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Establishing post-pueruli growout data for western rock lobsters

Final FRDC Report - Project No. 2003/213

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ROCK LOBSTER ENHANCEMENT & AQUACULTURE

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Objectives:

1. Determine optimal flow rates for pueruli and juvenile western rock lobsters held at high densities in flow through tanks.

2a. Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (*Panulirus cygnus*) under two levels of biomass and two shelter types.

2b. Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (*Panulirus cygnus*) at two temperatures (ambient and 23°C) and under two feeding regimes.

3. Estimate the expected survival rate and period required to produce a marketable sized animal from post-puerulus.

4. Provide biological data to assist in assessing the economic potential for growing out western rock lobsters from post-puerulus to marketable size.

5. Compare the biochemical profiles of three western rock lobster year classes subjected to different treatments under aquaculture conditions, with corresponding year classes caught in the wild.

Non Technical Summary:

Outcomes Achieved to Date

Through a series of tank experiments we have determined that western rock lobsters have many biological attributes that are consistent with their suitability for aquaculture. Most significantly, post-pueruli, year 1 and year 2 post-settlement juveniles can be stocked at very high densities (up to 100 m^{-2} for post-pueruli) without adverse effects on growth. This species tolerates low flow rates and high ammonia concentrations and has significantly faster growth rates at 23°C compared to ambient temperatures, without impacting the survival of post-pueruli and only minimally impacting the survival of older juveniles. Male pueruli held at 23°C can potentially reach legal size (76 mm CL) within 2.3 years, whereas females can reach legal size within 2.5 years. These are substantially faster growth rates than wild lobsters which reach legal size in approximately 4 years. Lobsters consumed formulated pellet diets reasonably well, but superior growth was achieved when supplemented with fresh mussels. A novel rigid plastic mesh shelter design significantly improved survival compared with the brick shelters that have been used in many other growout studies. Finally the biochemical composition of aquacultured and wild caught lobsters have been investigated in relation to their size and aquaculture holding treatments.

A solid foundation of biological data and recommendations for culture parameters are now available for potential investors within the aquaculture and fishing industry to maximise survival, growth and production of western rock lobster post-pueruli and juveniles in future commercial operations. There has been significant interest by investors in this potential aquaculture industry and an application by one group for the collection and growout of pueruli has been made. A policy paper on puerulus allocation, collection and licensing is currently under development by the WA Fisheries Pearling and Aquaculture Branch to improve existing policy arrangements that would underpin any harvesting and ongrowing of western rock lobster pueruli.

In addition, a number of areas require further work before *P. cygnus* culture would be a commercial proposition. Development of a more palatable, nutritionally complete diet specific for *P. cygnus* is essential for the economic viability of commercial operations. Commercial scale trials need to be conducted to determine optimum tank specifications for commercial culture. An economic analysis needs to be undertaken to identify those parameters that are most sensitive to the profitability of a western rock lobster growout venture, so that it can be used to focus future research on the economically critical issues.

Previous studies have shown that large numbers, potentially of commercial quantities, of pueruli and post-pueruli can be harvested from Western Australian waters. At this stage, this appears to be unique in Australia. The same research concluded that harvesting of post-pueruli was likely to have little impact on the commercial fishery and that there could be ways to make post-pueruli removal biologically neutral. There is commercial interest in ongrowing western rock lobster post-pueruli to a marketable size, but basic data on growth and survival under a range of culture conditions was lacking. This project provides much of the essential biological information (growth rates, survival, feed consumption, food conversion, health) under a range of culture variables (flow rates, density, shelter, temperature, feed frequency) to assist potential investors in assessing the economic potential of western rock lobster growout.

A series of experiments were conducted to determine the flow rates, densities, shelter type, temperature and feed frequency that would maximise growth and survival of three size classes of western rock lobster (post-pueruli, year 1 and year 2 juveniles) in flow through onshore tank systems. Results and conclusions are summarised below:

- Levels of free ammonia NH_3-N up to 0.2 mg L^{-1} at stocking densities between 20-25 kg m^{-3} did not hinder feed consumption or affect survival of 3 year post settlement western rock lobsters.
- Western rock lobsters tolerate high ammonia levels associated with low flow rates, provided densities are suitable for culture.
- Stocking density has a significant effect on western rock lobster survival, with post-pueruli the most sensitive to density as they prefer a solitary habitat. Survival after six months for post-pueruli held at densities of 50 m^{-2} (90%) and survival for year 1 and 2 animals held at 10 m^{-2} (90-95%) was significantly higher than post-pueruli held at higher densities of 100 m^{-2} (78%) and year 1 and 2 juveniles held at 20-22 m^{-2} (86% and 88%, respectively).
- A novel rigid plastic mesh shelter design promoted significantly higher survival after six months of post-pueruli (92%) compared with conventional brick shelters (76%), with a similar trend exhibited by year 1 and 2 juveniles.
- Ambient water temperatures slowed growth in winter months and it is clear that temperatures need to be consistently higher than ambient in winter to optimise growth and production.
- A diet regime of a formulated pellet diet supplemented with fresh blue mussels achieved growth rates at ambient temperatures similar to wild lobsters and provides an acceptable diet for *P. cygnus* culture in the short term. However, further research on pellet formulations is required as the provision of mussels is unlikely to be cost effective for commercial operations.
- Survival of post-pueruli did not differ between ambient and 23°C, although survival was significantly higher at ambient temperatures for year 1 and 2 juveniles.
- All lobsters, irrespective of age class, grew significantly faster at 23°C than those held at ambient temperatures with the most significant impact on post-pueruli, where growth rates were almost double at 23°C.
- The faster growth rates of year 1 and 2 juveniles at 23°C may potentially offset their lower survival at this temperature and warrants a cost benefit analysis for commercial production.
- Feeding frequency (same ration delivered once versus three times per night) did not affect the survival of post-pueruli and year 2 juveniles, but survival was 9% higher for year 1 juveniles fed 3 times per night.
- Multiple feeds per night did not significantly improve the growth of post-pueruli or year 1 and 2 juveniles.
- Growth of post-pueruli was significantly higher at ambient temperatures when fed three times per night, whereas at 23°C growth was significantly greater when fed once per night.
- Western rock lobsters kept under aquaculture conditions for 12 months were monitored for signs of disease using histology and prophenoloxidase and serum protein levels in the haemolymph. There was no evidence that these lobsters were diseased or were suffering from undue stress.

- Males held at 23°C can potentially reach legal size (76 mm CL) within 2.3 years after settlement, whereas females can reach legal size within 2.5 years. These are substantially faster growth rates than wild lobsters, which reach legal size three to four years after settlement.
- Several year 2 females held at 23°C were tarspotted (had spermatophores) and some produced eggs, indicating that western rock lobsters held continuously at elevated temperatures mature precociously.
- The biochemical profiles of hepatopancreas and muscle tissue from the three aquaculture – held year classes were compared to wild caught animals corresponding to these cohorts. Major lipid classes were triacylglycerols (TAG), polar lipids (PL) and sterols (ST). There were elevated levels of TAG in the tissue of aquacultured animals.
- Fatty acid composition of lobsters held under aquaculture conditions differed significantly to those caught in the wild. Furthermore those held under aquaculture conditions showed significant differences in fatty acid composition in relation to age and diet, but not frequency of feed delivery or temperature or culture conditions.

The biological data reported here clearly demonstrates that *P. cygnus* is well suited to aquaculture. Most significantly, post-pueruli, year 1 and year 2 juveniles can be stocked at very high densities (up to 100 m^{-2} for post-pueruli) without adverse effects on growth or captivity-related health problems. This species can also tolerate very low flow rates and resultant high concentrations of ammonia, a considerable advantage for reducing pumping costs for culture systems. *P. cygnus* also responds favourably to elevated temperatures of 23°C with significant increases in growth rates and only a moderate reduction in survival. The time to achieve legal size is reduced significantly from approximately 4 years (wild lobsters) to 2.3 and 2.5 years for males and females, respectively, an essential requirement for successful culture. Ambient water temperatures slowed growth in winter months and it is clear that temperatures need to be consistently higher than ambient in winter to optimise growth and production. Although the consumption of formulated *P. ornatus* pellet diets by *P. cygnus* was not optimal, it has provided a basis for the development of commercial diets specific to western rock lobsters.

We recommend the use of rigid mesh shelters and stocking densities of 50 m^{-2} for post-pueruli and between 20 and 25 m^{-2} for year 1 and 2 juveniles, to maximise survival and production of *P. cygnus*. Heating water is unlikely to be economically viable so potential commercial operations should be situated where water temperatures average 23°C to maximise growth and shorten the length of growout time to legal size. There is no benefit of feeding *P. cygnus* multiple times at night in terms of growth or survival, although feeding 3 times per night is recommended for maximising growth of post-pueruli held at elevated temperatures (23°C). A diet regime of *P. ornatus* pellet supplemented with fresh mussels two days per week will promote good growth and survival, although in the long term a pellet only diet is the only cost effective option. Feeding should be to excess once daily before dusk to co-incide with nocturnal feeding behaviour.

While this project has generated a solid foundation of biological data and recommendations for culture parameters are now available, a number of areas require further research before *P. cygnus* culture could be a commercial reality. Development of a more palatable, nutritionally complete diet specific for *P. cygnus* is essential for the economic viability of commercial operations. There is a need to improve survival rates during culture. Commercial scale trials need to be conducted to determine optimum tank specifications for commercial culture. A comprehensive economic analysis is required to validate whether *P. cygnus* growout will be

economically viable. This analysis should utilise the biological data provided in this report to assess the production costs and profitability of a range of potential culture scenarios. Market research is essential to assess the potential demand and market price achievable for an aquaculture product. Cheap commercial scale puerulus collectors also need to be designed to ensure that pueruli do not represent a significant cost hurdle for aquaculture operations.

Most importantly, WA Fisheries Aquaculture and Pearling Branch has begun on the process to develop a new policy that will review the arrangements (Fisheries Management Paper 122 of 1998 and Ministerial Policy Guideline 20 of 2004) that underpin the allocation, harvesting and ongrowing of western rock lobster pueruli.

Keywords: Pueruli, post-pueruli, density, shelter, temperature, feeding frequency, growth, survival, diet, ***Panulirus cygnus***, western rock lobster, lipid, fatty acid.

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1.0 Background

Western Australia has a rock lobster aquaculture development plan which has been endorsed by the Rock Lobster Industry Advisory Committee. The first step of the plan has been to establish methods for capturing large quantities of pueruli for ongrowing and to develop mechanisms which would enable the capture of large numbers of pueruli to be biologically neutral. This objective has been achieved (Phillips et al., 2003a). The next step in the aquaculture development plan was to undertake experimental pueruli growout trials and obtain preliminary figures on the viability of this potential new industry.

The potential exists to on-grow pueruli, juvenile and undersized ornate rock lobster (*Panulirus ornatus*) to marketable sized animals, as evidenced by existing industries in Vietnam, which are currently producing over 1,000 tonnes per annum (Tuan et al., 2000; Hair et al., 2003). Recent unpublished production figures have been estimated to be 4000 tonnes per annum. Production from other Asian countries is difficult to ascertain, but it is known that pueruli/ juvenile harvesting of ornate lobsters for aquaculture purposes is being developed in several other countries, for example Sri Lanka, Singapore and the Philippines.

Australia has been slow to develop rock lobster aquaculture, but Western Australia is uniquely placed with its abundant supply of pueruli and reliable techniques to capture pueruli of western rock lobsters. As a result, this state appears to have the best potential for on-growing juveniles to a marketable (not necessarily legal) size. This project is seen as an important step in the process of developing methods and evaluating the economic viability, of this potential new industry.

Rock lobsters are attractive candidates for aquaculture because of their high value (average annual beach price $18-$33/kg over the last decade) and the ability to hold them at very high short-term holding densities (190 kg/m^3). Considerable progress has also been made with food development. Western Australia has the advantage of a relatively undeveloped coastline with few estuaries within the western rock lobster fishery area. These factors have led to the availability of low cost sites outside of populated areas, as well as excellent oceanic water quality. The rock lobster industry is very successful and provides a large potential pool of investors in rock lobster culture.

Since the commencement of this project, a Ministerial Policy Guideline (Department of Fisheries, 2004) was developed to assist with the orderly development of a rock lobster aquaculture industry in Western Australia. The Department of Fisheries has recently embarked on the development of new policy that will supersede the MPG. As a first step in this process, a scoping paper (Department of Fisheries, 2006) has been produced to scope matters relevant to the development of a sustainable allocation and growout model for western rock lobster pueruli.

1.1 Need

Research has been completed showing that it is possible to harvest large, possibly commercial quantities of pueruli/post-pueruli, which at this stage, appears to be unique in Australia. The same research has shown that harvesting of post-pueruli would have little impact on the commercial fishery and that there are ways to make post-pueruli removal biologically neutral. There is commercial interest in ongrowing western rock lobster post-pueruli to a marketable size, but basic data have yet to be obtained on growth and survival rates at different stocking densities. This project will provide the biological information (growth rates, food consumption etc) to assist potential investors to assess the economic potential of this form of aquaculture. It should be noted that this proposed research will be equally relevant should it become possible in the future to produce pueruli from hatcheries, rather than by harvesting wild caught pueruli and post-pueruli.

1.2 Objectives

The objectives of the original project were:

1. determine optimal flow rates for pueruli and juvenile western rock lobsters held at high densities in flow through tanks;
2. evaluate growth rates and survival of pueruli to market sized lobsters with and without refuges and under two levels of biomass per unit volume of water;
3. estimate the expected survival rate and period required to produce a marketable sized animal from post-puerulus;
4. provide biological data to assist in assessing the economic potential for growing out western rock lobsters from post-puerulus to marketable size.

Objective 2 was modified for a number of reasons. The original trial was to be run twice, each trial of 12 months duration (block 1 and 2), however, results of the first scheduled trial after 6 months sufficiently answered the scientific questions posed. A second repeat trial (block 2) was not conducted, as statistical power in the initial trial was sufficient to allow trends to be analysed effectively (block 2 was to be conducted only to increase statistical power during data analysis). Results from this trial also revealed that although the appropriate density and shelter for excellent survival of post-puerulus, year 1 and year 2 lobsters was achieved, growth was not optimal due to ambient water temperatures (growth slowed dramatically during winter months). Previous studies have shown that *P. cygnus* growth is optimal at 23°C and that multiple feeds overnight may increase growth rates of spiny lobsters. Therefore an additional objective was included to address these issues, which were believed to be important to this project by providing additional information pertinent to optimising growout of western rock lobster. The modifications were accepted by the Rock Lobster Enhancement and Aquaculture Subprogram steering committee and are as follows:

2a. Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (*Panulirus cygnus*) under two levels of biomass and two shelter types.

2b. Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (*Panulirus cygnus*) at two temperatures (ambient and 23°C) and under two feeding regimes.

This project achieved savings and permission was sought and granted for the savings to be used to compare the biochemical profiles of lobsters held under a number of different aquaculture treatments during this project, with wild caught animals corresponding to the same cohorts as the aquacultured lobsters. This objective is as follows:

5. Compare the biochemical profiles of three western rock lobster year classes subjected to different treatment under aquaculture conditions with corresponding year classes caught in the wild.

1.3 Reporting format

Much of the research reported on in this document has either been, or is in the process of being submitted for publication in the peer reviewed scientific literature. Four of the project objectives have been reported as separate chapters and where those chapters have been prepared for journal submission, the publication authorship has been provided at the start of the chapter. The fifth objective was undertaken after this report had been written and for publishing convenience, has been included as an appendix rather than as a chapter in this report.

