cycle culture whereby phyllosoma hatched from captive broodstock are reared to puerulus and ongrown to a marketable size, the capture and subsequent growout of wild post-pueruli and juveniles and the ongrowing and value-adding of adults in land based or seacage systems (Phillips and Liddy, 2003). Although considered the most sustainable option, attempts to mass culture spiny lobsters from eggs to puerulus have been hampered by the provision of unsuitable diets and microbial infection during the long larval phase (Cox and Johnston, 2003). It also remains unclear how economically viable commercial larval culture would be, due to the extended larval period of up to 18 months. For this reason research efforts are focusing on the tropical spiny lobster, Panulirus ornatus, which has a larval phase between 6 and 8 months.

The most feasible culture option, in the short term, is to capture wild pueruli on specialised collectors and ongrow them either in seacage or land based systems. In New Zealand the possibility of expanding to commercial ventures has been promoted by offering entrepreneurs the option of exchanging the right to collect tens of thousands of pueruli for ongrowing in exchange for commercial quota (Booth and Kittaka, 2000). The legislation to collect pueruli has now lapsed and currently there are no commercial collections being undertaken. Trial permits to collect southern rock lobster, Jasus edwardsii, pueruli were also issued in Tasmania and led to the successful collection of pueruli for commercial ventures (Gardner et al., 2004), however, these short duration permits were not renewed and commercial collections have ceased. The cultivation of tropical spiny lobsters, P. ornatus, from captured pueruli and juveniles has become an important industry in Vietnam, and annual exports have reached a value of US\$ 25 million in seven years with production of over 1,000 tonnes per annum (Tuan et al., 2000; Hair et al., 2003). Recent

90 unpublished production figures have been estimated to be 4000 tonnes per annum. Preliminary investigations on small-scale commercial aquaculture of Panulirus argus, based on puerulus capture, have also commenced recently in the Caribbean (Power et al., 2005).

Recent research has shown that it is possible to harvest large numbers of western rock lobster, Panulirus cygnus, pueruli from coastal waters off Western Australia for aquaculture or stock enhancement (Phillips et al., 2001). Removal of P. cygnus post-  
100 pueruli has also been shown to have little impact on the commercial fishery, ensuring biological neutrality (Phillips et al., 2003a,b). However, despite the abundant supply and reliable techniques to capture pueruli, basic data on parameters to ensure optimal growth and survival of P. cygnus in captivity have yet to be obtained.

Shelter is known to be very important in the natural habitat of spiny lobsters (MacDiarmid et al., 1998), and has been shown to affect survival and growth in cultured species (Chittleborough, 1974b; 1976; Zimmer-Faust and Spanier, 1987; Spanier and Zimmer-Faust 1988; Crear et al., 2000; James et al., 2001). For example, shelters significantly enhanced survival of J. edwardsii, but had little effect on growth (Crear et al., 2000; James et al., 2001). The importance of shelter in the reduction of  
110 mortality is also recognised by the placement of artificial shelters in coastal waters of many spiny lobster fisheries (Butler and Herrnkind, 1997; Losada-Tosteson and Posada, 2001). Despite this fact, there is very little information on the effect of different types of shelter on growth and survival of captive spiny lobsters.

Stocking densities are also important for growth and survival of spiny lobsters, although the effects appear to vary between species. In general, mortalities are highest under crowded conditions with post-pueruli being the most susceptible to cannibalism compared with larger juveniles (Booth and Kittaka, 2000). Most mortality is generally due to cannibalism, with recently moulted animals the most vulnerable (Crear et al., 2000; James et al., 2001). Tong (1993) found that for young juvenile J. edwardsii,  
120 weight increase was 40% less for those at 200 m<sup>-2</sup> than those at 50 m<sup>-2</sup>, even though survival was high in all treatments. James et al. (2001) and Rayns (1991) also reported slower growth at higher densities with the same species. Nevertheless, spiny lobsters are gregarious and can be held at relatively high densities compared with other

cultured crustaceans, with individuals held in isolation exhibiting slower growth or lower survival than when in groups (Booth and Kittaka, 2000).

130 Density-dependent mortality of *P. cygnus* in the wild is estimated to be very high (80-98%) during the time between puerulus settlement on inshore reefs and offshore migration by juveniles recruiting into the fishery (Phillips et al., 2003a). Coupled with the fact that post-puteruli are solitary in the wild and only become gregarious as they become larger juveniles (>20 mm carapace length) (Fitzpatrick et al., 1989), it is clear that stocking densities will be particularly important for the successful culture of *P. cygnus*.

140 The aims of this study were twofold: 1) to investigate the effect of two levels of stocking density and 2) two shelter types, on the growth and survival of western rock lobster *P. cygnus* post-puteruli, year 1 and year 2 juveniles. This study also examined the effects of these parameters on feed intake and health status. Based on these results we have provided a preliminary assessment of the feasibility of *P. cygnus* as an aquaculture candidate.

## Methods

Lobsters were collected from waters off Seven Mile Beach, Western Australia, using sandwich collectors (Phillips et al., 2001) and baited mesh pots between November 2003 and January 2004. Animals were transported to the Western Australian Marine Research Laboratories, Perth and held in 250L tanks with shelter until the trial commenced in March 2004. The trial continued for 6 months until September 2005. Post-puteruli (mean  $2.14 \pm 0.07$  g,  $13.2 \pm 0.1$  mm CL); year 1 post settlement juveniles (mean  $57.1 \pm 1.1$  g,  $38.7 \pm 0.28$  mm CL) and year 2 post settlement juveniles (mean  $138.2 \pm 2.26$  g,  $51.9 \pm 0.25$  mm CL) were randomly stocked at two densities into 60 L, 250 L and 350 L tanks respectively, each containing either bricks or novel shelters designed out of rigid plastic mesh, hereafter termed 'mesh shelters' (total of twelve tanks for each size class with all experimental treatments run in triplicate) (Fig 1). The stocking densities were as follows: post-puteruli: 50 and 100 m<sup>-2</sup>; year one: 11 and 23 m<sup>-2</sup>; year two: 10 and 19 m<sup>-2</sup> (10 or 20 lobsters in each tank respectively for each size class). These densities translated to a final predicted biomass after one year's growth

(Chittleborough, 1974a; 1976; Phillips et al., 1977) of: post-pueruli 5.8 and 11.6 kg m<sup>-3</sup>; year one 5.2 and 10.5 kg m<sup>-3</sup> and year two 7.2 and 14.3 kg m<sup>-3</sup>. All tanks received flow-through ambient temperature seawater at a flow rate of 60 L h<sup>-1</sup>. Wet weight of all lobsters was measured using an electronic balance to the nearest 0.1 g after blotting excess water with absorbent towel. Carapace length (CL) was measured using vernier callipers to the nearest 0.1 mm.

The mesh shelters were made of folded rigid mesh (between 2 and 4 folds vertically) attached by cable ties to 4 vertical rods and a plastic lid (Fig 1a, b). Short pieces of plastic tubing were placed over the vertical rods as spacers between the mesh layers. Cable ties were attached in the middle of each mesh fold in post pueruli shelters to reduce the height of each layer and create small crevices for pueruli to hide (Fig 1a). The length of these shelters and the height of the mesh crevices (post pueruli 2-4 cm; year 1 and 2 juveniles 10-15 cm) increased with each lobster size class, so that the available surface area of the shelter to lobsters remained relatively constant between size classes. The brick shelters for post-pueruli consisted of small individual bricks with circular holes ranging in diameter from 2 cm to 5 cm. Bricks with the smallest holes were used for recently settled animals and were replaced throughout the trial with bricks with larger holes as the animals increased in size (Fig 1c). Besser bricks with two large individual holes and 3mm thick rectangular plastic sheets were used for year 1 and 2 lobsters. The Besser bricks were placed on two smaller bricks to raise them off the floor of the tank and a single plastic sheet was extended from one side to provide additional shelter. There were two of these shelters per tank (Fig 1d).