2.0 Objective 1

Determine optimal flow rates for pueruli and juvenile western rock lobsters held at high densities in flow through tanks

Feeding and survival of juvenile western rock lobster (Panulirus cygnus) at high densities in tanks with low water exchange

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2.1 Abstract

Wild-caught western rock lobsters (*P. cygnus*) can be held in tanks at very high biomasses using fast flow rates (150 kg m^{-3} or 60 L h^{-1} per kg of lobsters). Future land-based aquaculture of *P. cygnus* is likely to use lower flow rates to reduce pumping costs. High biomass coupled with lower flow rates may lead to rapid ammonia accumulation for fed *P. cygnus*. Juvenile *P. cygnus* were subjected to different flow rates and high biomasses to see if accumulated ammonia affected survival or feed consumption.

Lobsters of three age classes: 1 year (1+); 2 year (2+); and 3 year (3+) post puerulus were stocked at 213, 64, and 20 individuals m^{-2} into triplicate 60 L, 250 L and 350 L tanks (respectively) to give a range of biomasses from 19 – 26 kg m^{-3}. Water was supplied at 4 flow rates (ranging from 6 – 59 L h^{-1}) for each age class. Survival was measured over the duration of the experiment. Consumption of a pelleted rock lobster diet fed *ad-libitum* was measured daily. Free ammonia concentrations were measured daily.

Slower flow rates resulted in higher free ammonia concentrations. These ranged between 0.02 - 0.15 mg L^{-1} in tanks of 3+ and 1+ lobsters, but they did not affect survival. Free ammonia concentrations ranged from 0.01 – 0.25 mg L^{-1} in tanks of 2+ lobsters. The survival of 2+ lobsters was higher at higher flow rates. Food consumption of 3+ lobsters was not affected by flow rate. Food consumption by 1+ and 2+ lobsters was confounded because undetected mortalities during the course of the experiment, which probably arose because of predation during moulting, caused lower apparent feed rates to what occurred. Accordingly, the effect of flow rate and ammonia concentrations on food consumption by smaller lobsters is not known.

P. cygnus are amenable to culture in tanks with low flow rates, and can tolerate the increased ammonia concentrations that arise from high biomasses. However, high biomasses may cause mortality, particularly of younger juveniles during moulting when they are vulnerable to predation.

Keywords: western rock lobster, flow rates, ammonia, feeding, survival, stocking density.

2.2 Introduction

The success of using larvae to culture rock lobster has been limited in Australia and elsewhere (Kittaka, 2000). An alternative is to on-grow wild-caught pueruli (Department of Fisheries, 2004). It has been decided to concentrate efforts in this area for the culture of western rock lobster *(P. cygnus)* in Western Australia. The on-growing of pueruli is assisted by methods that have been developed to harvest them in large numbers (Phillips et al., 2001), and protocols to ensure that this will not impact the large (11,000 t p.a.) wild capture fishery (Phillips et al., 2003a, b).

Pueruli are hardy and have good growth and survival when cultured individually in tanks (Chittleborough, 1974b, 1976). However, successful aquaculture will depend upon determining the appropriate biomass for communally held lobsters under different water quality conditions. Commercial-sized western rock lobster are routinely communally held in tanks without shelters at biomasses of 150 kg m^{-3} (Crear and Allen, 2002). Water quality is maintained by supplying very large volumes of un-aerated seawater (60 L kg^{-1} h^{-1}) to unfed lobsters.

These conditions seem unsuitable for farming smaller lobsters, because of the high costs of pumping, and likely water quality problems arising from feeding and holding them at such high biomasses over long periods. The gregarious nature of *P. cygnus* suggests that relatively high biomasses may still be possible. But, the lower flow rates may cause a deterioration of water quality that may affect the survival and feed consumption of lobsters.

As flow rates decrease, particulate and excretory wastes can increase free ammonia concentrations. These reduce the survival and food conversion ratio of prawns (Allan et al., 1990) and feed consumption in fish (Beamish and Tandler, 1990; Person-Le Ryet et al., 1997). Allan et al. (1990) showed that the survival of *Metapenaeus macleayi* and *Penaeus monodon* prawns declined by 50% and 66% when free ammonia concentrations were 1.2 mg L^{-1} and 1.6 mg L^{-1} (respectively). The food conversion ratio of *P. monodon* decreased significantly when more than 0.8 mg l^{-1} of free ammonia was present (Allan et al., 1990). When *P. cygnus* is fed, ammonia excretion increases 6-fold and remains at least double resting concentrations for more than 10 hours (Crear and Forteath, 2002). Crear et al. (2003) suggested that the "safe" concentration of free ammonia for commercial-size lobsters were less than approximately 0.08 mg L^{-1} (equivalent to <2 mg L^{-1} of total ammonia nitrogen). It is possible that ammonia concentrations will exceed this when juvenile lobsters are grown in high biomasses in tanks with low water exchange, and that this will affect survival and food consumption.

In this study we investigated the influence of flow rates on the accumulation of ammonia, and the effect this had on feed consumption and survival of *P. cygnus*. This was conducted at high biomasses; equivalent to those anticipated for the culture of these animals from pueruli to commercial size.

2.3 Methods

Experimental design

Three age classes of western rock lobster, representing animals 1, 2 and 3 years post-puerulus, were stocked at densities of 19 – 26 kg m^{-3} in three different-sized sets of tanks (60 L, 250 L and 350 L) for 28 days. The age classes were selected to simulate the maximum size of lobsters, grown in sequentially larger tanks, from immediately following post-puerulus to 3 year old (commercial sized) lobsters.

The tanks were supplied with ambient temperature, flow-through seawater and ample aeration. After 6 days' acclimation at a flow rate of 120 L h^{-1}, flows were adjusted to provide four progressively lower rates (fast, medium, slow and very slow) to each replicate set of tanks (n = 3) in each age class (Table 1). The flow rates were selected in an attempt to expose lobsters to total ammonia nitrogen concentrations of 1.4 - 16 mg L^{-1} (Table 1). These concentrations were calculated from hourly ammonia excretion rates for *P. cygnus* (after Crear and Forteath, 2002) and effective tank exchange rates (after Sprague, 1969).

Feed consumption, mortality, moult frequency, pH, temperature, dissolved oxygen, conductivity and total ammonia nitrogen concentrations were measured in each tank over 21 days. The tanks were inspected daily, prior to feeding, for evidence of moults and mortalities. Mortalities, where observed, were replaced with reserve lobsters of a similar size to maintain biomass. Survival data excluded the animals used to maintain biomass.

Table 1. Tank and water parameters for juvenile *P. cygnus* held in 60 L, 250 L and 350 L tanks, subjected to four water flow rates. Projected ammonia concentrations are derived from data from Sprague, (1969) and Crear and Forteath, (2002).

Parameter	60 L tank	250 L tank	350 L tank
Stocking Rate (lobsters/m^2)	213	64	20
Tank Base (m^2)	0.2	0.89	1.05
Lobster Numbers	40	57	21
Average Wt Lobster (g)	39	116	316
Wet Biomass/tank (g)	1546	6270	6642
	Very Slow Flow		
Flow Rate (L/h)	6	12.5	8.8
Predicted TAN max., min. (mg/L)	16, 10.5	14, 11.4	13, 10.5
	Slow Flow		
Flow Rate (L/h)	10	19.2	14
Predicted TAN max., min. (mg/L)	9.5, 5.6	10, 7.1	9, 6.7
	Medium Flow		
Flow Rate (L/h)	19	31.3	26.9
Predicted TAN max., min. (mg/L)	4.9, 2.5	6, 4.3	4.8, 3.5
	Fast Flow		
Flow Rate (L/h)	35	50	58.3
Predicted TAN max., min. (mg/L)	2.7, 1.4	4, 2.3	2.5, 1.4

Lobsters

Lobsters were collected using baited pots from sites at Seven Mile Beach and Lancelin, Western Australia, between November 2003 and January 2004. Lobsters were transported to Western Australian Marine Research Laboratories, Perth (WAMRL), where they were held and graded for the experiment.

Three age classes of lobster were chosen, according to the work of Chittleborough (1970, 1974b, 1976) and Fitzpatrick et al. (1989) to represent cohorts of one year (1+), two years (2+) and three years (3+) after larval settlement (puerulus). The age classes were graded by carapace length (CL) into the groups: 1+ = 25 – 39 mm CL, modal size 35 mm, 2+ = 40 – 59 mm CL, modal size 50 mm, 3+ = 60 – 74 mm CL, modal size 70 mm.

Holding Conditions

The 1+ and 2+ lobsters were held in 60 L rectangular grey polyethylene and 250 L black fibreglass tanks, respectively, in a temperature-controlled room (25°C) with a 12:12 light:dark photoperiod. The 3+ lobsters were held in 350 L rectangular black polyethylene tanks, in a shaded compound outdoors, with a natural 14:10 light:dark photoperiod and ambient air temperatures (18°C - 42°C). Oceanic water, filtered to 100 µm, was supplied to all tanks at ambient temperatures (20 - 25°C). Water temperatures in all tanks did not differ by more than 1.5°C at any time during the experiment. Water salinities remained steady at 36 - 37 ppt.

The 250 L and 350 L tanks were covered with 6 mm black polyethylene oyster mesh in timber frames. Translucent plastic lids covered the 60 L tanks. Shelters were provided in all tanks; those in the 350 L and 60 L tanks consisted of multiple folds of 6 mm polyethylene oyster mesh at spacings appropriate to the height of the animals, mounted on a frame with a solid 5 x 300 x 300 mm PVC cover (See Johnston et al., 2006). Animals in the 250 L tanks were provided with hides made from concrete blocks and bricks, with 5 × 800 × 500 mm PVC sheets leaning against the blocks. The bases of the 350 L polyethylene tanks were fitted with weighted sheets of 6 mm oyster mesh to give lobsters traction on the tank floor. The bases of other tanks were sufficiently rough to allow lobsters to move freely.

Aeration was adjusted daily to maintain oxygen at 80% saturation. On day 5 of the experiment, it was necessary to fit additional aeration and filtration to the 60 L and 250 L tanks with slow and very slow flow rates. Water flow rates on tanks were checked manually and adjusted daily (Table 1).

Water Quality

Total ammonia nitrogen concentrations in tanks were recorded under two regimes; peak measurements taken once per day for 21 days, and a semi-continuous profile taken every 2 hours over a 24 hour period. A Hach Sension 4® meter with an ISE ammonia probe was used to measure total ammonia. The meter was calibrated immediately prior to the daily peak measurements, and between each 2-hourly set of the continuous samples. Temperature, salinity, pH and dissolved oxygen in tanks were determined daily, immediately following feeding. Un-ionised (free) ammonia nitrogen during peak measurements was calculated using total ammonia nitrogen (TAN), temperature, pH and salinity according to the formulae:

$$[NH_3\_N] = [TAN] \times 10^{-pK_a} \div 10^{-pH}$$

where $pK_a = -0.0321 \times \text{temperature} + 10.153$ at salinities between 32 –40 ppt (after Bower and Bidwell, 1978).

Feed and Feeding

Prior to the acclimation lobsters were fed live blue mussels (*Mytilis edulis*). During acclimation and the experiment lobsters were fed 20 × 2 mm strands of a tropical rock lobster feed (Smith et al., 2005) at 1.5%, 1% and 0.5% of initial biomass/day, for the 1+, 2+ and 3+ groups, respectively. To maximise feed consumption by lobsters the feed was distributed *ad libitum* to tanks at 15-minute intervals over a 2-hour session (09:00 h – 11:00 h) each day. Following each feed session, all uneaten feed from each tank was collected on a 100 µm screen, washed in fresh water and dried overnight at 90°C, then weighed to determine the amount of wasted food. These data were adjusted using data for the loss of weight because of disintegration during immersion, and they were used to correct the daily feed consumption.

Daily feed consumption per tank = Total dry weight feed distributed per tank – Dry weight waste feed collected per tank x 100/(100 - % weight loss feed by immersion).

Statistical Analyses

Two-way analysis of variance was used for survival data, which was tested against flow rate, year class and the interaction between these two factors. Feed consumption for the 3+ lobsters was analysed using a general linear model that incorporated experiment days, ammonia concentrations, water flow rates, tank oxygen concentration, temperature and residuals as factors and covariates. A linear equation was developed for feed consumption over time that incorporated only significant factors or covariates in the general model.

2.4 Results

Flow rates and ammonia concentrations were inversely related (Figures. 1 – 4). Free ammonia concentrations were greatest at the slowest flow rates, with 0.1 – 0.2 mg L^{-1} occurring for the 3+ lobsters (Figure 1), and 0.05 – 0.12 mg L^{-1} occurring for 1+ lobsters (Figure 3) throughout most of the experiment. In the tanks housing the 2+ lobsters the free ammonia concentrations initially showed a similar relationship with flow rate (Figure 2). However, over the course of the experiment the differences in ammonia concentrations between flow rates declined as different concentrations of mortality and biomass occurred at different flow rates (Table 2). Although lobsters were added to maintain the biomass in tanks experiencing mortality, in the 2+ tanks some undetected mortalities occurred that were not rectified. This affected the amount of ammonia produced, particularly for lobsters held under very slow, slow and medium flow rates (Figure 2).

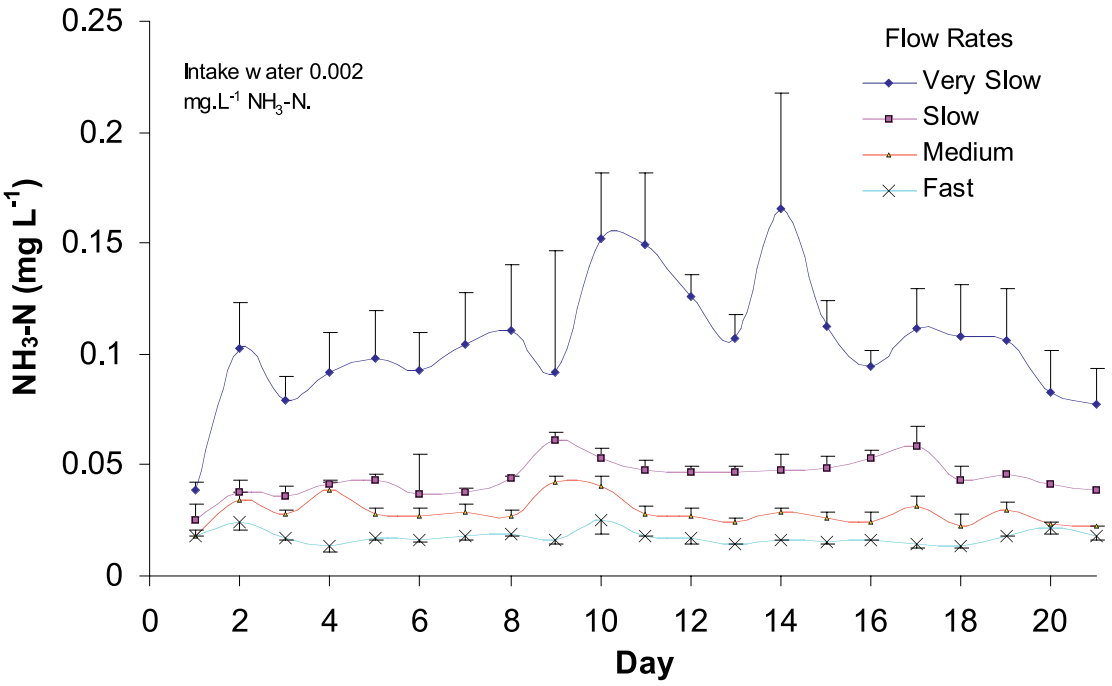

Figure 1. Daily free ammonia (NH_3-N) concentrations (mean ± s.e., n = 3) in 350 L tanks containing 3+ *P. cygnus* lobsters stocked at 20 kg m^{-3}, subjected to 4 water flow rates. Water samples were taken 6 hours following feeding.

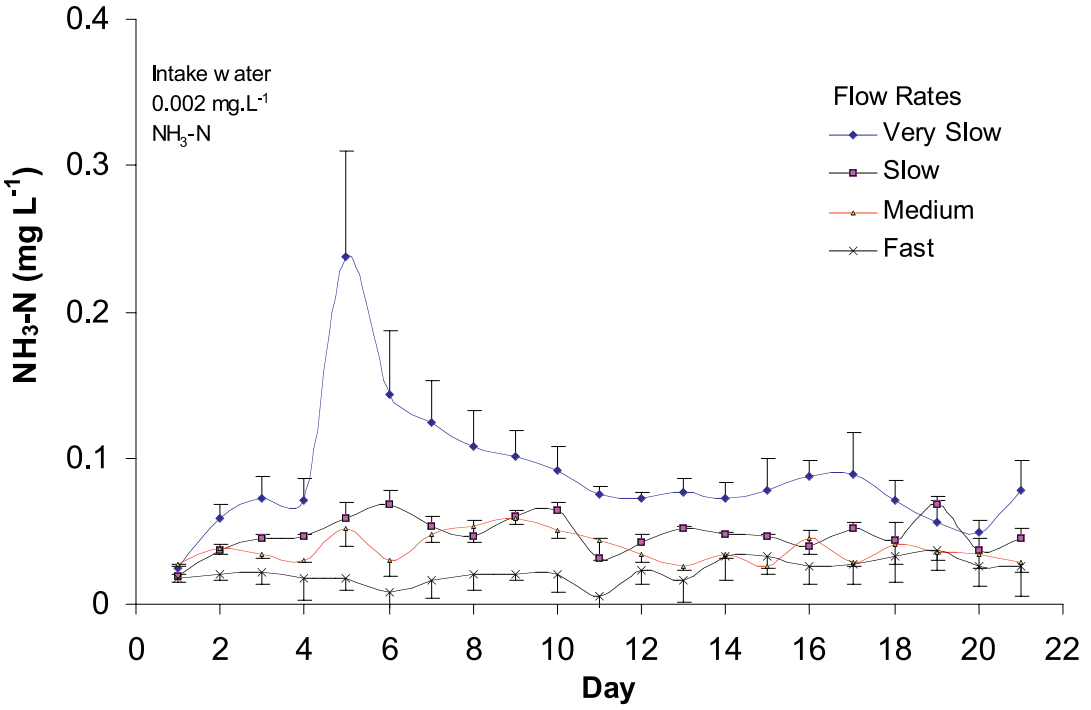

Figure 2. Daily free ammonia (NH_3-N) concentrations (mean ± s.e., n = 3) in 250 L tanks stocked with 2+ *P. cygnus* lobsters at 25 kg m^{-3}, subjected to 4 water flow rates. Water samples were taken 6 hours after feeding.

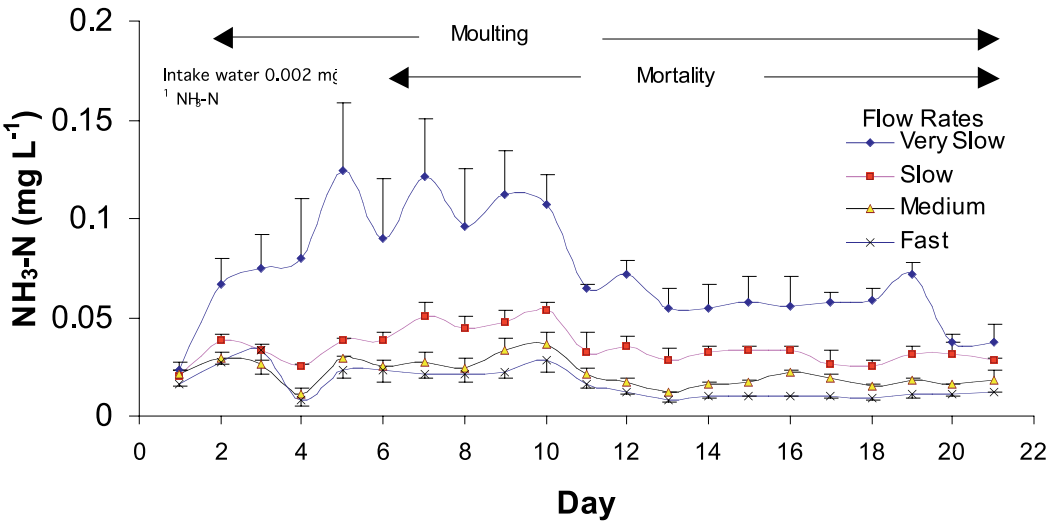

Figure 3. Daily free ammonia (NH_3-N) concentrations (mean ± s.e.) in 60 L tanks stocked with 1+ *P. cygnus* lobsters at 26 kg m^{-3}, subjected to 4 water flow rates. Water samples were taken 6 hours after feeding.

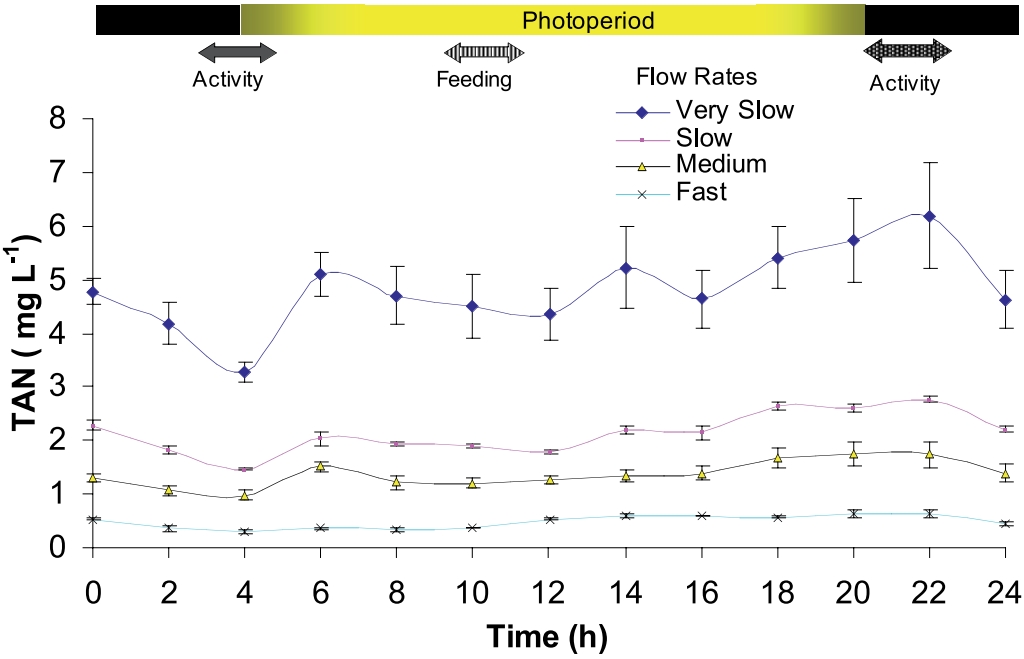

Figure 4. Total ammonia nitrogen (TAN) concentrations (mean ± s.e., n = 3) of water in 350L tanks containing 3+ *P. cygnus* lobsters stocked at 20 kg m^{-3}, subjected to 4 water flow rates. Samples were taken 2-hourly, from midnight (0 h) on day 2 to midnight (24 h) on day 3. Similar readings occurred in tanks of 2+ and 1+ lobsters.

Total ammonia nitrogen production was also inversely related to flow rates for 3+ lobsters (Figure 4).The most prominent peak in total ammonia nitrogen production for these lobsters occurred immediately after dusk (Figure 4).Minor peaks occurred around dawn, and 4 – 6 h after the onset of feeding (Figure 4). Similar trends were shown for the other age groups. For example, the maximum total ammonia nitrogen production of ~4 mg L^{-1} for 1+ and ~6 mg L^{-1} for 2+ lobsters occurred with the slowest flows during the evening (18:00 h – 22:00 h).

Flow rates did not affect the survival of 1+ and 3+ lobsters (Table 2). The survival of 2+ lobsters in the highest flow rate was greater than in all other flow rates for this age group ($p < 0.05$). A similar trend was shown for the 1+ lobsters, but this was not significant. Survival increased with the age of lobsters, but there were no interactions between flow rates, age and survival.

Table 2. Percentage survival (mean ± s.e., n = 3) over 21 days of year class 3+, 2+ and 1+ *P. cygnus* lobsters in rectangular tanks, provided with four water flow rates. Values that carry the same superscript are not significantly different ($p > 0.05$).

	Flow Rate			
Year Class	**Very Slow**	**Slow**	**Medium**	**High**
3+	100 ± 0[a]	100 ± 0[a]	98 ± 2[a]	100 ± 0[a]
2+	78 ± 12[b]	82 ± 6[b]	87 ± 7[b]	97 ± 1[a]
1+	56 ± 2[c]	58 ± 1[c]	53 ± 1[c]	60 ± 4[c]

Survival appeared to be related to the incidence of moulting, which was indicated by presence of exuviae in tanks. The 1+ lobsters, that had the lowest survival, had the highest concentrations (18 – 33%) of moulting (Figure 5, Table 3). The high survival of the 3+ lobsters was accompanied by very little (~ 3%) moulting (Table 3). Little moulting (0.6 – 2%) or mortality (0.6 – 1%) was detected by daily inspection of the 2+ tanks (Table 3), but lower survival (78 – 97%) was evident when animals were counted at the end of the experiment (Table 2).

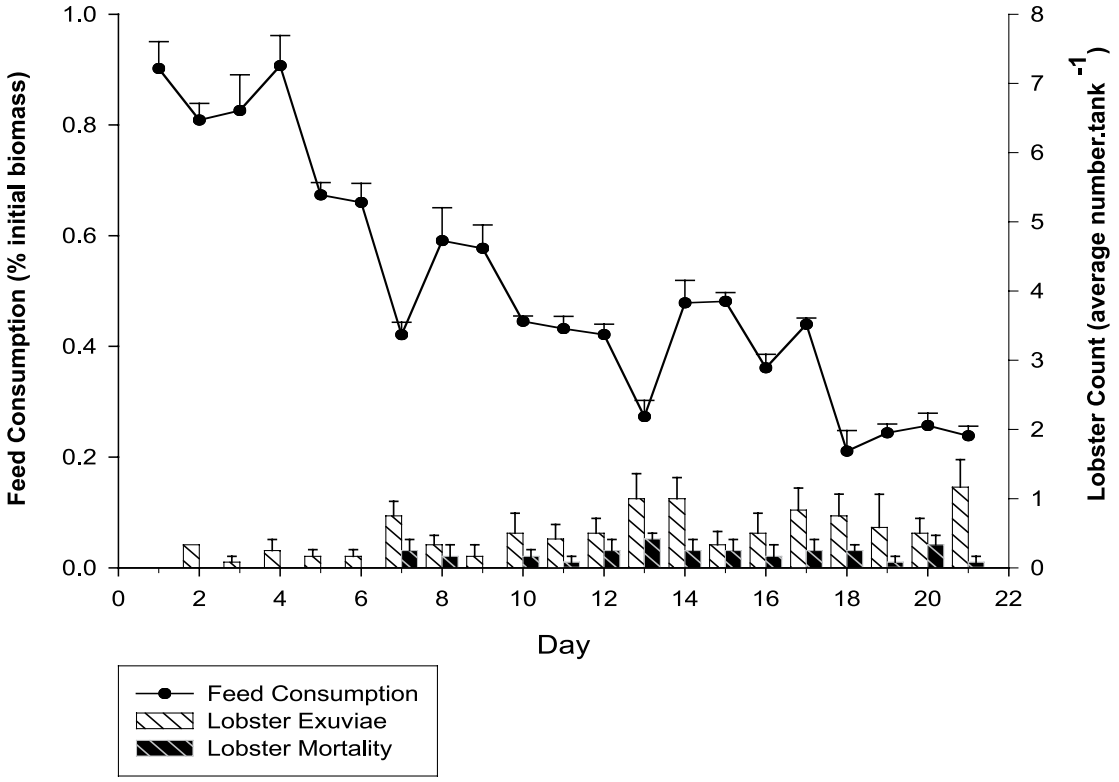

Figure 5. Consumption of pelleted feed, observed numbers of moults (exuviae), and mortalities of 1+ *P. cygnus* lobsters (means ± s.e., n = 12) over 21 days.

Table 3. Moulting as indicated by the presence of exuviae and mortalities (mean % of initial number of stocked lobsters ± s.e., n = 3) that were detected by daily observation of year class 3+, 2+ and 1+ *P. cygnus* lobsters in rectangular tanks, provided with four water flow rates over 21 days.

		Flow Rate			
Year Class		**Very Slow**	**Slow**	**Medium**	**High**
3+	exuviae (%)	0	3 ± 1.6	3 ± 1.6	3 ± 3.0
	mortality (%)	0	0	3 ± 1.6	0
2+	exuviae (%)	2 ± 2	2 ± 1.2	2 ± 1.0	0.6 ± 0.6
	mortality (%)	0.6 ± 0.6	1 ± 1.0	0	0
1+	exuviae (%)	33 ± 6.5	21 ± 0.8	18 ± 3.0	31 ± 3.6
	mortality (%)	7 ± 0.8	6 ± 2.2	8 ± 3.8	10 ± 1.4

Feed consumption remained high (0.3 – 0.7% of initial tank biomass) in the tanks containing 3+ lobsters across all flow rates (Figure 6). A generalised linear model analysis of these data showed that flow rates and ammonia concentrations did not alter consumption ($p = 0.24$ and $p = 0.20$ respectively, $r^2 = 0.21$). The optimal linear equation with parameters (and standard error) describing the factors that influenced consumption was as follows:

Consumption = 0.6±0.4 + 0.008±0.003 × Time + 0.02±0.01 × Temperature – 0.008±0.003 × Dissolved Oxygen

As the 3+ animals were kept outdoors at ambient temperatures, feed consumption correlated positively with average water temperature ($p = 0.03$, $r^2 = 0.17$), and negatively with average dissolved oxygen concentrations ($p = 0.01$, $r^2 = 0.17$). Dissolved oxygen concentrations were lower (4.4 – 4.7 mg L^{-1} or 65 - 70% saturation) in the tanks with very slow flow rates on days when air temperatures were >38°C causing water temperatures to become elevated from 23.5°C to 25°C. There was also a slight increase in consumption over time ($p = 0.03$, $r^2 = 0.17$) (Figure 6, Table 4).

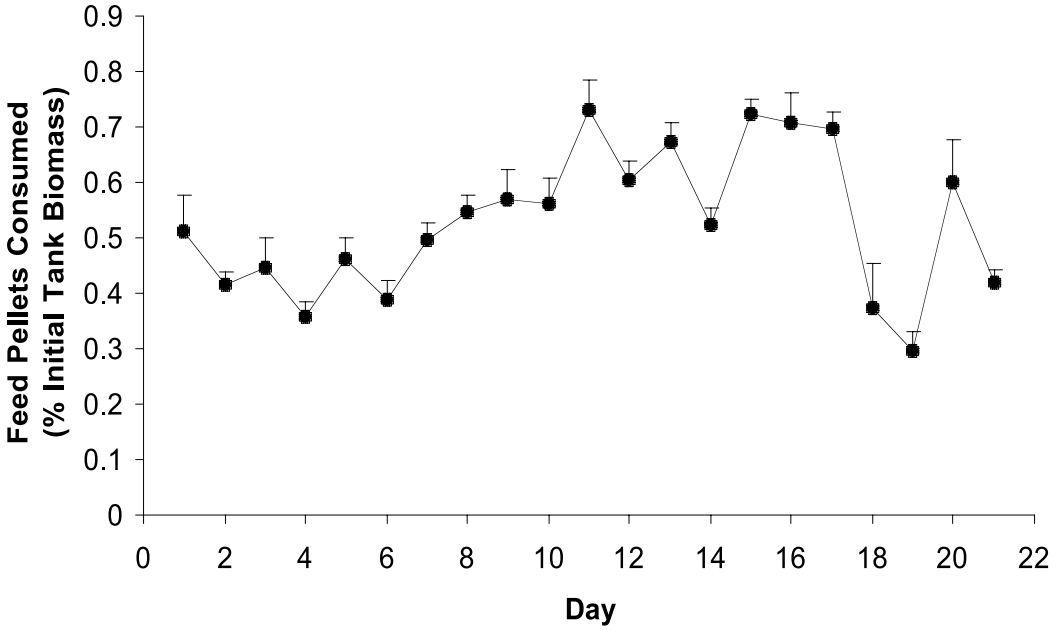

Figure 6. Consumption (mean ± s.e., n = 12) of pelleted feed by 3+ *P. cygnus* lobsters in all flow rate treatments over 21 days.