Following stocking, lobsters were acclimated in the treatment tanks for 1 week and fed with a rock lobster pelleted diet. This diet has been specially formulated for the tropical rock lobster *P. ornatus* (Smith et al., 2005) and was made in monthly batches at the Western Australian Department of Fisheries nutrition laboratory during the experiment, using a pasta maker followed by oven drying at 70°C. The proximate composition of the diet on a % dry matter basis was protein 55%, lipid 10% and ash 11%. Any mortalities during the acclimation week were replaced with similar sized animals which had been held under similar conditions. During this acclimation period satiation feed rates were determined for each tank and a feed rate per biomass (expressed as % body weight day<sup>-1</sup>) calculated and fed during the trial (90% of the

feed rate where satiation was reached during the acclimation period). During the experiment mortalities were not replaced due to potential long-term differences between lobsters in holding versus treatment tanks over the 6 month period.

Lobsters were fed the pelleted diet daily in the late afternoon (1600 h) during the week (about 10% BW day<sup>-1</sup> for post pueruli, 2.5% BW day<sup>-1</sup> year 1 juveniles and 2% BW day<sup>-1</sup> for year 2 juveniles) and supplemented with fresh mussels (Mytilus edulis) on the weekends. Supplementation with mussels was implemented to address any possible nutritional deficiencies in the pellet diet and hence maximise growth and survival. Feeding prior to dusk was implemented as the majority of feeding occurs by lobsters at the start of the dark photophase (Fielder, 1965). The following morning, the amount of feed left uneaten (as a percentage of food fed) was assessed visually and the feed rate adjusted so that >90% of the feed was consumed each day. All uneaten pellets were then removed from tanks. Once a month for six consecutive days, apparent feed intake was accurately measured by siphoning uneaten feed the morning after the 1600 h feed onto a mesh screen, washing with fresh water to remove salt (Brunson et al., 1997) and drying overnight for 18 hours at 70°C. Apparent feed intake (g pellet dry matter day<sup>-1</sup>) was calculated taking into account leach rates of the diet. Estimates of feed intake refers only to that of pellet feed and for 2 out of the 7 days of the week (28% of the time) the spiny lobsters were fed on fresh mussels, the intake of which was not quantified. Consequently calculations of food conversion ratios were not included in this paper.

Each morning, moults and mortalities were removed and recorded. Water quality parameters, temperature, dissolved oxygen, pH and salinity were monitored weekly. Photoperiod was 12 h fluorescent light per day for the indoor 60 L and 250 L tanks and natural photoperiod for the 350 L tanks which were located outside. Lobster weight and carapace length was measured every 5 weeks and data used to calculate the mean tank specific growth rate (SGR) and body weight increase (% weight gain). Adjustments to feed allocations for the increase in biomass were made every month following the weight measurements. At each weighing the tanks were thoroughly cleaned.



### *Data Processing*

Apparent feed intake was calculated by subtracting the dry weight of uneaten feed from the dry weight of pellet fed, after taking into account the proportion of feed lost into the water. The percentage of pellet lost into the water was calculated by immersing samples into 3 replicate tanks with lightly agitated/aerated water for 19 h. 230 The feed remaining was collected into a sieve, washed with fresh water to remove salt (Brunson et al., 1997) and dried in an oven overnight at 70°C. A mean 20% of pellet weight was lost into water (80% of pellet was available for ingestion by the lobsters). Mussel consumption was not included in the calculation of feed intake, because mussels were not fed in weeks that accurate feed intakes were measured.

To compensate for mortalities the number of lobster days of feeding was calculated (based on survivors at each weighing) and used to calculate daily weight gain (weight of dead lobsters subtracted from the tank biomass, lobster weight based on mean weight of lobsters in the tank) and feed intake for each lobster.

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Apparent Feed Intake (g dry matter DM lobster day<sup>-1</sup>) = (Dry weight of pellet fed (g) \* 0.8) – (dry weight of uneaten pellet (g)) / lobster days of feeding

Growth rates were calculated using specific growth rates (SGR) to overcome problems associated with exponential growth rates (Hopkins, 1992; Crear et al., 2000). SGR as % body weight per day (% BW day<sup>-1</sup>), percentage weight gain (% WG) and growth coefficient were calculated as follows:

SGR (% BW day<sup>-1</sup>) = (ln final mean lobster weight – ln initial mean lobster weight) \* 100 / number of days 250

Percentage weight gain (% WG) = (final mean lobster weight – initial mean lobster weight) \* 100 / initial mean lobster weight

Growth Coefficient = 100 x (time 2 tank total weight<sup>1/3</sup> – time 1 tank total weight<sup>1/3</sup>) / number of days

### *Statistical Analyses*

Two-way analysis of variance (ANOVA) was used to test for differences in measured variables between the treatments at the completion of the trial (significance level  $P < 0.05$ ). For each analysis, the assumptions of ANOVA were checked using residual plots. Tukey's HSD *post hoc* test was used to identify differences between means for days and treatment. To compare how measured variables for different treatments varied over time throughout the trial, a split-plot design (Insightful, 2001) was used. This design is suitable for a repeated measures experiment when the "circulatory condition" holds, as well as the usual conditions required for ANOVA. The circulatory condition means that the variances of all pair-wise differences of the observations at each point in time are equal (Insightful, 2001). Estimating the variance of all pair-wise differences and comparing using multiple F-tests assessed the validity of this assumption. To test the effects of density and shelter type on the proportion of lobsters surviving over time, a logistic regression was used:  $S_i(t) = \exp(-At)$ , where  $S_i(t)$  is the proportion of lobsters (necessarily between 0 and 1) that have survived over time  $t$  in tank  $i$  and  $A$  is a linear combination of dummy variables that have been used to model the main effects and interaction terms of the variables being considered. A logistic regression was seen as being appropriate since it forces predicted values (and their confidence intervals) to be between 0 and 1, whereas the split-plot design does not. Regressions were also used to assess significant differences in survival as the trend in survival over time was seen as important, rather than just the final survival data at the completion of the trial.

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### *Health Monitoring*

Visual assessments of lobster condition (fouling on shell and gills, lesions, tail fan necrosis) were undertaken weekly. To assess the ability of lobsters to fight infection, nine post-*pueruli*, year 1 and 2 lobsters were sampled (3 replicates x 3 tanks) on 23 June 2004 for prophenoloxidase analysis (Norton et al., 2001). Each lobster was measured (weight and CL) and sexed and 2 ml of haemolymph withdrawn from the 3<sup>rd</sup> walking leg via a 21 gauge needle into a 5 ml disposable syringe containing anticoagulant solution at pH 4.6. Samples were kept chilled until centrifuged at 800 g for 25 min at 4°C. The supernatant was collected for protein determination using a BCA Protein Assay Kit (Pierce). The pellet was washed in anticoagulant and then washed with 10 mM sodium cacodylate buffer (pH 7.0) before being homogenised.

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Each sample was then made up to 9 ml in a centrifuge tube with fresh buffer and centrifuged at 13500 rpm (20 000 g) for 20 min at 4°C. Supernatant (50 µl) was pre-incubated with 50 µl of inducer (1 mg ml<sup>-1</sup> trypsin made up in deionised water) for 30 mins at 20°C. Fifty µl of enzyme substrate L-DOPA (3 mg ml<sup>-1</sup>) was added with 850 µl of deionised water to slow the reaction (1 ml in total) at 20 °C. For a control, 0.45 M NaCl was used instead of supernatant. Prophenoloxidase activity was measured in a spectrophotometer after 0, 5, 10, 20 and 60 min by absorbance at 490 nm (the formation of the red pigment DOPA-chrome). Spontaneous oxidation (control) was measured by incubating L-DOPA only with 0.45M NaCl. Results are expressed as change in prophenoloxidase per min per ml of sample.

Nine post-pueruli, year 1 and year 2 lobsters were sampled (3 replicates x 3 tanks) for histological analysis to ascertain whether lobsters showed evidence of pathology. Following dissection, the digestive glands of lobsters were fixed in 10% formalin in seawater and processed routinely for histology. Transverse sections (6 µm) of the gland were taken and stained in haemotoxylin and eosin.

## Results

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Mean survival after 6 months ranged from 75.8% to 95.0% (Table 1). The two levels of density tested significantly affected the survival of all size classes, with considerably lower survival in higher density tanks. Post-pueruli were the most sensitive to density, exhibiting the greatest decline in survival. Post-pueruli survival decreased dramatically in the first 80 days, particularly in the high density treatment, and then plateaued for the remainder of the trial (Fig. 2). Year 1 and 2 lobster survival was constant in the first 60 days but steadily declined through to 188 days, except for year 2 lobsters held at high density when all the losses were in the final month (Fig. 2). Most mortality was due to cannibalism of recently moulted lobsters.