Table 4. Fully reduced ANOVA of feed consumption of 3+ *P. cygnus* lobsters modelled against various significant factors and covariates in tanks. R-square = 0.17.

Factor	DF	SS	MS	F	p
Time (days)	1	0.1	0.1	4.61	0.03
Water Temp	1	0.10	0.10	4.75	0.03
Diss. O_2	1	0.15	0.15	6.87	0.01
Residuals	80	1.71	0.02		

The optimal linear equation describing feed rate over time for 3+ lobsters is given by
Feed = 0.6078 (± 0.4359) + 0.0075 (± 0.0030) x Time + 0.0229 (± 0.0136) x Temp – 0.0084 (± 0.0032) x Diss.O_2

The feed consumption of the 1+ and 2+ lobsters generally declined (0.3 – 1.0% initial tank biomass) over the course of the experiment regardless of flow rates (Figures 5, 7). Lower consumption of the 1+ lobsters coincided with observed onset of moulting and mortality (Figure 5). Undetected mortalities in the 1+ and 2+ age classes over the experiment confounded the calculation of the feed consumption data, because changes in the biomass, predation, and the consumption of exuviae affected the data for consumption rates of individual lobsters. No statistical analysis of the feed consumption data from 1+ and 2+ animals was attempted.

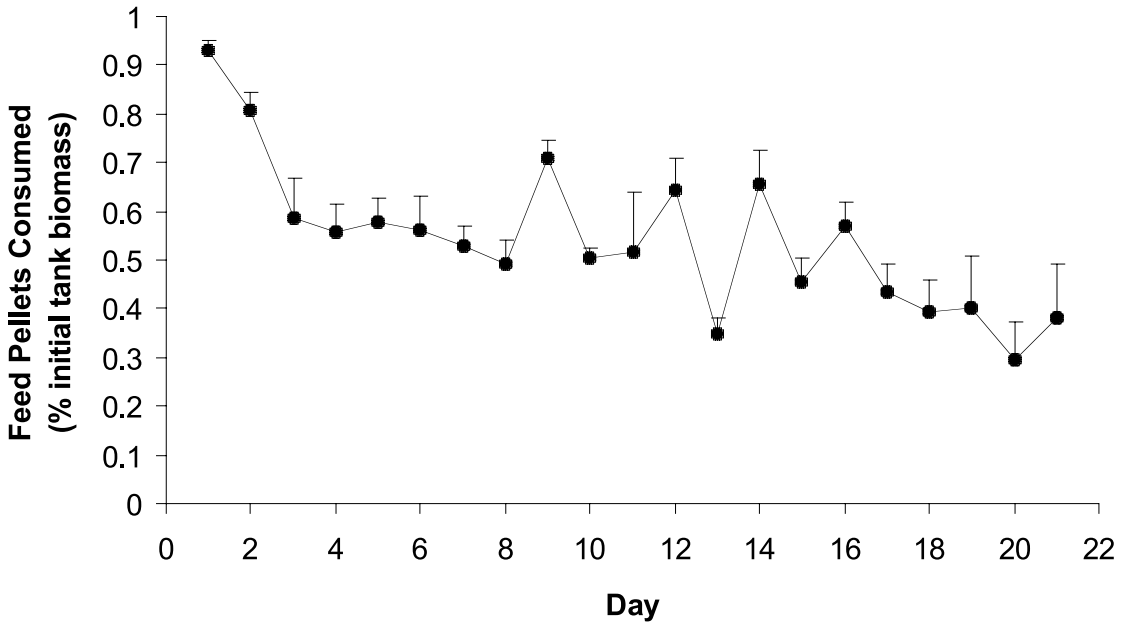

Figure 7. Consumption of pelleted feed (mean ± s.e., n = 12) by 2+ *P. cygnus lobsters* in all flow rate treatments over 21 days.

2.5 Discussion

Lower flow rates resulted in higher ammonia concentrations. However, higher ammonia concentrations did not significantly affect the survival of the 1+ and 3+ lobsters in this experiment. The maximum concentrations of ammonia were 25 – 70% greater than those considered appropriate for holding commercial-sized lobsters (Crear et al., 2003), suggesting that smaller *P. cygnus* are relatively tolerant to elevated ammonia concentrations. The highest ammonia concentrations occurred in tanks housing the 2+ lobsters with slow flow rates. These lobsters had significantly higher mortality, which may have been due to ammonia toxicity. A similar trend was shown for 1+ lobsters, however the ammonia concentrations were generally lower, and the differences in mortality were not significant.

It is difficult to conclude if the cause of mortality at lower flow rates was due to ammonia toxicity or predation. Whether predation occurred after senescence arising from ammonia toxicity, or because of aggression arising from the high biomasses is uncertain. Mortality was closely related with moulting. The greater occurrence of mortality of 2+ lobsters towards the end of the experiment, when similar concentrations of ammonia occurred for all flow rates, suggests that the cause of mortality was more likely to be due to predation during moulting, rather than different levels of ammonia toxicity. This inference is further supported by the rapid

and voracious consumption of exuviae and recently moulted individuals suggesting that the presence of ammonia did not induce morbidity or suppress their appetites.

The feed consumption of 3+ lobsters was not influenced by the ammonia concentrations that arose from the combination of low flow rates and high biomasses. The maximum concentrations of free ammonia (0.1 – 0.2 mg L^{-1}) in the tanks housing the 3+ lobsters were less than those shown to affect the food conversion ratio of prawns (0.8 mg L^{-1}) (Allan et al., 1990) suggesting that sub-lethal levels were not reached. However the levels were five times those recommended for holding of commercial size lobster (Crear et al., 2003). This suggests that feed consumption of 3+ *P. cygnus* may not be readily affected by the concentration of ammonia that is likely to arise during culture.

However, feed consumption of the 3+ lobsters was positively affected by temperature and negatively affected by low concentrations of oxygen. Higher temperatures increase metabolic rates in lobsters that increase food consumption, up to at least 25°C (Chittleborough 1974b, 1976; Crear and Forteath, 2001). Food consumption in this experiment followed this trend as the temperature increased from 19°C to 23°C. However, higher temperatures appeared to cause low oxygen concentrations in some tanks. Despite heavy aeration, high air temperature (>38°C) caused high water temperature in the tanks with very low flow rates, resulting in dissolved oxygen concentrations of 70% saturation or 4.7 mg L^{-1}. These are below recommended concentrations of 80% saturation (Crear and Allen, 2002). It is likely that a large amount of particulate matter, increased biological oxygen demand, and increased respiratory demand by lobsters also contributed to the low oxygen concentration in the tanks, which suppressed food consumption. However, oxygen concentrations remained satisfactory in the faster flow rate treatments, showing that they can be remediated easily. The relationship between flow rate, temperature and oxygen concentrations needs to be addressed with respect to feeding lobsters cultured under commercial conditions.

It is uncertain if feed consumption by 1+ and 2+ lobsters is unaffected by ammonia concentrations as shown for the 3+ lobsters. As noted, the evaluation of feed consumption by the 1+ and 2+ lobsters was confounded by mortalities that were undetected during the course of the experiment. This altered the biomass within the tanks, preventing an accurate estimation of food consumption per lobster. At the same time, the predation of recently moulted individuals and the consumption of exuviae are likely to have affected the consumption of the pellets. As a consequence, assessment of the impact of reduced flow rates on feed consumption was not possible for these lobsters.

The ammonia concentrations for each flow rate were consistently about two thirds lower than predicted in the design of the experiment, regardless of the size of the lobsters. This consistency for different sized lobsters suggests that the formulae developed by Crear and Forteath (2002) to explain excretion rates for different sized lobsters greater than 400 g is also able to reliably predict excretion rates of lobsters between 27 – 400 g. The lower than predicted ammonia concentrations most likely arose because the removal of ammonia from tanks was more rapid than anticipated by our *a-priori* calculations. It is also possible that considerable oxidation of ammonia occurred in tanks through bacterial degradation. The very high aeration, warm temperatures, restricted flows, high particulate concentrations, and high ammonia concentrations in tanks are conducive for the production of denitrifying bacteria and the oxidation of ammonia.

P. cygnus showed a diurnal pattern in ammonia excretion, with a peak concentration of ammonia excretion immediately after dusk. A notable rise in ammonia excretion also occurred immediately after dawn. This coincides with periods of peak activity in other lobsters such as *Jasus edwardsii* (Crear and Forteath, 2000). In contrast, Crear and Forteath (2002) did not find a clear daily pattern in ammonia excretion in *P. cygnus*, but rather noted that peak ammonia excretion occurred following feeding. In this experiment a rise in ammonia production occurred following feeding, but it was less than that observed at dawn and dusk. There are large differences in treatment of lobsters in this experiment and that conducted by Crear and Forteath (2002) who used respirometers to measure ammonia excretion. In our experiment the lobsters were left undisturbed, with the pelleted feed being added directly to the tanks. Crear and Forteath (2002) removed their lobsters from the respirometers to feed them a diet of squid, before replacing them to measure ammonia excretion. Higher stress levels due to handling may have contributed to a greater concentration of ammonia excretion from the lobsters used by Crear and Forteath (2002). Also, the excretion of ammonia from lobsters fed squid may have been higher than those fed the pellet in our experiment, because squid is unlikely to meet the nutritional needs of lobsters as effectively. The normal foraging activity of the lobsters removed from respirometers may have also been affected because of handling, although this is likely to have reduced food consumption and ammonia excretion rates. As previously mentioned, bacterial oxidation of ammonia may also have substantially reduced concentrations in our experiment. Despite these differences it is likely that peak ammonia production in cultured lobsters is likely to occur at immediately following dusk and dawn, and after feeding. This should be considered with respect to higher flow rates at these times to reduce the risk of ammonia toxicity.

The results from this experiment show that *P. cygnus* are able to feed and survive well in the short term with very low water exchange rates. However, large losses during moulting have demonstrated that stocking densities that reach 25 – 26 kg m^{-3} are impractical, at least for younger juveniles with higher rates of moulting. Their ability to tolerate low flow rates and associated high ammonia concentrations reveals that this species is robust to poor water quality. Whilst such conditions are not recommended for their holding, they are encouraging characteristics for those exploring future culture possibilities for the species.

3.0 Objective 2A

Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (Panulirus cygnus) under two levels of biomass and two shelter types

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

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3.1 Abstract

The growth, survival and feed intake of three size classes of wild caught western rock lobster, *Panulirus cygnus* (post-pueruli, mean 2.14 ± 0.07 g, 13.2 ± 0.1 mm CL; year one post-settlement juveniles, 57.1 ± 1.1 g, 38.7 ± 0.28 mm CL; and year two post settlement juveniles, mean 138.2 ± 2.26 g, 51.9 ± 0.25 mm CL) were examined at combinations of two stocking densities (post-pueruli: 50 and 100 m^{-2}; year one: 11 and 23 m^{-2}; year two: 10 and 19 m^{-2}) 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 (92%) than brick shelters (76%) 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^{-1}) ($p<0.05$) 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 $lobster^{-1}$ day^{-1}) was not significantly different between densities. This study has shown that *P. cygnus* is well suited for aquaculture based on the collection and ongrowing of wild caught pueruli, as this species exhibits good survival at high densities (up to 100 m^{-2}) without adverse effects on growth, and shows no captivity-related health problems. We recommend mesh shelters, with stocking densities of 50 m^{-2} for post-pueruli and between 20 and 25 m^{-2} for year 1 and 2 juveniles, to maximise survival and growth.

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

3.2 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 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-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 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 survival and growth 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*, weight increase was 40% less for those at 200 m^{-2} than those at 50 m^{-2}, 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 after the post-puerulus stage 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).

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., 2003b). Coupled with the fact that post-pueruli 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*.

The aims of this study were to investigate 1) the effect of two levels of stocking density and 2) the effect of two shelter types, on the growth and survival of western rock lobster *P. cygnus* post-pueruli, 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.

3.3 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 250 L tanks with shelter until the trial commenced in March 2004. The trial continued for 6 months until September 2005. Post-pueruli (mean 2.14 ± 0.07 g, 13.2 ± 0.1 mm CL); year 1 post settlement juveniles (mean 57.1 ± 1.1 g, 38.7 ± 0.28 mm CL) and year 2 post settlement juveniles (mean 138.2 ± 2.26 g, 51.9 ± 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) (Figure 1). The stocking densities were as follows: post-pueruli: 50 and 100 m^{-2}; year one: 11 and 23 m^{-2}; year two: 10 and 19 m^{-2} (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^{-3}; year one 5.2 and 10.5 kg m^{-3} and year two 7.2 and 14.3 kg m^{-3}. All tanks received flow-through ambient temperature seawater at a flow rate of 60 L h^{-1}. 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 (Figure 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 (Figure 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 (Figure 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 (Figure 1d).

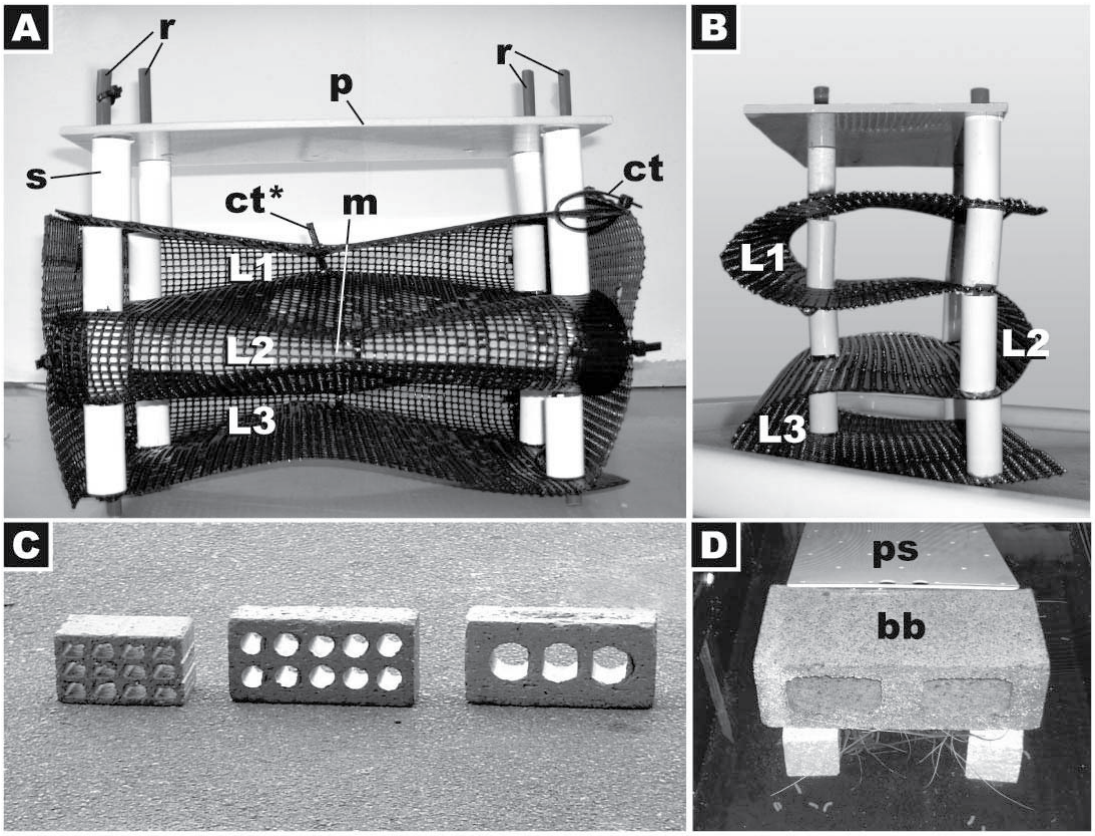

Figure 1. Photographs illustrating the structure of mesh and brick shelters. (A) Front view of mesh shelter showing 3 layers of folded oyster mesh. Cable ties used in post-pueruli shelters to reduce the height of each layer and create small crevices are shown (ct*). (B) Side view of mesh shelter with the side mesh panel removed to show the inner layers of folded mesh. (C) Small brick shelters used in post-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.

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%. During this acclimation period satiation feed rates were determined for each tank and a feed rate per biomass (expressed as % body weight day^{-1}) calculated and fed during the trial (90% of the feed rate where satiation was reached during the acclimation period). Any mortalities during the acclimation week were replaced with similar sized animals which had been held under similar conditions. 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^{-1} for post-pueruli, 2.5% BW day^{-1} year 1 juveniles and 2% BW day^{-1} 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^{-1}) 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. 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.

Apparent Feed Intake (g dry matter (DM) lobster day^{-1}) = (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^{-1}), percentage weight gain (% WG) and growth coefficient were calculated as follows:

SGR (% BW day^{-1}) = (ln final mean lobster weight – ln initial mean lobster weight) *100 / number of days

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

Growth Coefficient = 100 x (final tank total weight$^{1/3}$ – initial tank total weight$^{1/3}$) / 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: $Si(t) = \exp(-At)$, where $Si(t)$ 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.

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 3rd 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. 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^{-1} trypsin made up in deionised water) for 30 mins at 20°C. Fifty µl of enzyme substrate L-DOPA (3 mg ml^{-1}) was added with 850 µl of deonised 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 0.45 M 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.

3.4 Results

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 (Figure 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 low density when all the losses were in the final month (Figure 2). Most mortality was due to cannibalism of recently moulted lobsters.

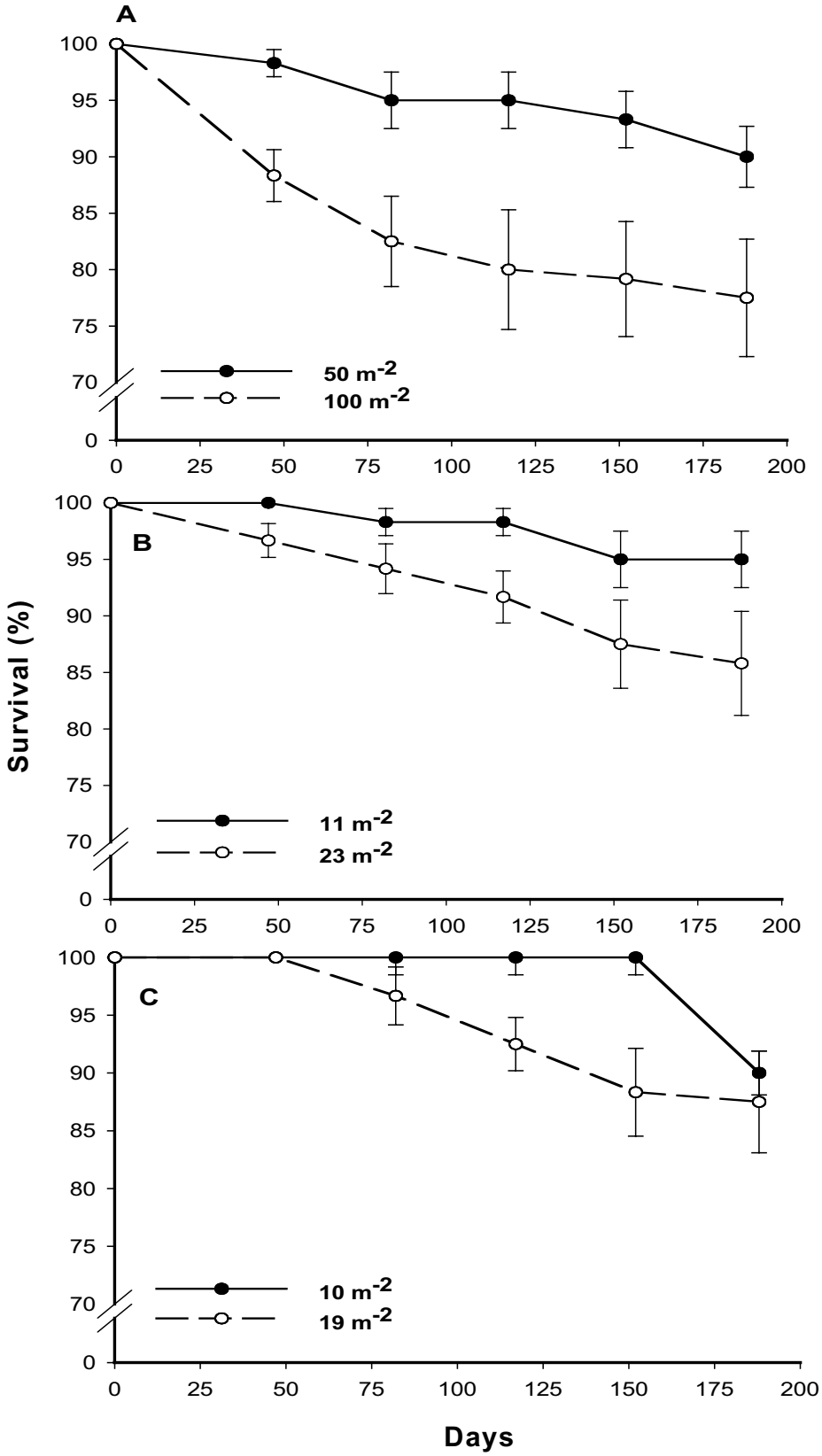

Figure 2. Differences in survival of *Panulirus cygnus* post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density for each size class after 6 months. Data are mean and standard error and analysed by logistical regression (see Table 1). Differences in lobster survival with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

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.

Table 1. Survival (mean % ± standard error) of *P. cygnus* at two densities and shelter types for different size classes after 6 months. Low and high densities (lobsters m^{-2}) 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
Post-Pueruli	90.0 ± 3.3	77.5 ± 5.2	75.8 ± 4.2	91.7 ± 3.7
	p<0.01		p<0.01	
Year 1 Juveniles	95.0 ± 3.1	85.8 ± 4.6	89.2 ± 3.7	91.7 ± 4.2
	p<0.01		p=0.34	
Year 2 Juveniles	90.0 ± 1.9	87.5 ± 4.4	89.2 ± 4.2	95.0 ± 3.1
	p<0.01		p=0.3	

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 (Figure 3 a,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.

The two levels of density tested (post-puerulus - 50 or 100 m^{-2}; year 1 juveniles - 11 or 23 m^{-2}; and year 2 juveniles - 10 or 19 m^{-2}) 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; Figure 4). There was no significant difference in the growth of year 1 and year 2 lobsters between mesh and brick shelters (Table 2).

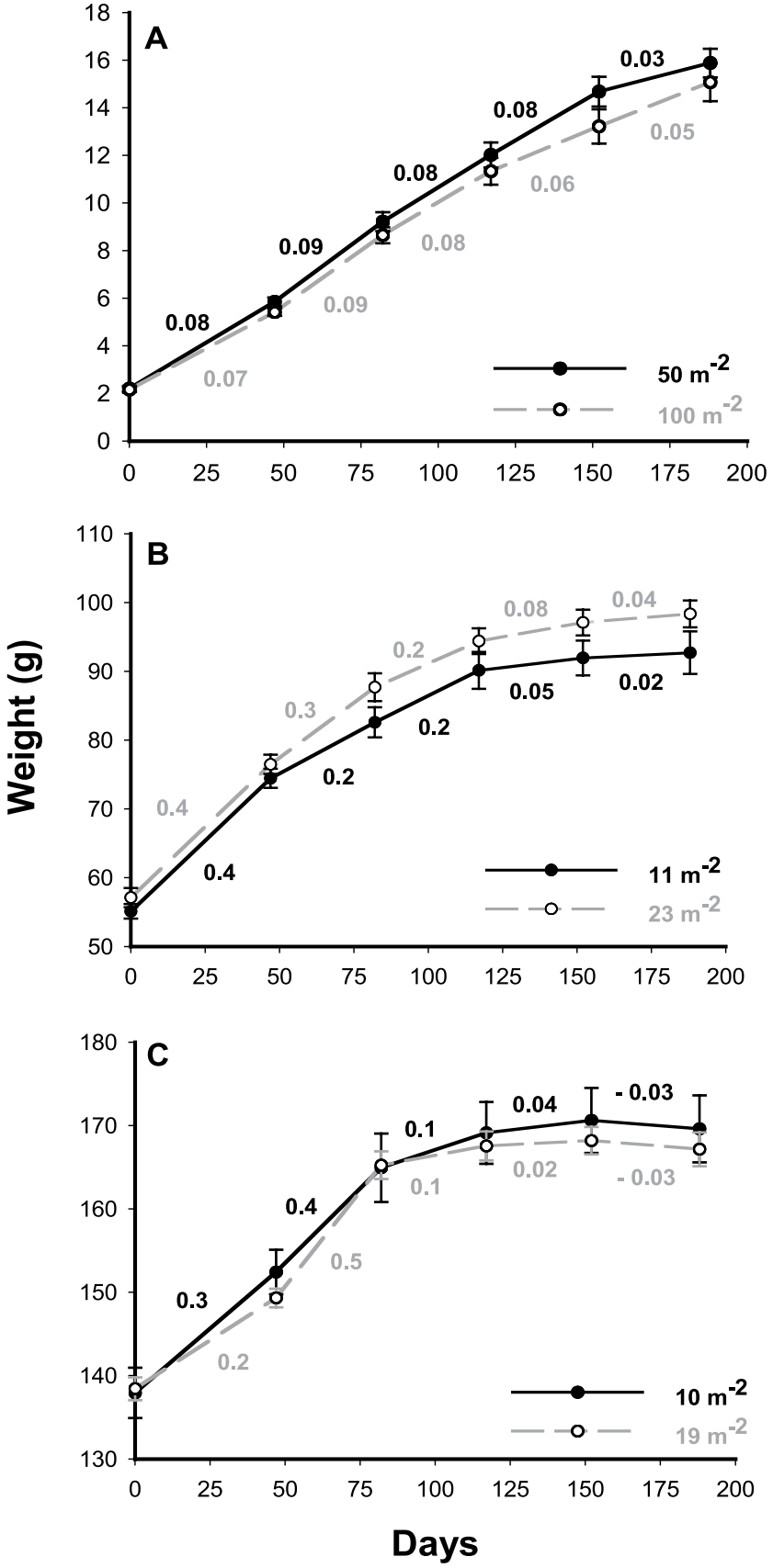

Figure 3. Growth rates of three size classes of *Panulirus cygnus* post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density after 6 months. Data are mean weight and standard error. Weight gain in grams per day is indicated between time periods.

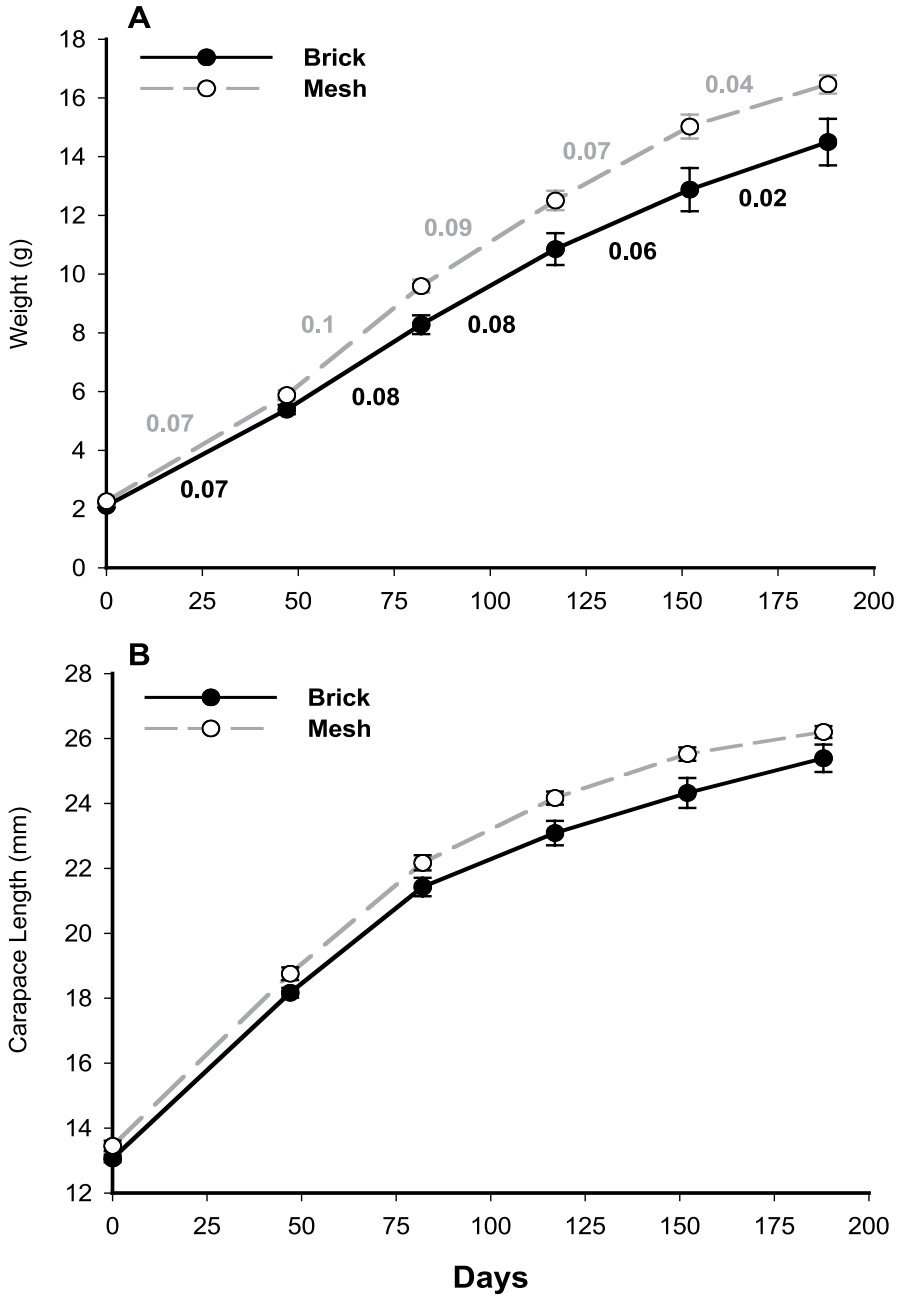

Figure 4. Growth rates of *Panulirus cygnus* post-pueruli in tanks with either mesh or brick shelters after 6 months. Data are mean weight (A) or carapace length (B) and standard error. Weight gain in grams per day is indicated between time periods on Figure A.

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 (Figure 5).