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Survival of post-pueruli was significantly higher in tanks with mesh shelters than brick shelters (Table 1). A similar trend was evident for year 1 and 2 lobsters, although not statistically significant.

Growth rates of lobsters in each size class were high in the first 30 to 60 days of the trial (March and April) but slowed significantly after 100 days (July, August and September), with almost negligible growth in the last 60 days for year 1 and year 2 lobsters (Fig. 3a,b,c). The reduced growth rates are attributed to seasonal changes in temperature, which declined from 21.9 °C in March to 15.9 °C in August.

330 The two levels of density tested (post-puerulus - 50 or 100 m<sup>-2</sup>; year 1 juveniles - 11 or 23 m<sup>-2</sup>; and year 2 juveniles - 10 or 19 m<sup>-2</sup>) did not significantly affect lobster growth (final weight, SGR, % weight gain, growth coefficient) in any size class (Table 2). Shelter type significantly affected growth of post-pueruli (final weight -  $F_{(1,8)} = 5.49$ ;  $p = 0.047$ ; SGR -  $F_{(1,7)} = 5.3$ ;  $p = 0.05$ ; % weight gain -  $F_{(1,7)} = 5.7$ ;  $p = 0.05$ ; growth coefficient -  $F_{(1,6)} = 13.34$ ;  $p = 0.01$ ), with considerably higher growth in tanks with mesh shelters (Table 2; Fig. 4). There was no significant difference in the growth of year 1 and year 2 lobsters between mesh and brick shelters (Table 2).

340 Apparent feed intake (AFI) was greatest in year 2 lobsters and lowest in post-puerulus (Table 2). AFI declined significantly throughout the trial (from March to September) for all size classes, but mostly for year 2 juveniles, and reflects a reduction in metabolic rate consistent with seasonal declines in water temperature (Fig. 5). The greatest decline was during the last 30 days of the trial (August-September) and for year 2 lobsters. Density did not significantly affect AFI for post-puerulus or year 2 lobsters, although it was significantly greater for year 1 lobsters held at high density ( $F_{(1,6)} = 6.08$ ,  $p = 0.05$ ) (Table 2). There was no significant difference in AFI by post-puerulus in tanks with brick versus mesh shelters, but AFI was significantly lower in tanks with mesh shelters for year 1 ( $F_{(1,6)} = 16.95$ ;  $p = 0.01$ ) and year 2 lobsters ( $F_{(1,6)} = 7.54$ ,  $p = 0.03$ ) (Fig. 6).

350 Histological analysis of lobsters sampled during the trial revealed no pathology of the digestive glands that was consistent with infection or stress in any lobster size class. All lobsters had high numbers of reserve cells in digestive gland tubules. Prophenoloxidase levels increased with lobster size, although one individual had a considerably lower level than equivalent sized animals (Table 3).

## Discussion

This study has shown that P. cygnus is well suited for aquaculture based on the collection and on-growing of wild caught pueruli, as this species exhibits good survival at high densities (up to 100 m<sup>-2</sup>) without adverse effects on growth, and shows no captivity-related health problems. Post-pueruli in particular can be stocked at very high densities of 50 and 100 m<sup>-2</sup> achieving between 77.5% and 90% survival, respectively, whereas year 1 and 2 lobsters achieved between 85.8% and 95% survival at densities between 10 and 23 m<sup>-2</sup>. Such high survival at these densities can be attributed in part to the generally gregarious nature of spiny lobsters. The higher mortalities for post-pueruli compared with year 1 and 2 lobsters may be attributed to the fact that recently settled P. cygnus post-pueruli are solitary up to approx 20 mm CL, whereas year 1 and 2 lobsters are more gregarious (Fitzpatrick et al., 1989).

Growth rates in this study are consistent with those of *P. cygnus* lobsters held in aquaria at ambient temperatures (Phillips et al., 1977; Glencross et al., 2001). Growth of all lobsters declined throughout the trial, with negligible growth for year 1 and 2 lobsters in the months of July, August and September. The reduced growth rates are attributed to a seasonal reduction in tank water temperatures from 21.9°C in March to 15.9°C in September, revealing the close relationship between spiny lobster growth and water temperature. In contrast, growth rates of lobsters held at constant 23°C temperatures were considerably higher and did not exhibit the same degree of fluctuation (Phillips et al., 1977). Optimal growth of P. cygnus has been achieved at 23°C (Phillips et al., 1977), and 25°C (Chittleborough, 1974a,b; 1976), hence it is clear that to maximise growth of P. cygnus in culture, water temperatures need to be elevated above winter ambient temperatures. Feed intake also declined significantly from March to September reflecting seasonal declines in water temperature and metabolic rates of lobsters. Due to these low winter temperatures feed intake is generally lower than has been reported for both J. edwardsii and P. cygnus fed pellet diets (Crear et al., 2000; Glencross et al., 2001).

It has been shown that growth of P. cygnus when fed only the pellet diet is considerably lower than when fed in combination with fresh mussels, indicating that the current pellet diet is not nutritionally complete (Johnston et al. unpublished data). Glencross et al. (2001) also reported a similarly poor performance of P. cygnus post-

390 pueruli fed solely pelleted diets compared with fresh mussels and suggested that low feed intake of pellets rather than grossly inadequate supply of nutrients was responsible. It is clear that considerable research is required to increase pellet consumption and optimise nutrient specifications for the long-term viability of P. cygnus culture.

#### *Density Effects*

Density clearly had a significant effect on survival of P. cygnus during the six month trial, with lobsters stocked at the higher densities exhibiting the lowest survival. Post-  
400 pueruli were the most sensitive to density due to rapid moulting and associated cannibalism, compared with the slower growing year 1 and 2 lobsters. P. cygnus post-  
pueruli settlement densities are estimated to be between 0.11 and 1.93 m<sup>-2</sup> at Seven Mile Beach (Fitzpatrick et al., 1989) and between 1 and 4 individuals m<sup>-2</sup> on inshore  
reefs (Chittleborough and Phillips, 1975) with extremely high density-dependent mortality (80-98%) between settlement and offshore migration by juveniles recruiting into the fishery (Phillips et al., 2003a, b). This study confirms that density-dependent mortality is also prevalent in tank culture and confirms the importance of maintaining appropriate stocking densities for optimal survival of post-  
410 pueruli. Although survival of P. cygnus appears to be significantly affected by density, it is not necessarily the case for other spiny lobster species. P. ornatus post-  
pueruli were not strongly influenced by density, although substantial mortality occurred over the duration of their 9 month experiment (mean survival 52.5%) (Jones et al., 2001). Furthermore, it is possible that densities in their study (14, 29, 43 m<sup>-2</sup>) were not high enough to show  
a pronounced density effect on survival. Nevertheless, James et al. (2001) also found  
410 that much higher densities of 50, 100, 150 and 200 m<sup>-2</sup> had no effect on survival of J. edwardsii after 118 days, although maximum survival was recorded at 50 and 100 m<sup>-2</sup>. There are clearly species-specific differences in the response of spiny lobsters to density, however, the present study demonstrates and supports the well accepted belief that under crowded conditions spiny lobster post-  
420 pueruli are highly susceptible to cannibalism and mortality (Booth and Kittaka, 2000).

In contrast to survival, the two levels of density tested (post-  
420 pueruli - 50 or 100 m<sup>-2</sup>; year 1 juveniles - 11 or 23 m<sup>-2</sup>; and year 2 juveniles - 10 or 19 m<sup>-2</sup>) did not significantly affect lobster growth (final weight, SGR, % weight gain, growth

coefficient) for all size classes. In contrast it has been demonstrated that growth of P. cygnus in the wild is most suppressed at localities where the density of juveniles is highest and is considerably better at localities with low densities (Chittleborough, 1976). Unfortunately densities were not stated so it is difficult to directly compare these wild trends with the tank studies. However, Chittleborough (1976) conceded that it was possible that factors other than density, such as limited food supply, may be responsible for the depressed growth rates. Food abundance has since been found to be responsible for differences in growth rate of wild lobsters at these locations (Joll and Phillips, 1984; Edgar, 1990). In this study food was in surplus, so under  
430 conditions of unlimited food supply, it is likely that density may play a less important role in influencing growth for P. cygnus. Indeed density-dependent growth implies a limited supply of food (Morrissy, 1992). At higher densities social hierarchies and agonistic behaviour between conspecifics are generated whereby aggressive individuals consume a disproportionate share of the group's meal and subsequently grow faster than subordinates (Thomas et al., 2003). This situation has been discouraged in the present study by feeding lobsters to satiation, a technique that is commonly applied in many culture situations to maximise growth.