Table 2. Growth response (mean ± standard error) and diet utilisation (mean ± 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
Post-Pueruli	Initial Weight (g)	2.21 ± 0.11	2.16 ± 0.09	2.11 ± 0.05	2.26 ± 0.13
	Final Weight (g)	15.88 ± 0.60	15.07 ± 0.79	14.49 ± 0.79*	16.46 ± 0.31*
	SGR (% BW day-1)	1.06 ± 0.03	1.04 ± 0.03	1.08 ± 0.06*	1.25 ± 0.03*
	% Weight Gain	628.3 ± 41.0	601.9 ± 38.7	588.5 ± 34.7*	641.7 ± 42.5*
	Growth Coefficient	1.40 ± 0.09	1.41 ± 0.13	1.31 ± 0.12*	1.50 ± 0.08*
	AFI (g DM lobster day-1)	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Year 1	Initial Weight	55.13 ± 1.06	57.11 ± 1.39	55.79 ±1.15	56.44 ± 1.42
Juveniles	Final Weight	92.71 ± 3.09	98.34 ± 1.95	97.80 ± 1.69	93. 25 ± 3.37
	SGR	0.28 ± 0.02	0.29 ± 0.01	0.32 ± 0.02	0.33 ± 0.01
	% Weight Gain	68.2 ± 4.7	72.5 ± 3.3	75.6 ± 3.9	65.0 ± 3.1
	Growth Coefficient	0.73 ± 0.05	0.73 ± 0.05	0.78 ± 0.10	0.71 ± 0.1
	AFI	0.23 ± 0.02*	0.27 ± 0.01*	0.28 ± 0.01*	0.21 ± 0.02*
Year 2	Initial Weight	137.94 ± 3.02	138.43 ± 1.36	138.59 ± 2.47	137.77 ± 2.19
Juveniles	Final Weight	169.61 ± 4.02	167.15 ± 2.03	167.86 ± 3.32	168.90 ± 3.12
	SGR	0.11 ± 0.002	0.10 ± 0.006	0.10 ± 0.003	0.11 ± 0.006
	% Weight Gain	22.93 ± 0.39	20.78 ± 1.37	21.09 ± 0.63	22.62 ± 1.35
	Growth Coefficient	0.38 ± 0.07	0.13 ± 0.16	0.15 ± 0.14	0.36 ± 0.12
	AFI	0.31 ± 0.02	0.36 ± 0.04	0.39 ± 0.03*	0.29 ± 0.02*

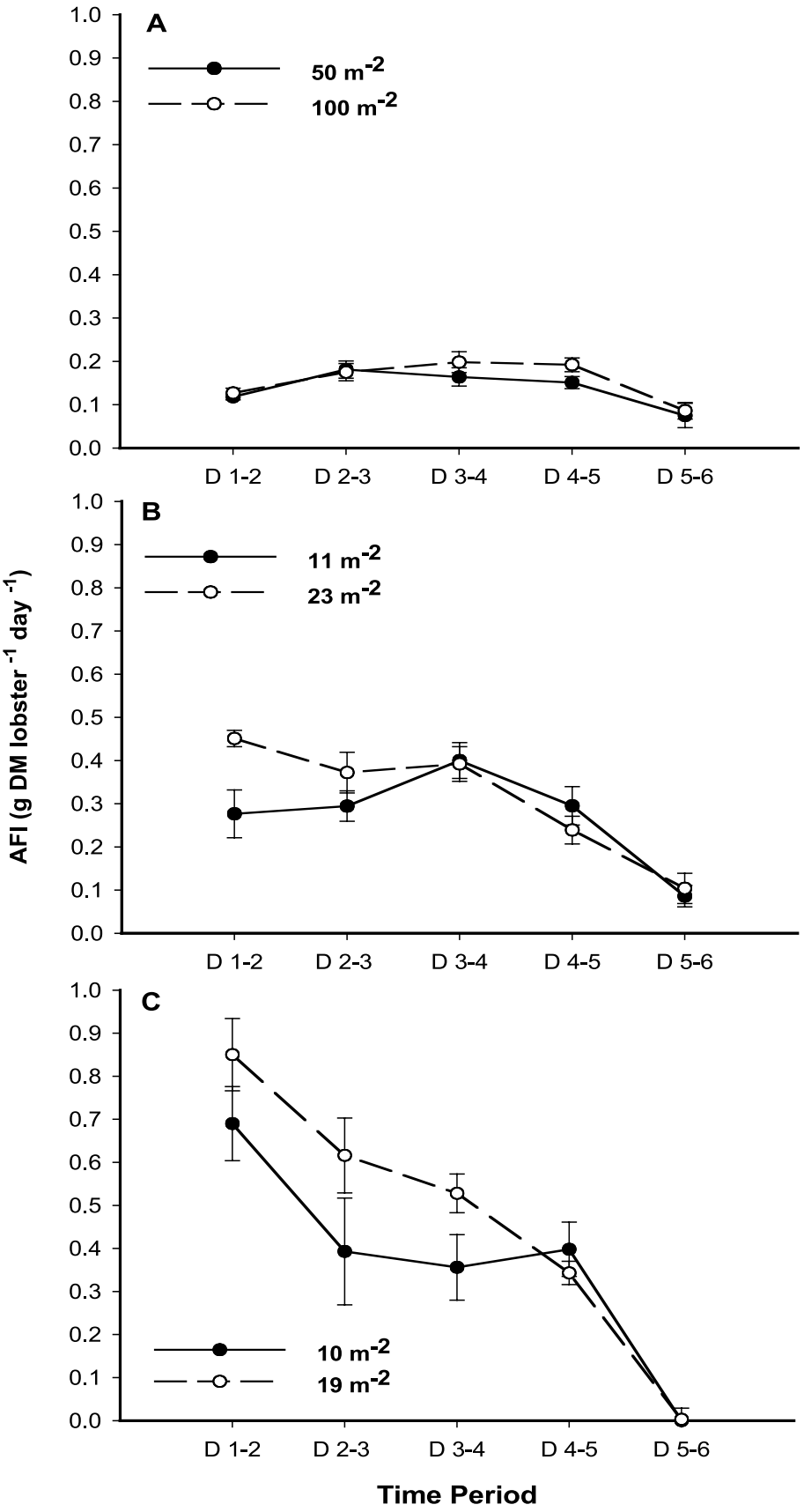

Figure 5. Apparent feed intake of *Panulirus cygnus* post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), at two levels of density after 6 months. Data are mean and standard error. Time period: D1-2, 5 week period between consecutive tank drains and measurements.

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) (Figure 6).

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).

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

Carapace length (mm)	ProPO (min ml^{-1})	Protein (mg ml^{-1})	Comment
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	

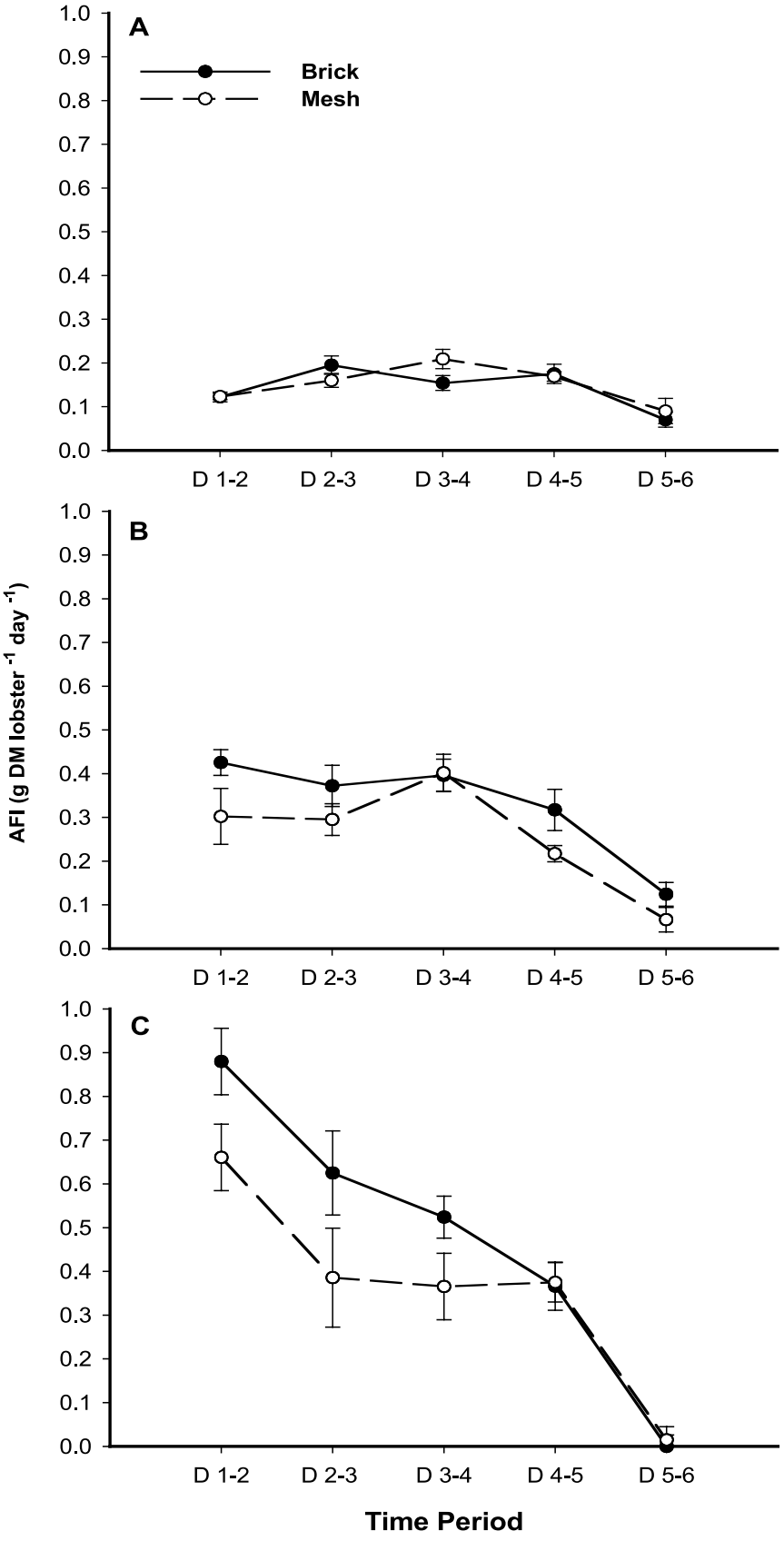

Figure 6. Apparent feed intake of *Panulirus cygnus* post-pueruli (A), year 1 juveniles (B) and year 2 juveniles (C), using brick or mesh shelters after 6 months. Data are mean and standard error. Time Period D1-2: five-week period between first and second consecutive tank drains and measurements.

3.5 Discussion

This study has shown that *P. cygnus* is well suited for aquaculture based on the collection and ongrowing of wild caught pueruli, as this species exhibits good survival at high densities (up to 100 m^{-2}) 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^{-2} achieving between 77% and 90% survival, respectively, whereas year 1 and 2 lobsters achieved between 86% and 95% survival at densities between 10 and 23 m^{-2}. 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-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-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 0.1 and 2 m^{-2} at Seven Mile Beach (Fitzpatrick et al., 1989) and between 1 and 4 individuals m^{-2} 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-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 a 9 month experiment (mean survival 52.5%) (Jones et al., 2001). Furthermore, it is possible that densities in their study (14, 29, 43 m^{-2}) were not high enough to show a pronounced density effect on survival. Nevertheless, James et al. (2001) also found that much higher densities of 50, 100, 150 and 200 m^{-2} had no effect on survival of *J. edwardsii* after 118 days, although maximum survival was recorded at 50 and 100 m^{-2}. 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-pueruli are highly susceptible to cannibalism and mortality (Booth and Kittaka, 2000).

In contrast to survival, the two levels of density tested (post-pueruli - 50 or 100 m^{-2}; year 1 juveniles - 11 or 23 m^{-2}; and year 2 juveniles - 10 or 19 m^{-2}) 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 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 43 m^{-2}) (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^{-2} having the highest mean CL and wet weight and those at 200 m^{-2} the lowest (James et al., 2001). These authors did, however, recommend that maximum growth rates and survival of *J. edwardsii* would be achieved at 50-100 m^{-2}, 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.

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^{-2}) compared with the lowest density (30 m^{-2}) (Moyle, 2005). Therefore it appears that *P. cygnus* post-pueruli are able to be held at relatively high densities, up to 100 m^{-2}, 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 for economical production only being achieved at densities of 3-5 m^{-2} for

marron in ponds (Morrissy et al., 1995a) and 1 m^{-2} for yabbies unfed in ponds with zero water exchange. Growth rates of freshwater crayfish are curvilinear, where at low densities (1-2 m^{-2}) crayfish grow rapidly, at high densities (10 m^{-2}) their growth curve flattens out and at very high densities (20 m^{-2}) 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 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 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 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 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 apparently 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 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 and Haffner 2000) and they, together with the level of protein in the hemolymph, have been used as an indicator of lobster health (Chang, 1995; Floreto et al., 2000; Ozbay and 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.

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 with lobsters fed pellet and fresh mussels (Johnston et al., unpublished data).

3.6 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-pueruli, year 1 and year 2 lobsters should be cultured using mesh shelters, with stocking densities of 50 - 80 m^{-2} for post-pueruli and between 20 and 25 m^{-2} 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*.

4.0 Objective 2b

Evaluate growth rates and survival of post-pueruli, year 1 and year 2 western rock lobsters (Panulirus cygnus) at two temperatures (ambient and 23°C) and under two feeding regimes

Growth rates and survival of western rock lobster (Panulirus cygnus) at two temperatures (ambient and 23°C) and two feeding frequencies

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4.1 Abstract

Wild caught post-pueruli, year 1 and year 2 post settlement juvenile western rock lobster, *Panulirus cygnus,* were held at ambient temperatures or at 23°C, and fed the same ration of a formulated pellet diet either once per night, or 3 times per night, over 12 months, to determine whether elevated temperatures and multiple feeds per night would stimulate growth through increased metabolism and feed utilisation without significant negative impacts on survival. Survival of post-pueruli (mean 63%) did not differ between ambient and 23°C. Survival of year 1 and 2 juveniles was higher at ambient temperatures ($p < 0.01$ ambient: year 1 juveniles, 68%; year 2 juveniles, 88%; 23°C: 57% and 74%, respectively). Feeding frequency did not affect survival of post-pueruli and year 2 juveniles (mean 63%, 81% respectively), but survival was 9% higher for year 1 juveniles fed three times per night (58% versus 67%; $p < 0.01$). All lobsters grew faster at 23°C than at ambient temperatures ($p < 0.05$), with the growth of post-pueruli almost double at 23°C (weight gain at ambient vs 23°C: post pueruli, 18,438% vs 9,915%; year 1 juveniles 259% vs 165%; year 2 juveniles 23% vs 21%). Feed frequency did not influence the growth of year 1 and 2 juveniles. However, there was an interaction effect of temperature and feed frequency on post-pueruli where weight and carapace length were significantly higher at ambient temperatures when post-pueruli were fed three times a day, whereas at 23°C weight and carapace length were significantly greater when fed once per day ($p < 0.05$). Feed intake (g dry matter lobster^{-1} day^{-1}) of pellets was higher at 23°C for all lobsters ($p < 0.05$), but was the same between lobsters fed 3 times per night versus once per night. This study has shown that increasing temperatures to 23°C significantly improved the growth of *P. cygnus* post-pueruli without any adverse effects on survival. The faster growth rates exhibited by year 1 and 2 juveniles at 23°C may potentially offset their lower survival by significantly reducing culture period. There is no benefit of feeding *P. cygnus* multiple times at night in terms of growth and survival. The implications for *P. cygnus* culture are that temperatures should be maintained close to 23°C during the entire growout period, with due care taken to minimise mortalities through adequate provision of food and shelter. Feeding *P. cygnus* once daily to excess just prior to dusk to coincide with nocturnal feeding behaviour is recommended.

Keywords: rock lobster, temperature, feed frequency, survival, growth, aquaculture.