Growth of P. ornatus juveniles was not significantly affected by density (14, 29 and  
440 43 m<sup>-2</sup>) (Jones et al., 2001), which is consistent with the results of this study. In contrast, growth was depressed at high densities for J. edwardsii, with lobsters held at 50 m<sup>-2</sup> having the highest mean CL and wet weight and those at 200 m<sup>-2</sup> the lowest (James et al., 2001). These authors did, however, recommend that maximum growth rates and survival of J. edwardsii would be achieved at 50-100m<sup>-2</sup>, which is consistent with this study. It has been found that growth of spiny lobsters is depressed at very low densities or when animals are held in isolation (Chittleborough, 1974b; 1975; Rayns, 1991) confirming that growth performance is better at higher densities for these gregarious animals.

450 It is possible that densities in this study, and that of Jones et al. (2001), were not high enough to inhibit growth, particularly for year 1 and 2 lobsters. A recent study examining the growth and survival of P. cygnus post-pueruli at a wide range of densities found that growth was significantly lower at the highest density (150 m<sup>-2</sup>) compared with the lowest density (30 m<sup>-2</sup>) (Moyle, 2005). Therefore it appears that P.

*cygnus* post-pueruli are able to be held at relatively high densities, up to 100 m<sup>-2</sup>, without any effect on growth, but beyond this growth is inhibited. This characteristic of *P. cygnus* suggests that it would be an excellent species for culture as production per area would be extremely high compared with some other cultured crustaceans. For example, density significantly inhibits the growth of freshwater crayfish with optimal growth rates and production only being achieved at densities of 3-5 m<sup>-2</sup> for marron in ponds (Morrissy et al., 1995a) and 1 m<sup>-2</sup> for yabbies unfed in ponds with zero water exchange. Growth rates of freshwater crayfish are curvi-linear, where at low densities (1-2 m<sup>-2</sup>) crayfish grow rapidly, at high densities (10 m<sup>-2</sup>) their growth curve flattens out and at very high densities (20 m<sup>-2</sup>) there is almost no growth (Morrissy, 1992; Morrissy et al., 1995 a, b).

Density did not significantly affect feed intake of post-pueruli and year 2 lobsters, although it was higher in year 1 lobsters held at high densities. It is likely that feeding to excess ensured that all post-pueruli and year 2 juveniles were able to consume similar quantities of pellet, irrespective of density. It is not clear why year 1 lobsters at high density had higher feed intake given that feed was also given to excess in these tanks.

#### *Shelter Effects*

This study confirms that shelter significantly affects the growth and survival of spiny lobsters, a trend consistent with other wild and culture lobster studies (Chittleborough, 1974b; 1975; Zimmer-Faust and Spanier, 1987; Spanier and Zimmer-Faust, 1988; Crear et al., 2000; James et al., 2001). It has been clearly demonstrated that the presence of shelters in tanks significantly increased the survival of *J. edwardsii*, but had little effect on growth (Crear et al., 2000; James et al., 2001). With the absence of predators in a cultured situation this reduction in mortalities can be attributed to shelter being used primarily by subordinate animals to avoid interactions with dominant lobsters during vulnerable stages such as moulting, subsequently reducing the likelihood of cannibalism.

There have been very few studies on the effects of different types of shelters on growth or survival, with most studies examining presence versus absence. The type of shelter or habitat provided needs to accommodate the complex social behaviour of



spiny lobsters (Atema and Cobb, 1980). Based on their different behaviours during  
490 development it is likely that shelter needs may vary between different sized lobsters.  
This study demonstrated that shelter type significantly influences the survival of P. cygnus, with considerably higher survival of all lobsters with mesh shelters than brick shelters with this trend significant for post-*pueruli*. The folding mesh stack configuration provided greater refuge and protection against cannibalism at all sizes. This was especially important for post-*pueruli* as it allowed them to climb away from conspecifics during moulting. Provision of a vertical mesh design has also been shown to make better use of tank space for J. edwardsii (Rayns, 1991; James et al., 2003). The greater impact of shelter type on the survival of P. cygnus post-*pueruli*, compared with year 1 and 2 lobsters, is attributed to their greater need for refuge on  
500 account of increased vulnerability during frequent moulting. This trend has also been reported for J. edwardsii (Kington, 1999; James et al., 2001). Despite their small size, post-*pueruli* are known to display aggressive behaviours to conspecifics, particularly in situations where space or food is limiting (Berrill, 1976; Thomas et al., 2003; Moyle, 2005). Hence it is vital particularly in the early stages of growout to not only provide shelter, but the correct type of shelter for post-*pueruli*.

Mesh shelters promoted significantly higher growth for post-*pueruli* but this trend was not evident for year 1 and year 2 juveniles. The folded mesh configuration facilitated minimal contact between post-*pueruli*, thereby reducing stress during frequent  
510 moulting and allowing maximum growth. This is consistent with other studies on P. cygnus where the provision of shelter resulted in higher rates of feed intake and better growth than those deprived of shelter (Chittleborough, 1974b; 1975). Older juveniles are less affected by conspecifics during growth and are therefore not as sensitive to shelter type compared with rapidly growing post-*pueruli*. This is consistent with J. edwardsii juveniles where growth did not change with shelter presence or absence (Crear et al., 2000; James et al., 2001). Nevertheless, other studies have reported a slowing of growth in the absence of shelters emphasising their importance in communal tanks (Booth and Kittaka, 2000). It is possible that shelter type may influence growth of these older lobsters in the presence of predators as it has been  
520 shown that shelter use and selection by adult lobsters is regulated by predation risk, group size and the relationship between lobster size and shelter size (Eggleston and

Lipcius, 1992). It is clear that provision of shelter and type of shelter is critical for maximising the survival and growth of *P. cygnus* post-*pueruli*, whereas it is only critical for maximising survival for older year 1 and 2 juveniles.

Shelter type significantly affected feed intake by older juveniles, but not post-*pueruli*, with feed consumption by year 1 and 2 lobsters lower in tanks with mesh shelters. The fact that this apparent increased feed consumption by lobsters in tanks with brick shelters did not correspond with a significant increase in growth rate suggests that this trend may be an anomaly. It is possible that this trend could be attributed to  
530 difficulties in siphoning, as pellets were often trapped in and under the larger mesh shelters in year 1 and 2 tanks. Although very few studies comment on feed utilisation with respect to shelter, Chittleborough (1974b, 1975) did observe higher feed consumption by *P. cygnus* when shelters were present indicating that when lobsters are protected and comfortable with their shelter provisions higher feed consumption and ultimately better growth is possible.

#### *Lobster Health*

Levels of prophenoloxidase in lobsters are known to fluctuate in response to environmental changes and stress (Moullac & Haffner 2000) and they, together with  
540 the level of protein in the hemolymph, have been used as an indicator of lobster health (Chang, 1995; Floreto et al., 2000; Osbay & Riley 2002). Comparison of prophenoloxidase levels between *P. cygnus* sampled in this study and a database of 119 *P. cygnus* lobsters (both wild caught and wild captive) has revealed that all but one of the test animals were within normal range for prophenoloxidase and similar to some of the larger wild caught lobsters (B. Jones, unpublished data). The 16.8 mm CL juvenile had low prophenoloxidase activity, but the haemolymph protein levels for that animal were in the normal range. Therefore there is no evidence to suggest that the lobsters in this study were unduly “stressed” and they appear to be as healthy as animals from the wild.

550 Histology revealed high numbers of reserve cells were present in the digestive gland tubules of *P. cygnus* lobsters in this study. Reserve cells are usually associated with lipid storage and transport, indicating that these lobsters consumed a high lipid diet. The fresh mussels fed to lobsters on weekends throughout the trial are high in lipid

compared with formulated pellet diet (Glencross et al., 2001; Smith et al., 2005). Therefore it is possible that lobsters in this experiment are storing lipid following ingestion of mussels and then mobilising lipid in the days thereafter for energy. This suggests that the pellet may not be nutritionally complete for P. cygnus, and is consistent with significantly slower growth by lobsters fed only pellets, compared  
560 with lobsters fed pellet and fresh mussels (Johnston et al., unpublished data).

### **Conclusions**

The spiny lobster P. cygnus exhibited excellent survival after six months in captivity and may be stocked at high densities with little adverse effect on growth. These attributes, together with no captivity-related health problems, make P. cygnus an ideal candidate for aquaculture, based on the growout of wild caught post-pueruli. Density and shelter type significantly impacted survival, particularly for post-pueruli, and this size class should therefore be carefully managed. This study has shown that post-  
570 pueruli, year 1 and year 2 lobsters should be cultured using mesh shelters, with stocking densities of 50 m<sup>-2</sup> for post-pueruli and between 20 and 25 m<sup>-2</sup> for year one and two juveniles, to maximise survival and production. Ambient water temperatures slowed growth in the winter months and it is clear that temperatures need to be consistently higher than ambient in winter to optimise growth of P. cygnus. The relatively good consumption of formulated pellet diets compared with other spiny lobster species offers potential for the further development of diets for P. cygnus.

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**Table 1.** Survival (mean %  $\pm$  standard error) of *P. cygnus* at two densities and shelter types for different size classes after 6 months. Low and high densities (lobsters m<sup>-2</sup>) are 50 and 100 for post-pueruli, 11 and 23 for year 1 and 10 and 19 for year two lobsters. P values for the logistic regressions are indicated and bold if significant. Logistic regressions were used to determine whether there were significant differences in survival over time (see Fig 1). There were no significant interactions between density and shelter type.

| Size Class              | Density          |                | Shelter Type     |                |
|-------------------------|------------------|----------------|------------------|----------------|
|                         | Low              | High           | Brick            | Mesh           |
| <b>Post-Pueruli</b>     | 90.0 $\pm$ 3.3   | 77.5 $\pm$ 5.2 | 75.8 $\pm$ 4.2   | 91.7 $\pm$ 3.7 |
|                         | <b>p&lt;0.01</b> |                | <b>p&lt;0.01</b> |                |
| <b>Year 1 Juveniles</b> | 95.0 $\pm$ 3.1   | 85.8 $\pm$ 4.6 | 89.2 $\pm$ 3.7   | 91.7 $\pm$ 4.2 |
|                         | <b>p&lt;0.01</b> |                | p=0.34           |                |
| <b>Year 2 Juveniles</b> | 90.0 $\pm$ 1.9   | 87.5 $\pm$ 4.4 | 89.2 $\pm$ 4.2   | 95.0 $\pm$ 3.1 |
|                         | <b>p&lt;0.01</b> |                | p=0.3            |                |

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**Table 2.** Growth response (mean  $\pm$  standard error) and diet utilisation (mean  $\pm$  standard error) of three size classes of *P. cygnus* at two levels of density and two shelter types after 6 months (March – September 2004). Asterisks indicate parameters that are significantly different between either density or shelter type. Refer to text for statistical results. Data analysed using split plot analyses to determine significant changes with density or shelter over time of trial (SGR, %WG, growth coefficient, AFI), and two way ANOVA to determine significant differences between final data (initial weight, final weight, FCR). There were no significant interactions between density and shelter type. FCR was calculated using mean weight of lobsters.

| Size Class              | Parameter                                  | Density           |                   | Shelter Type      |                   |
|-------------------------|--|-------------------|-------------------|-------------------|-------------------|
|                         |  | Low               | High              | Brick             | Mesh              |
| <b>Post-Pueruli</b>     | <b>Initial Weight (g)</b>                  | 2.21 $\pm$ 0.11   | 2.16 $\pm$ 0.09   | 2.11 $\pm$ 0.05   | 2.26 $\pm$ 0.13   |
|                         | <b>Final Weight (g)</b>                    | 15.88 $\pm$ 0.60  | 15.07 $\pm$ 0.79  | 14.49 $\pm$ 0.79* | 16.46 $\pm$ 0.31* |
|                         | <b>SGR (% BW day<sup>-1</sup>)</b>         | 1.06 $\pm$ 0.03   | 1.04 $\pm$ 0.03   | 1.08 $\pm$ 0.06*  | 1.25 $\pm$ 0.03*  |
|                         | <b>% Weight Gain</b>                       | 628.3 $\pm$ 41.0  | 601.9 $\pm$ 38.7  | 588.5 $\pm$ 34.7* | 641.7 $\pm$ 42.5* |
|                         | <b>Growth Coefficient</b>                  | 1.40 $\pm$ 0.09   | 1.41 $\pm$ 0.13   | 1.31 $\pm$ 0.12*  | 1.50 $\pm$ 0.08*  |
|                         | <b>AFI (g DM lobster day<sup>-1</sup>)</b> | 0.12 $\pm$ 0.01   | 0.14 $\pm$ 0.01   | 0.13 $\pm$ 0.01   | 0.14 $\pm$ 0.01   |
| <b>Year 1 Juveniles</b> | <b>Initial Weight</b>                      | 55.13 $\pm$ 1.06  | 57.11 $\pm$ 1.39  | 55.79 $\pm$ 1.15  | 56.44 $\pm$ 1.42  |
|                         | <b>Final Weight</b>                        | 92.71 $\pm$ 3.09  | 98.34 $\pm$ 1.95  | 97.80 $\pm$ 1.69  | 93.25 $\pm$ 3.37  |
|                         | <b>SGR</b>                                 | 0.28 $\pm$ 0.02   | 0.29 $\pm$ 0.01   | 0.32 $\pm$ 0.02   | 0.33 $\pm$ 0.01   |
|                         | <b>% Weight Gain</b>                       | 68.2 $\pm$ 4.7    | 72.5 $\pm$ 3.3    | 75.6 $\pm$ 3.9    | 65.0 $\pm$ 3.1    |
|                         | <b>Growth Coefficient</b>                  | 0.73 $\pm$ 0.05   | 0.73 $\pm$ 0.05   | 0.78 $\pm$ 0.10   | 0.71 $\pm$ 0.1    |
|                         | <b>AFI</b>                                 | 0.23 $\pm$ 0.02*  | 0.27 $\pm$ 0.01*  | 0.28 $\pm$ 0.01*  | 0.21 $\pm$ 0.02*  |
| <b>Year 2 Juveniles</b> | <b>Initial Weight</b>                      | 137.94 $\pm$ 3.02 | 138.43 $\pm$ 1.36 | 138.59 $\pm$ 2.47 | 137.77 $\pm$ 2.19 |
|                         | <b>Final Weight</b>                        | 169.61 $\pm$ 4.02 | 167.15 $\pm$ 2.03 | 167.86 $\pm$ 3.32 | 168.90 $\pm$ 3.12 |
|                         | <b>SGR</b>                                 | 0.11 $\pm$ 0.002  | 0.1 $\pm$ 0.006   | 0.10 $\pm$ 0.003  | 0.11 $\pm$ 0.006  |
|                         | <b>% Weight Gain</b>                       | 22.93 $\pm$ 0.39  | 20.78 $\pm$ 1.37  | 21.09 $\pm$ 0.63  | 22.62 $\pm$ 1.35  |
|                         | <b>Growth Coefficient</b>                  | 0.38 $\pm$ 0.07   | 0.13 $\pm$ 0.16   | 0.15 $\pm$ 0.14   | 0.36 $\pm$ 0.12   |
|                         | <b>AFI</b>                                 | 0.31 $\pm$ 0.02   | 0.36 $\pm$ 0.04   | 0.39 $\pm$ 0.03*  | 0.29 $\pm$ 0.02*  |

800 **Table 3.** Measurements of prophenoloxidase and protein for nine test lobsters of varying sizes. ProPO, prophenoloxidase.