4.2 Introduction

Temperature is one of the major environmental factors affecting the growth of crustaceans (reviewed by Hartnoll, 1982). Elevated (warm) temperatures are known to increase growth rates through reduced intermoult period and/or increased moult increment, whereas the reverse is true for low (cold) temperatures (Chittleborough, 1974; 1975; Serfling and Ford, 1975; Hazell et al., 2001). In a culture situation, temperature is particularly important for maximising growth rate and achieving market size in the shortest period possible. However, mortalities under these conditions are often higher due to the greater incidence of moult related cannibalism. Hence the relationship between temperature, growth and mortality rate is important for determining the economic viability of lobster ongrowing (Booth and Kittaka, 2000). Recent six-month growout trials for post-pueruli, year 1 and year 2 post-settlement juvenile *Panulirus cygnus* have shown good survival (76-95%) at high densities (up to 100 m^{-2}) without adverse effects on growth or captivity-related health problems (Johnston et al., 2006). However, growth was depressed during winter months due to low ambient water temperatures. A seasonal trend has also been observed in previous studies (Phillips et al., 1977). These depressed winter growth rates observed by Johnston et al. (2006) and Phillips et al. (1977), clearly indicate that *P. cygnus* is strongly influenced by temperature and that this is an issue which will need to be addressed if the species is to be successfully cultured in the future.

Growth rate of *P. cygnus* has been shown to increase with increasing temperature to a maximum, before declining near the upper thermal limits. The temperature range for optimum growth of *P. cygnus* is between 25°C and 26°C, above which both growth and survival declined (Chittleborough, 1974; 1975). However, Phillips et al. (1977) observed the fastest growth rate for this species occurred at 23°C after 450 days in culture. Faster growth rates at these temperatures were determined to be due to reduced intermoult period, rather than an increase in moult increment (Chittleborough, 1974, 1975; Phillips et al., 1977). Despite the slight discrepancy in optimum temperature, it is clear that elevated temperatures have the potential to significantly reduce the culture period of *P. cygnus*, which would be important for the long term economic viability of *P. cygnus* culture. However, while the effect of elevated temperature on feed consumption and feed conversion is unknown, it has important implications for culture in regards to feeding costs and systems management.

Ineffective feeding regimes are one of the factors responsible for poor growth rates of rock lobsters fed formulated feeds (Crear et al., 2000; Glencross et al., 2001; Thomas et al., 2003). When formulated prawn diets were divided into more frequent meals, marine prawn growth rate and feed utilization improved (Sedgwick, 1979; Wyban and Sweeney, 1989; Robertson et al., 1993). More frequent delivery of pellets reduces immersion time, theoretically increasing palatability, minimising leach rates and preventing significant pellet deterioration. As a result feed intake and utilization is improved, translating into faster growth rates. Few studies have evaluated optimal feeding regimes for rock lobster species, more specifically, the effectiveness of multiple day or night feeding to improve growth through improved palatability and feed intake. Recent studies on the development of a formulated diet for the tropical rock lobster, *Panulirus ornatus*, concluded that increasing feed frequency from twice to four times per day was a factor responsible for the improved growth rates and success of this formulated diet for this species (Smith et al., 2005). In contrast, a study investigating feeding frequency and ration level in juvenile (5-22 g) *Jasus edwardsii* found that, although feeding high ration levels of formulated feeds more than once daily reduces feed competition and incidence of agonistic behaviour, there appeared to be few benefits in terms of growth and survival (Thomas et al., 2003). Furthermore, Cox and Davis (2006) found that feeding juvenile *Panulirus argus* to

excess once as opposed to twice daily, resulted in greater weight gain after 28 days. Therefore, both studies recommended feeding a pelleted diet once daily to excess just prior to dusk, which coincides with nocturnal feeding behaviour.

P. cygnus requires daily feeding for optimum growth and survival (Chittleborough, 1975, 1976), however, no information is currently available on whether multiple day or night feeding will improve growth further through improved feed intake and utilisation. The potential may therefore exist to improve the growth performance of cultured *P. cygnus* through improved feeding regimes. Such data would be valuable in determining the amount and cost of diet required, one of the major considerations for culture operations.

The aim of this study was to determine the effect of elevated temperatures (23°C) and increased feeding frequency of a formulated pellet per night (same ration fed three times per night versus once per night) on the growth, survival, feed consumption and feed conversion of three size classes of western rock lobster *P. cygnus*.

4.3 Methods

Lobsters were collected from waters within one kilometre of Seven Mile Beach, Dongara, Western Australia, using sandwich collectors (Phillips et al., 2001) or baited mesh pots during November 2004. In December 2004, post-pueruli (mean 0.4 ± 0.006 g, 8.7 ± 0.03 mm CL), year 1 post settlement juveniles (60.9 ± 1.3 g, 38.3 ± 0.3 mm CL) and year 2 post settlement juveniles (142.9 ± 1.4 g, 52.2 ± 0.2 mm CL) were randomly stocked into 60 L, 250 L and 350 L tanks respectively, at the following densities: post-pueruli 50 m^{-2}; year 1 juveniles 23 m^{-2} and year 2 juveniles 19 m^{-2}. All tanks contained mesh shelters (Johnston et al., 2006) and received flow through seawater at 60 L h^{-1}. Six tanks for each size class had ambient temperature water and six tanks had seawater heated to 23°C ± 1°C. This temperature was selected as Phillips et al. (1977) found growth to be optimal at this level. Within each temperature treatment for each size class, three tanks were randomly allocated to feed frequencies of 1 feed per night at 1700h, or 3 feeds per night at 1800h, 0000h and 0500h. The same daily ration of pellet was provided to tanks within each size class irrespective of feeding regime. Wet weight of all lobsters was measured using an electronic balance to the nearest 0.1 g after blotting dry with absorbent towel. Carapace length was measured using vernier callipers to the nearest 0.1 mm.

Following stocking, lobsters were acclimated for 2 weeks and fed with the best available rock lobster pelleted diet, formulated for the tropical lobster *Panulirus ornatus* (Smith et al., 2005), with fresh mussels (*Mytilus edulis*) fed on weekends. Food pellets were made in two - monthly batches at the Western Australian Fisheries and Marine Research laboratories nutrition laboratory using a pasta maker followed by oven drying at 70°C. The proximate composition on a % dry matter basis was protein 55%, lipid 10%, carbohydrate 24%, ash 11%. Any mortalities during the acclimation weeks were replaced with similar sized animals held under similar conditions. During this acclimation period satiation feed rates for pellet and mussels were determined for each tank (90% of the feed rate where satiation was reached during the acclimation period) and a feed rate (expressed as % BW day^{-1}) was calculated. Mortalities were not replaced during the experiment, due to potential size differences between lobsters in holding versus treatment tanks over extended periods of time.

Following acclimation, automated feeders (to deliver 3 feeds per night) were calibrated and the feeding regimes implemented. The automated feeders consisted of a 25 cm long plastic cylinder with a circular plastic plate attached to a small motor at one end. Each feeder was suspended

upright above each tank and rested on a plastic cone fitted into the tank lid. The pellets were poured into the top of each cylinder and the plate rotated a set number of revolutions to distribute the pellet into the tank. The plate speed and number of revolutions were previously calibrated against the quantity of pellet to be delivered into each tank per feeding period. All tanks were supplemented with fresh blue mussels (*Mytilus edulis*) on the weekends to address possible nutritional deficiencies in the pellet diet (Johnston et al., submitted) and hence maximise growth and survival. Automated feeders were not used when feeding mussels, but mussels were fed in sufficient quantities to allow multiple feeding by lobsters throughout the night for both treatment groups. To minimise cannibalism in recently settled post-pueruli, this size group was fed mussels for the first two months of the trial before being weaned onto the pellets.

Each morning, the amount of feed left uneaten (as a percentage of food fed) was assessed visually and the ration adjusted so that >90% of the feed was consumed each day. All uneaten pellets or mussel were then removed from tanks. Once every two months for 7 consecutive days, the dry weight of pellet consumed (apparent feed intake) was accurately measured by siphoning uneaten food onto a mesh screen, washing with fresh water to remove salt (Brunson et al., 1997) and drying overnight at 70°C.

Each morning, moults and mortalities were removed and recorded. Water quality (pH, DO, salinity) was monitored weekly whereas temperature was monitored daily using dataloggers. Ambient water temperatures throughout the trial ranged between 15.6°C and 23.1°C (mean ± S.D., 19.0 ± 0.07°C). Heated water was held at 23.0 ± 0.35°C, apart from occasional instances when there were fluctuations to as low as 21.8°C and as high as 24.4°C. Photoperiod was maintained on a 12 h fluorescent light: 12 hour dark cycle. Growth and survival was measured every month for the first two months and then two-monthly for the remainder of the trial, which was conducted over a 12 month period between December 2004 and December 2005. Adjustments to feed allocations for the increase in biomass were made two-monthly following the weight measurements. At each weighing the tanks were thoroughly cleaned.

Data Processing

Specific growth rates (SGR) were used to overcome problems associated with exponential growth rates (Hopkins 1992; Crear et al., 2000).

SGR (% BW day^{-1}) = (ln final mean lobster weight – ln initial mean lobster weight) *100 / number of days

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 $weight^{1/3}$ – time 1 tank total $weight^{1/3}$) / number of days

Apparent feed intake was calculated by subtracting the dry weight of uneaten feed from the dry weight of pellet fed, 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 overnight. 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. Apparent feed intake (g DM day^{-1}) and food conversion ratio (FCR) calculations accounted for leach rates (stability) of the diet.

Apparent Feed Intake (g DM lobster day^{-1}) = (Dry weight of pellet fed (g) * 0.8) – (dry weight of uneaten pellet (g)) / lobster days of feeding

FCR = Estimated dry weight feed consumed per day (g) / lobster wet weight increase per day (g)

Statistical Analysis

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 each 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 variance using multiple F-tests assessed the validity of this assumption. To test the effects of diet on the proportion of lobsters surviving over time, a logistic regression was used: $Si(t) = \exp(-At) / 1 + \exp(-At)$, where $Si(t)$ 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.

4.4 Results

Mean survival across all temperature and feed frequency treatments after 12 months was highest for year 2 lobsters (81 ± 3.3 %), with no difference in survival between year 1 (63 ± 4.2 %) and post-pueruli (63 ± 7.3 %) (Table 1).

Table 1. Survival (mean % ± standard error) of *P. cygnus* at two temperatures and feeding frequencies for different size classes after 12 months. Temperatures were maintained at ambient seawater temperatures (ranging between 15.6 and 23.1°C, mean 19.0 ± 0.07°C), or heated seawater temperatures (ranging between 21.8 and 24.4°C, mean 23.0 ± 0.35°C). Feed frequency was carried out once at approximately 4pm daily, or three times at 6pm, midnight and 5am using autofeeders. Total amount fed per day was the same between treatments. 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).

Size Class	Temperature		Feed Frequency	
	23°C	Ambient	1	3
Post-Puerulus	58.3 ± 7.9	66.7 ± 12.8	60.0 ± 11.5	65.0 ± 9.9
	P = 0.51		P = 0.75	
Year 1	56.7 ± 5.6	68.3 ± 5.6	58.3 ± 6.2	66.7 ± 5.6
	P < 0.01		**P < 0.01**	
Year 2	74.2 ± 4.4	88.3 ± 2.8	82.5 ± 3.1	80.0 ± 6.1
	P < 0.01		P = 0.97	

Survival of post-pueruli did not differ with temperature ($p = 0.51$). However, survival of year 1 and 2 juveniles was higher at ambient temperatures (11% higher for year 1 $p < 0.01$; 14% higher for year 2, $p < 0.01$) (Table 1; Figure 1). Survival declined steadily throughout the 12 month period for all size classes across both temperature treatments. However, there appeared to be a subtle change in survival for ambient tanks which reflected changing water temperatures, where mortalities were higher in the warmer months and plateaued in winter months (Fig 1).

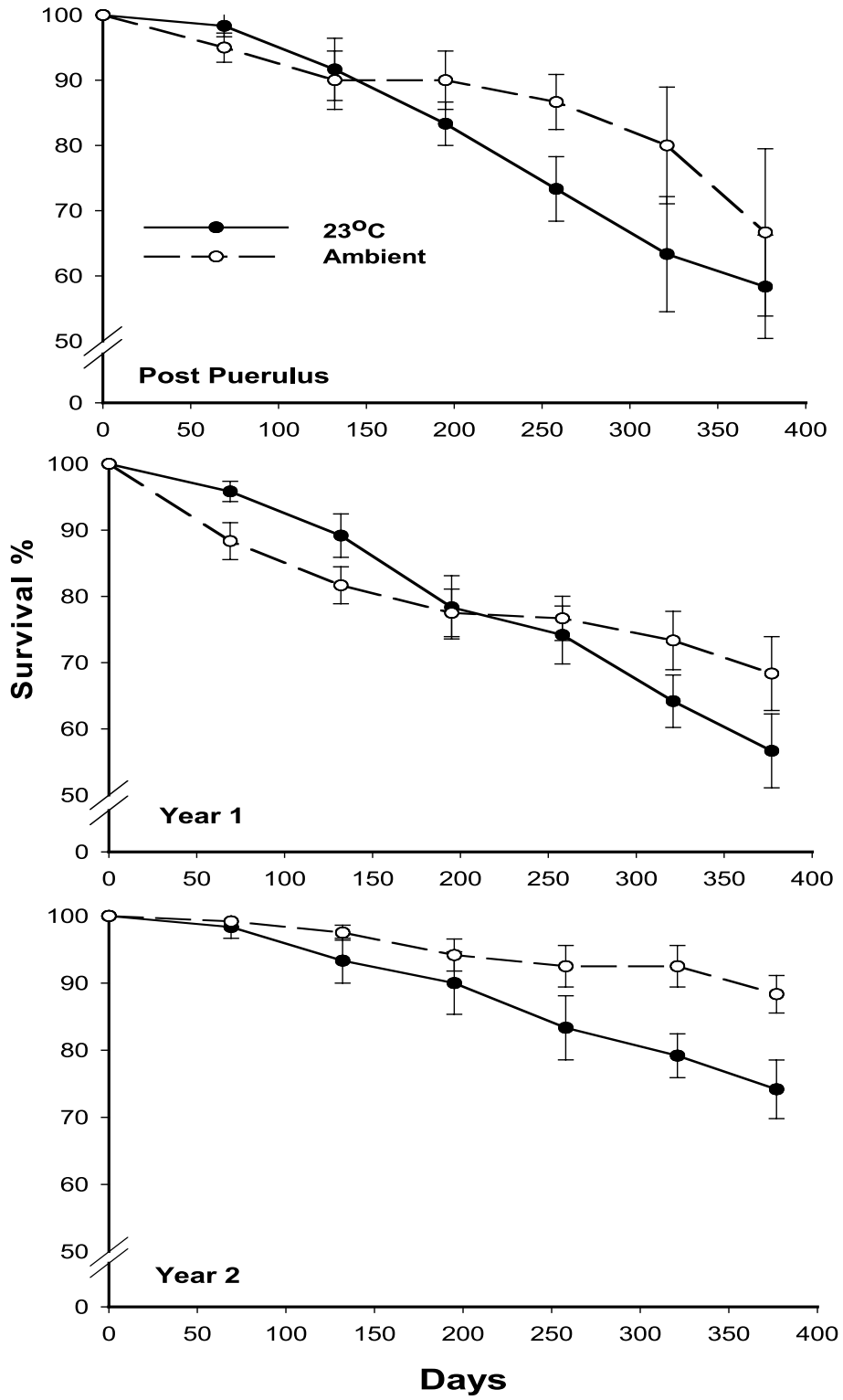

Figure 1. Differences in survival of *Panulirus cygnus* at two temperature regimes for each size class after 12 months (December 04 – December 05). Data are mean and standard error and analysed by logistic regression. Ambient temperatures ranged between 15.6 and 23.1°C, mean 19.0 ± 0.07°C.