| <b>Carapace length (mm)</b> | <b>ProPO (min ml<sup>-1</sup>)</b> | <b>Protein (mg ml<sup>-1</sup>)</b> | <b>Comment</b>       |
|-----------------------------|------------------------------------|-------------------------------------|----------------------|
| 13.8                        | 26.6                               | 0.34                                |                      |
| 16.8                        | 3.8                                | 0.35                                | low value for ProPO. |
| 17.3                        | 25.3                               | 0.25                                |                      |
| 38.7                        | 21.5                               | 0.65                                |                      |
| 50.5                        | 20.3                               | 1.51                                |                      |
| 52.4                        | 38.0                               | 0.5                                 |                      |
| 61.5                        | 55.7                               | 1.9                                 |                      |
| 65.7                        | 53.2                               | 3.1                                 |                      |
| 67.2                        | 76.0                               | 2                                   |                      |

## Figures

**Figure 1.** Photographs illustrating the structure of mesh and brick shelters. (A) Front view of mesh shelter showing 3 layers of folded oyster mesh. Cable ties used in post pueruli shelters to reduce the height of each layer and create small crevices are shown (ct\*). (B) Side view of mesh shelter with the side mesh panel removed to show the inner layers of folded mesh. (C) Small brick shelters used in post-  
 810 pueruli tanks. Bricks with the smallest holes were used for recently settled post-  
 pueruli and were replaced by bricks with larger holes as animals increased in size. (D) Besser bricks and plastic sheeting used as shelters in year 1 and 2 juvenile tanks. Annotation: bb, besser brick; ct, cable ties; L1, L2, L3, mesh layers one, two and three; m, mesh; p, plastic lid; ps, plastic sheet; r, rod; s, spacer.

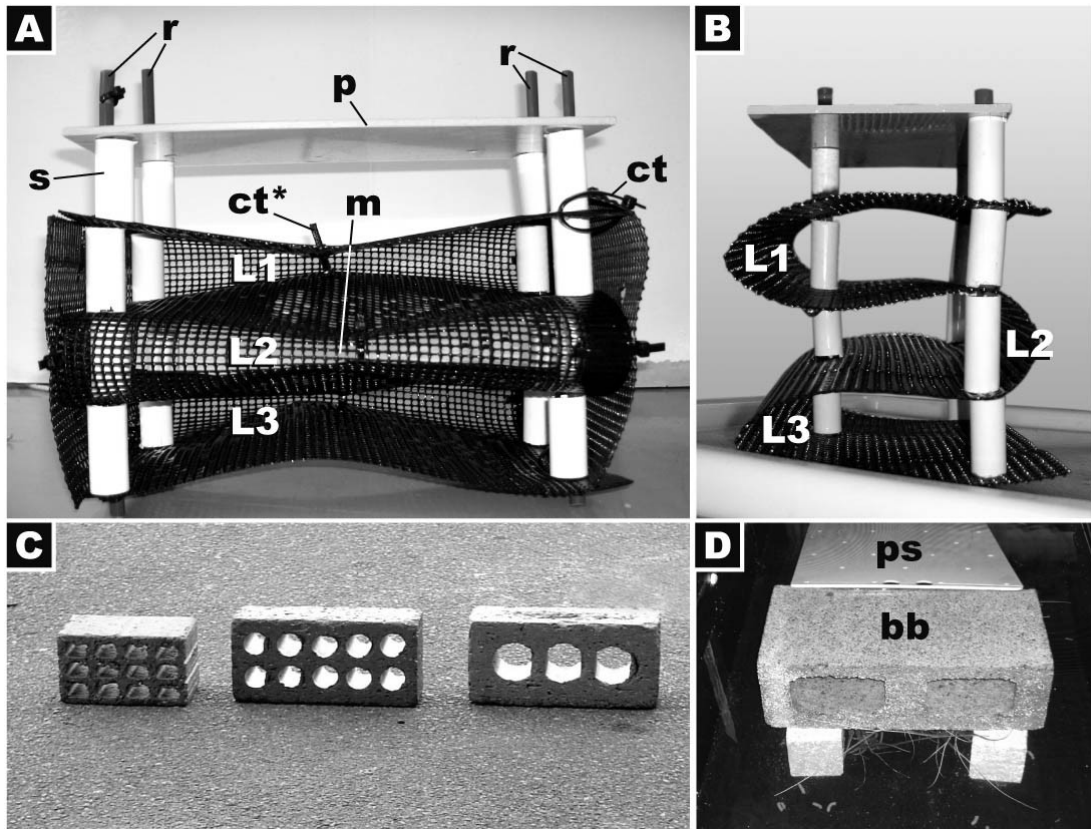
**Figure 2.** Differences in survival of Panulirus cygnus post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density for each size class after 6 months. Data are mean and standard error and analysed by logistical regression (see  
 820 Table 1). Differences in lobster survival with shelter type are not shown, as trends were only significant for post-pueruli.

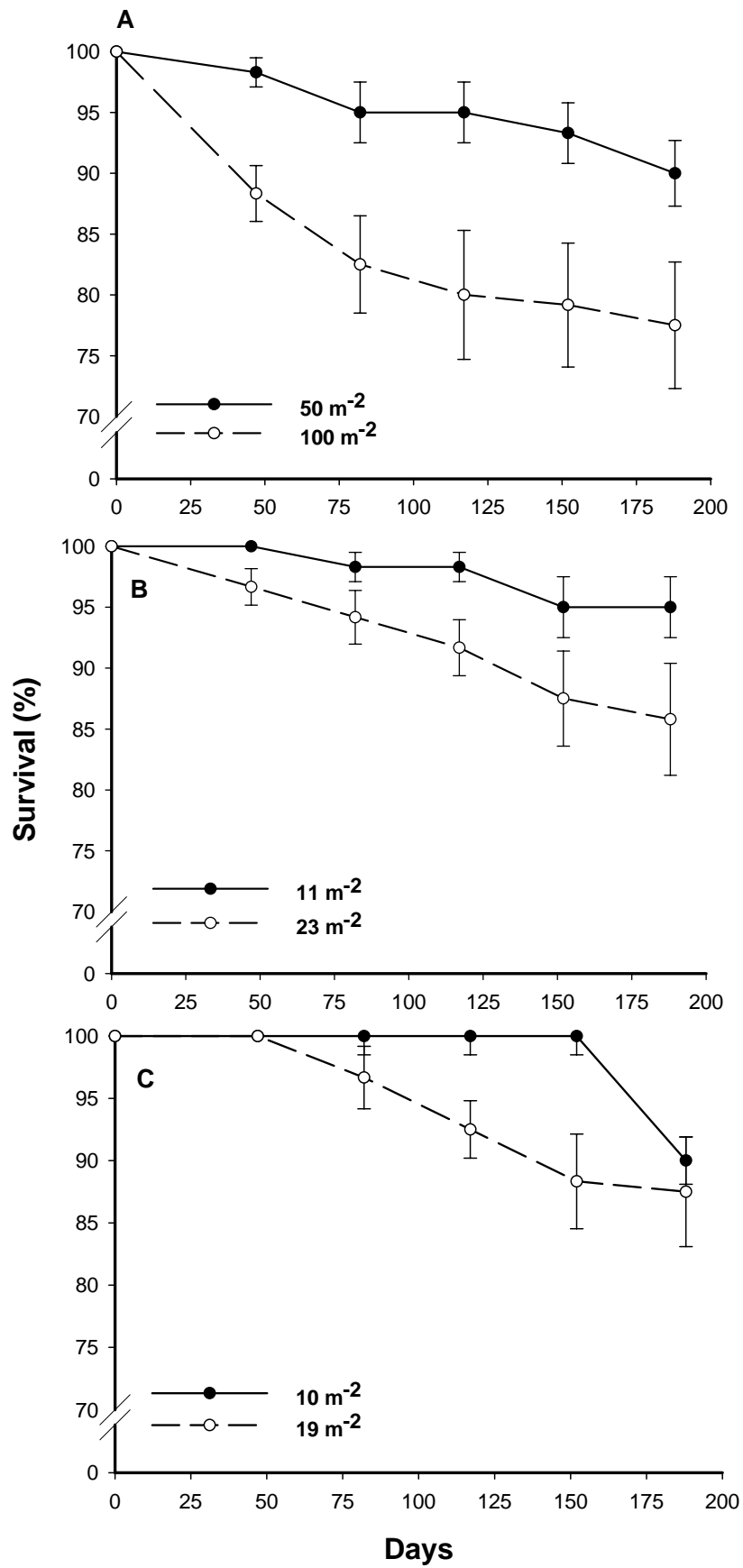
**Figure 3.** Growth rates of three size classes of Panulirus cygnus post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density after 6 months. Data are mean weight and standard error. Weight gain in grams per day is indicated between time periods.

**Figure 4.** Growth rates of Panulirus cygnus post-pueruli in tanks with either mesh or brick shelters after 6 months. Data are mean weight (A) or carapace length (B) and  
 830 standard error. Weight gain in grams per day is indicated between time periods on Figure A.

**Figure 5.** Apparent feed intake of Panulirus cygnus post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density after 6 months. Data are mean and standard error. Time period: D1-2, 5 week period between consecutive tank drains and measurements.

**Figure 6.** Apparent feed intake of Panulirus cygnus post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), using brick or mesh shelters after 6 months. Data are mean and standard error. Time Period D1-2: five-week period between first and second consecutive tank drains and measurements.





Figure

2

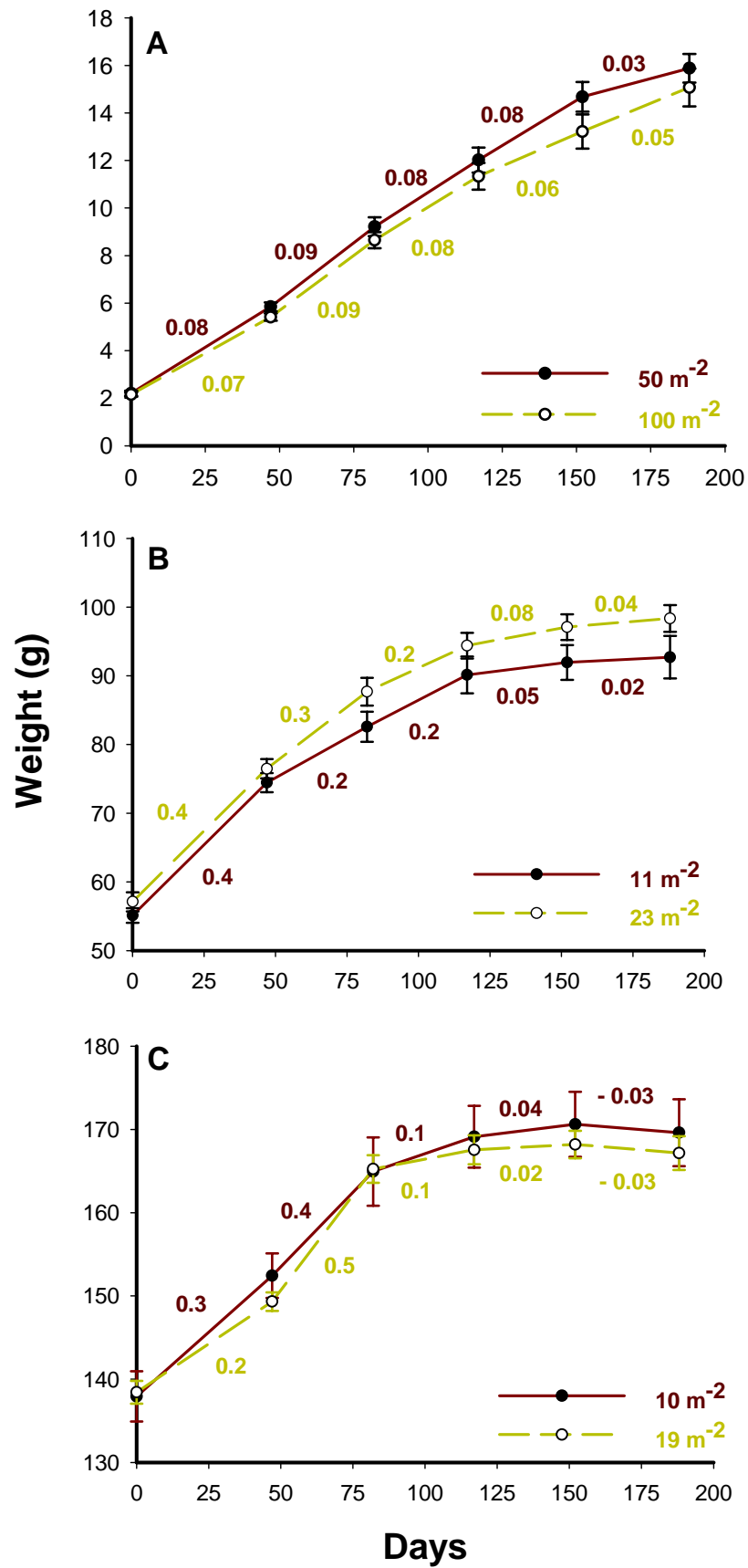
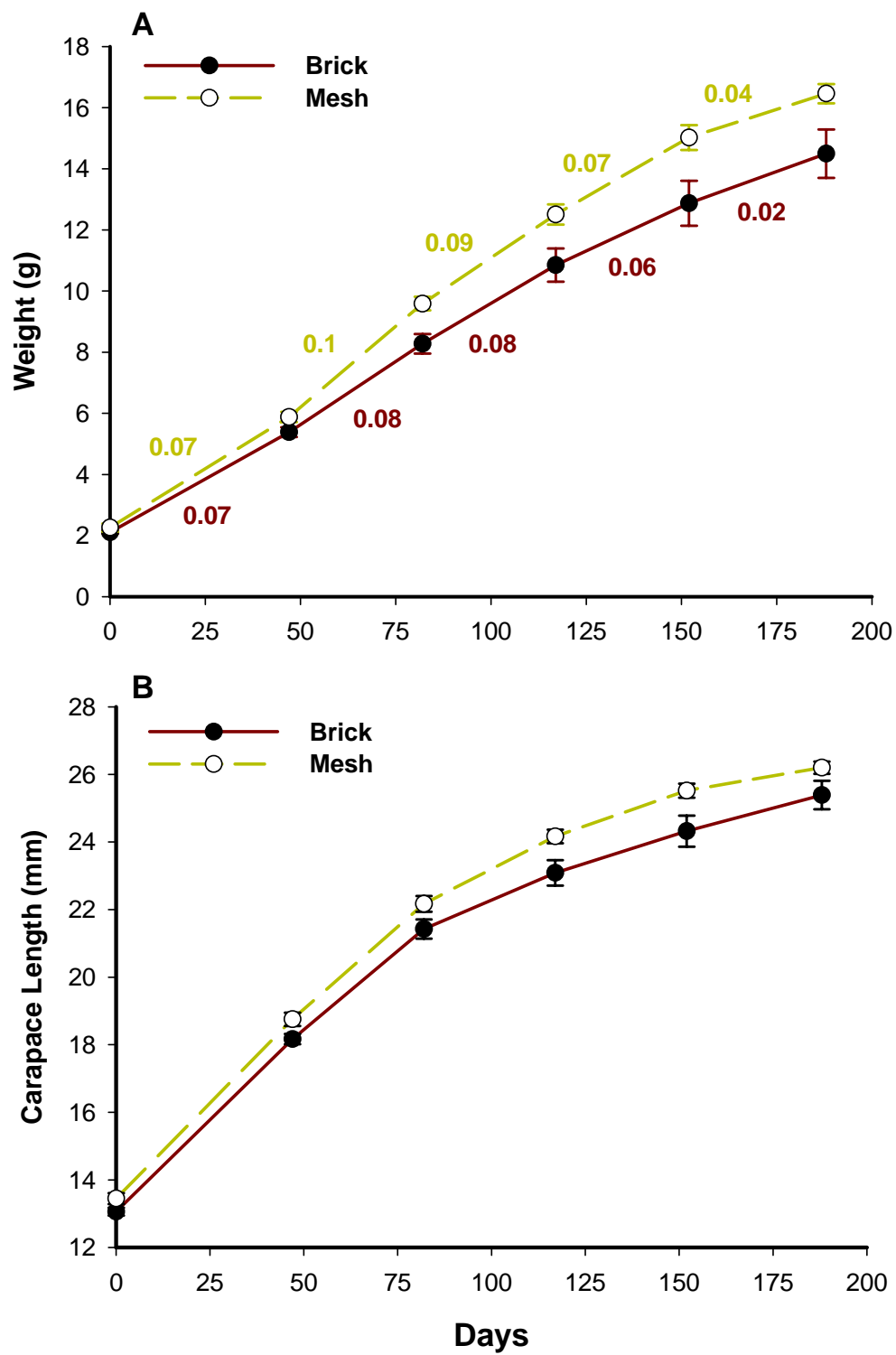


Figure 3





850 Figure 4

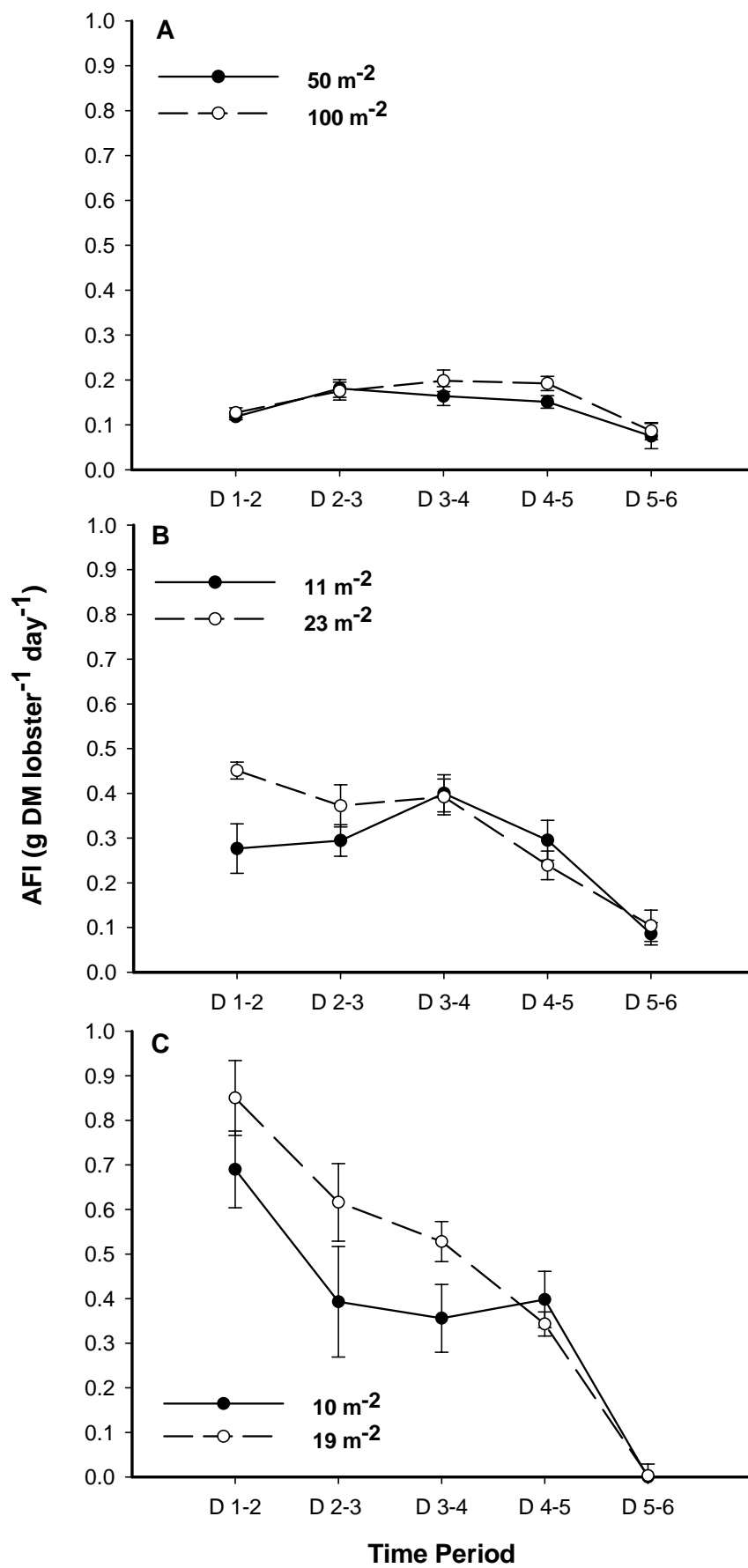


Figure 5

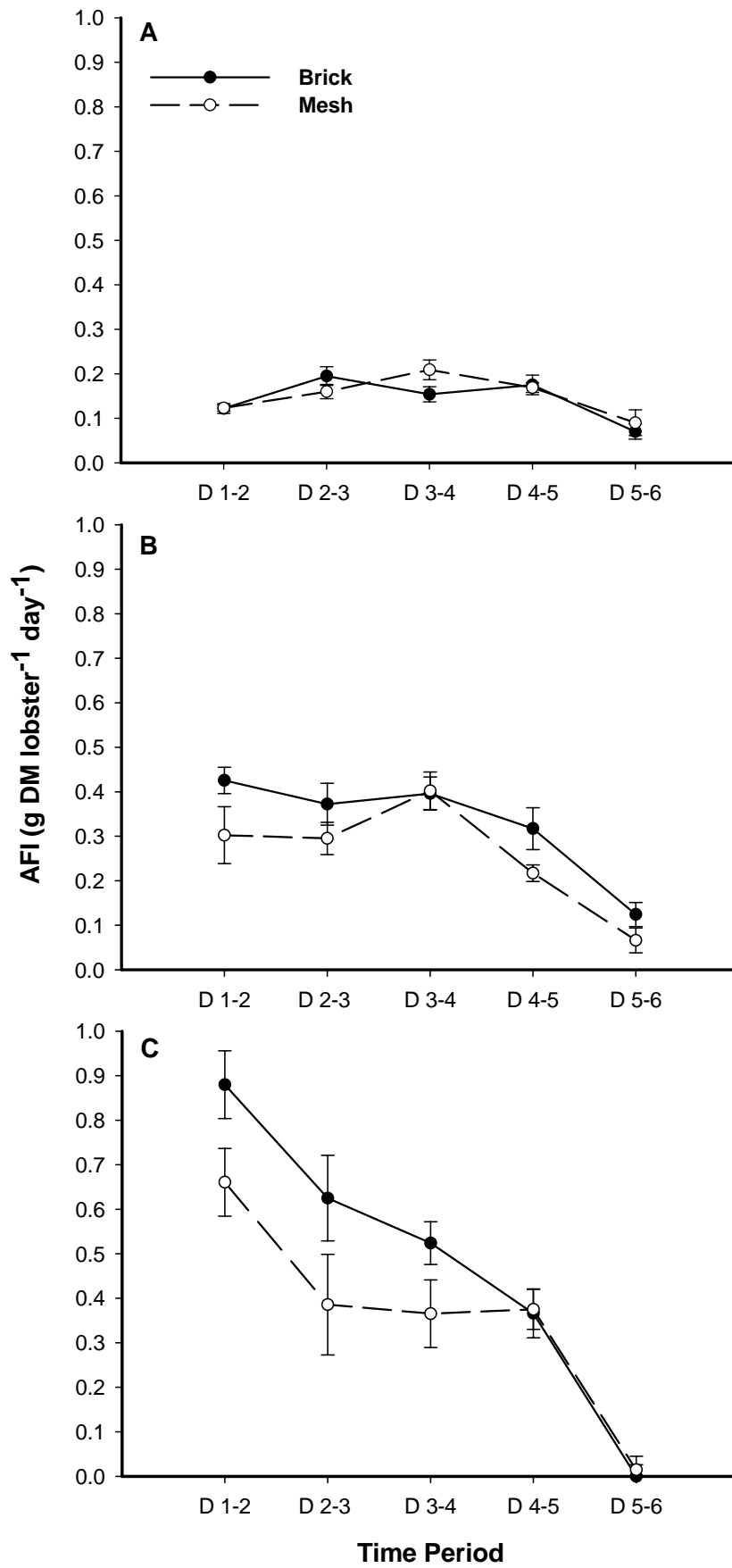


Figure 6