Feeding frequency (1 x per night, 3 x per night) did not affect survival of post-pueruli or year 2 lobsters after 12 months, although it was higher (9%, $p<0.01$) for year 1 juveniles fed three

times per night (Table 1; Fig 2). Survival declined steadily throughout the 12 month period irrespective of feed frequency for all size classes (Figure 2).

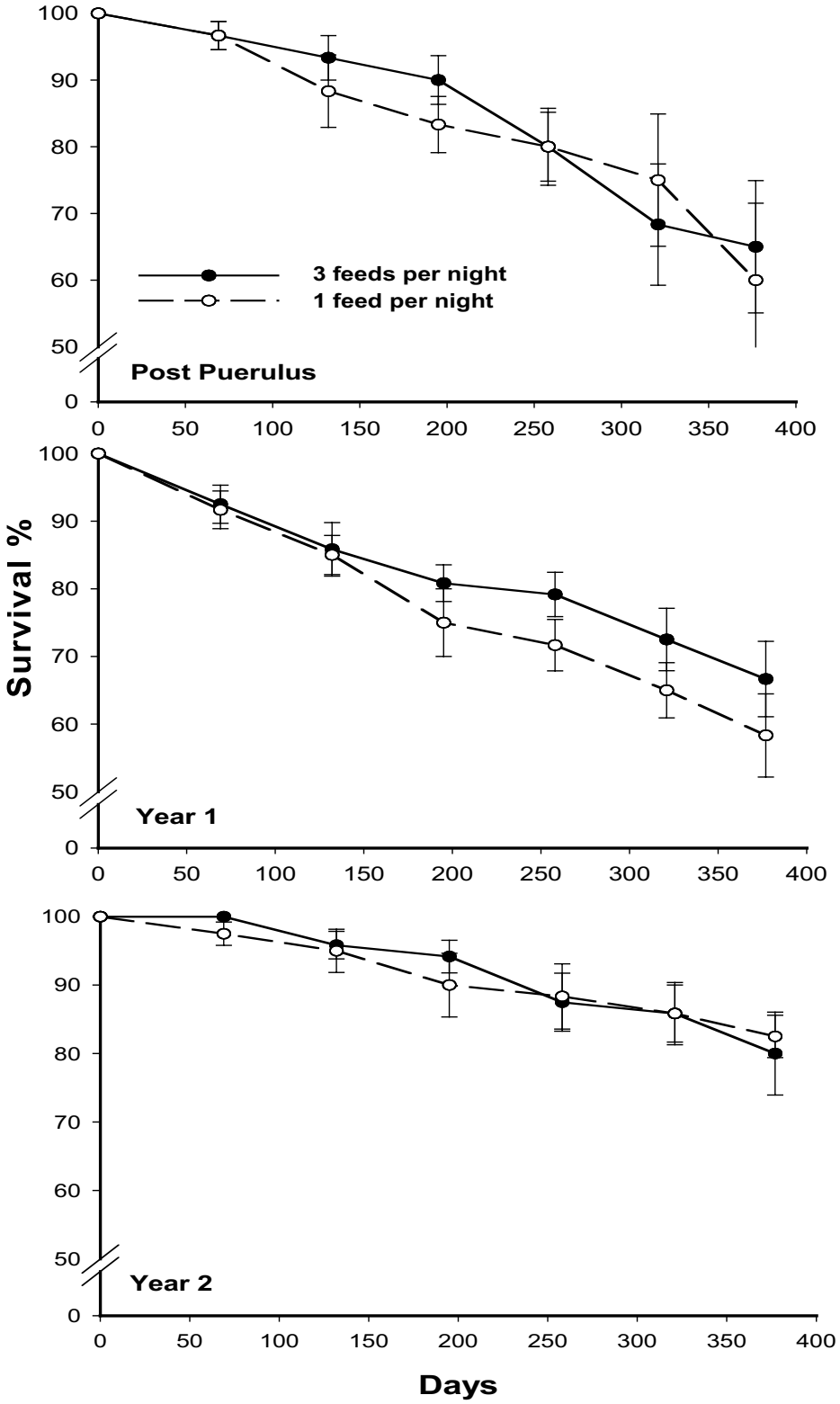

Figure 2. Differences in survival of *Panulirus cygnus* at two feeding frequencies for each size class after 12 months (December 04 – December 05). Data are mean and standard error and analysed by logistic regression.

All lobsters grew faster at 23°C than at ambient temperatures, with the most significant impact of temperature on post-pueruli and year 2 juveniles (split plot analysis post-pueruli: SGR $F_{(1,6)} = 37.28$, $p < 0.01$; % weight gain $F_{(1,6)} = 1.96$, $p = 0.02$; g/day $F_{(1,6)} = 46.07$, $p < 0.01$; Year 2: SGR $F_{(1,6)} = 7.16$, $p < 0.04$; % weight gain $F_{(1,6)} = 5.90$, $p = 0.05$, g/day $F_{(1,6)} = 6.53$, $p = 0.04$) (Table 2, Figure 3). In particular, growth (% weight gain and g/day) of post-puerulus at 23°C was double that of post-pueruli held at ambient temperatures. Growth rates of post-pueruli, year 1 and year 2 lobsters were similar initially between ambient and 23°C tanks, however growth in ambient tanks slowed during winter and was negligible for year 1 and 2 lobsters (Figure 3). Although growth increased significantly in the last 2 months of the trial, particularly for year 1 and 2 lobsters due to rising summer ambient temperatures, the size of all lobsters in 23°C tanks steadily increased throughout the 12 month trial (Figure 3).

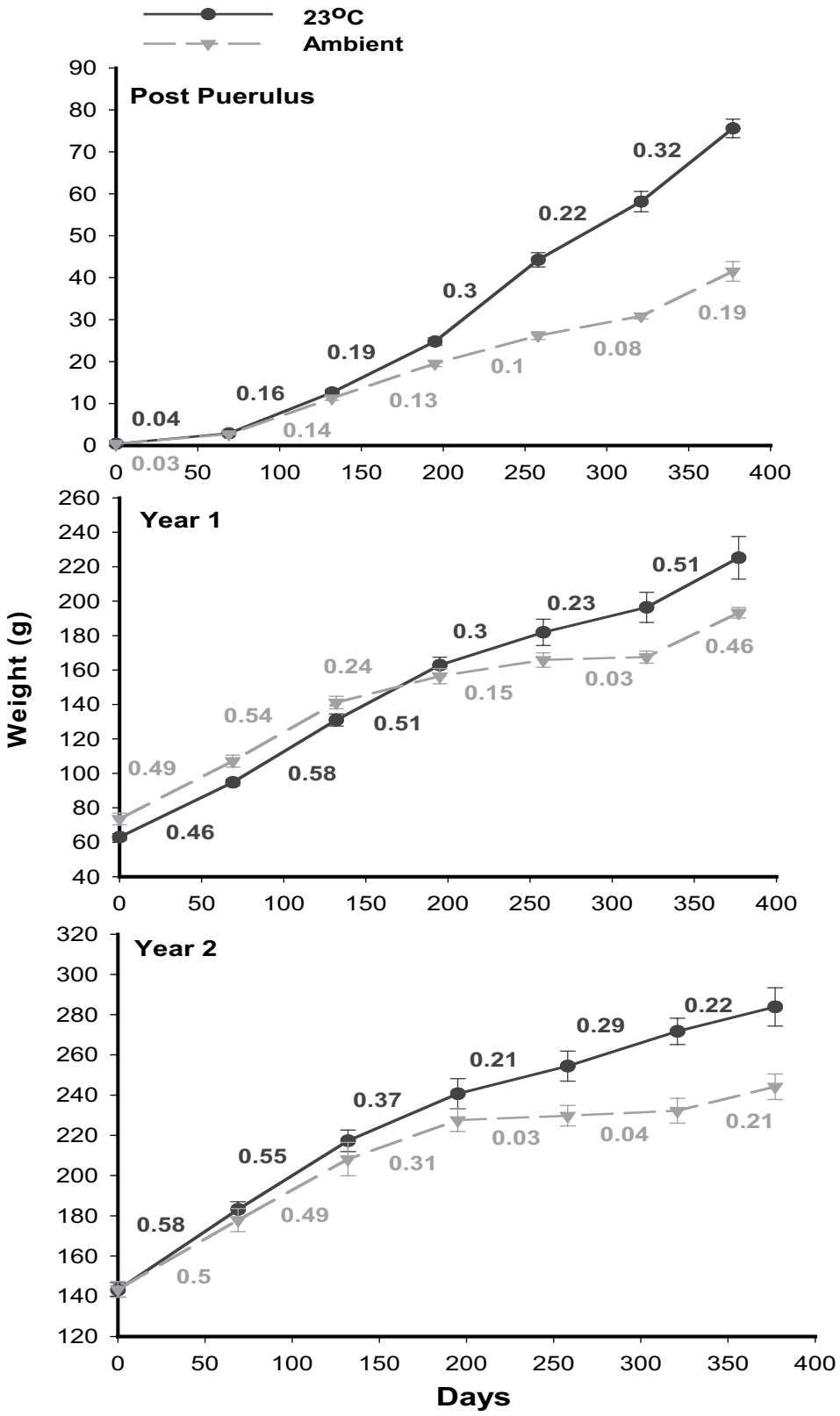

Figure 3. Growth rates of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two temperature regimes after 12 months (December 04 – December 05). Data are mean and standard error. Weight gain in grams per day between each time period is indicated. Ambient temperatures ranged between 15.6 and 23.1°C, mean 19.0 ± 0.07°C.

Feeding frequency did not influence the growth of year 1 and year 2 lobsters after 12 months (Table 2; Figure 4). However, there was a significant interaction effect of temperature and feeding frequency on post-pueruli where weight and carapace length were significantly higher at ambient temperatures when post-pueruli were fed three times a day, whereas at 23°C weight and carapace length were significantly greater when fed once a day (ANOVA Final weight $F_{(1,8)}$ = 3484.5; p = 0.000008; Final CL $F_{(1,8)}$ = 31.84; p = 0.00049; split plot analysis weight $F_{(1,6)}$ = 104.33, p < 0.01; carapace length $F_{(1,6)}$ = 18.79, p < 0.01) (Figure 5).

Mean apparent feed intake (AFI, g DM lobster^{-1} day^{-1}) of pellets by post-pueruli and year 2 lobsters was significantly higher at 23°C than at ambient temperatures (year 1 $F_{(1,8)}$=16.76, p = 0.003; year 2 $F_{(1,8)}$ = 19.14, p = 0.002; Table 2; Figure 6). This trend was also apparent for year 1 juveniles although not significant. Feed intake increased between December 2004 - March 2005 and November – December 2005 for all lobster size classes and reflected increasing ambient temperatures. Feed intake declined during winter months for all lobster size classes held at ambient temperatures and was negligible between July and October (tank drains 4 - 6) for post-pueruli and year 2 lobsters and July and November for year 1 lobsters (Figure 6). Feed intake of year 1 and 2 lobsters held at 23°C also declined in winter but not to the same extent, with a plateau in intake for post-pueruli (Figure 6). There was no difference in mean feed intake between lobsters fed 3 times per night versus once per night, for all size classes (Table 2; Figure 7).

Table 2. Growth response (mean ± standard error) and diet utilisation (mean ± standard error) of three size classes of *P. cygnus* at two temperatures and two feeding frequencies after 12 months (December 04 – December 05). Asterisks indicate parameters that are significantly different between either temperature or feeding frequency. # indicates a significant interaction effect between temperature and feed frequency. 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). FCR was calculated using mean weight of lobsters.

Size Class	Parameter	Temperature		Feeding Frequency	
		23°C	Ambient	1	3
Post-Puerulus	Initial Weight (g)	0.41 ± 0.009	0.42 ± 0.018	0.42 ± 0.018	0.41 ± 0.007
	Final Weight (g)	75.58 ± 2.21#	41.50 ± 2.36#	60.23 ± 8.25#	56.85 ± 7.57#
	Initial CL (mm)	8.78 ± 0.04	8.72 ± 0.06	8.78 ± 0.04	8.72 ± 0.07
	Final CL (mm)	42.04 ± 0.94#	35.97 ± 0.73#	40.08 ± 1.62#	37.92 ± 1.42#
	g/day	0.2 ± 0.006*	0.11 ± 0.006*	0.16 ± 0.02	0.15 ± 0.02
	SGR (% BW day^{-1})	1.39 ± 0.006*	1.22 ± 0.02*	1.30 ± 0.04	1.30 ± 0.04
	% Weight Gain	18438 ±406*	9915 ± 920*	14321 ± 2067	14032 ± 1999
	Growth Coefficient	1.57 ± 0.09	1.26 ± 0.12	1.39 ± 0.14	1.43 ± 0.12
	AFI (g DM lobster day^{-1})	0.36 ± 0.07*	0.22 ± 0.05*	0.29 ± 0.09	0.27 ± 0.04
	FCR	1.54 ± 0.31	1.75 ± 0.41	1.66 ± 0.45	1.63 ± 0.25
Year 1 Juveniles	Initial Weight (g)	63.03 ± 1.91	73.55 ± 3.41	67.01 ± 2.56	69.56 ±4.37
	Final Weight	225.15 ± 12.31*	193.22 ± 3.01*	214.12 ± 12.83	207.87 ± 8.07
	Initial CL (mm)	39.58 ± 0.47	40.93 ± 0.64	40.32 ± 0.56	40.19 ± 0.71
	Final CL (mm)	62.08 ± 1.19	58.81 ± 0.35	61.07 ± 1.35	60.19 ± 0.63
	g/day	0.43 ± 0.03	0.32 ± 0.012	0.36 ± 0.03	0.39 ± 0.04
	SGR	0.34 ± 0.02	0.26 ± 0.01	0.31 ± 0.02	0.29 ± 0.03
	% Weight Gain	259 ± 23	165 ± 12	222 ± 24	202 ± 30
	Growth Coefficient	-0.07 ± 0.71	0.46 ± 0.49	- 0.005 ± 0.71	0.39 ± 0.49
	AFI	0.55 ± 0.05	0.26 ± 0.05	0.45 ± 0.09	0.35 ± 0.07
	FCR	1.12 ± 0.14*	0.69 ± 0.13*	0.99 ± 0.20	0.81 ± 0.11
Year 2 Juveniles	Initial Weight (g)	143.17 ± 3.57	143.41 ± 3.88	148.36 ± 3.33	138.22 ± 2.53
	Final Weight	283.86 ± 9.51*	244.16 ± 6.36*	261.09 ± 6.93	266.92 ± 15.39
	Initial CL (mm)	51.65 ± 0.59	52.65 ± 0.17	52.46 ± 0.33	51.84 ± 0.57
	Final CL (mm)	66.54 ± 0.72*	63.59 ± 0.57*	65.02 ± 0.73*	65.11 ± 0.73*
	g/day	0.37 ± 0.03*	0.27 ± 0.013*	0.34 ± 0.04	0.30 ± 0.02
	SGR	0.182 ± 0.01*	0.14 ± 0.01*	0.15 ± 0.01	0.17 ± 0.01
	% Weight Gain	22.93 ± 0.39*	20.78 ± 1.37*	21.09 ± 0.63	22.62 ± 1.35
	Growth Coefficient	0.51 ± 0.06	0.55 ± 0.07	0.50 ± 0.07	0.55 ± 0.06
	AFI	0.57 ± 0.04*	0.38 ± 0.02*	0.47 ± 0.05	0.48 ± 0.06
	FCR	1.54 ± 0.13	1.44 ± 0.11	1.59 ± 0.15	1.39 ± 0.06

Food conversion ratios (FCR) of post-pueruli and year 2 lobsters over the 12 month period were not significantly different between 23°C and ambient temperatures (Table 2). FCR was significantly better for year 1 lobsters held at ambient (0.69) than at 23°C (1.12) ($F_{(1,6)} = 5.79$, $p < 0.05$). FCR was not significantly different between feed frequencies for any size class.

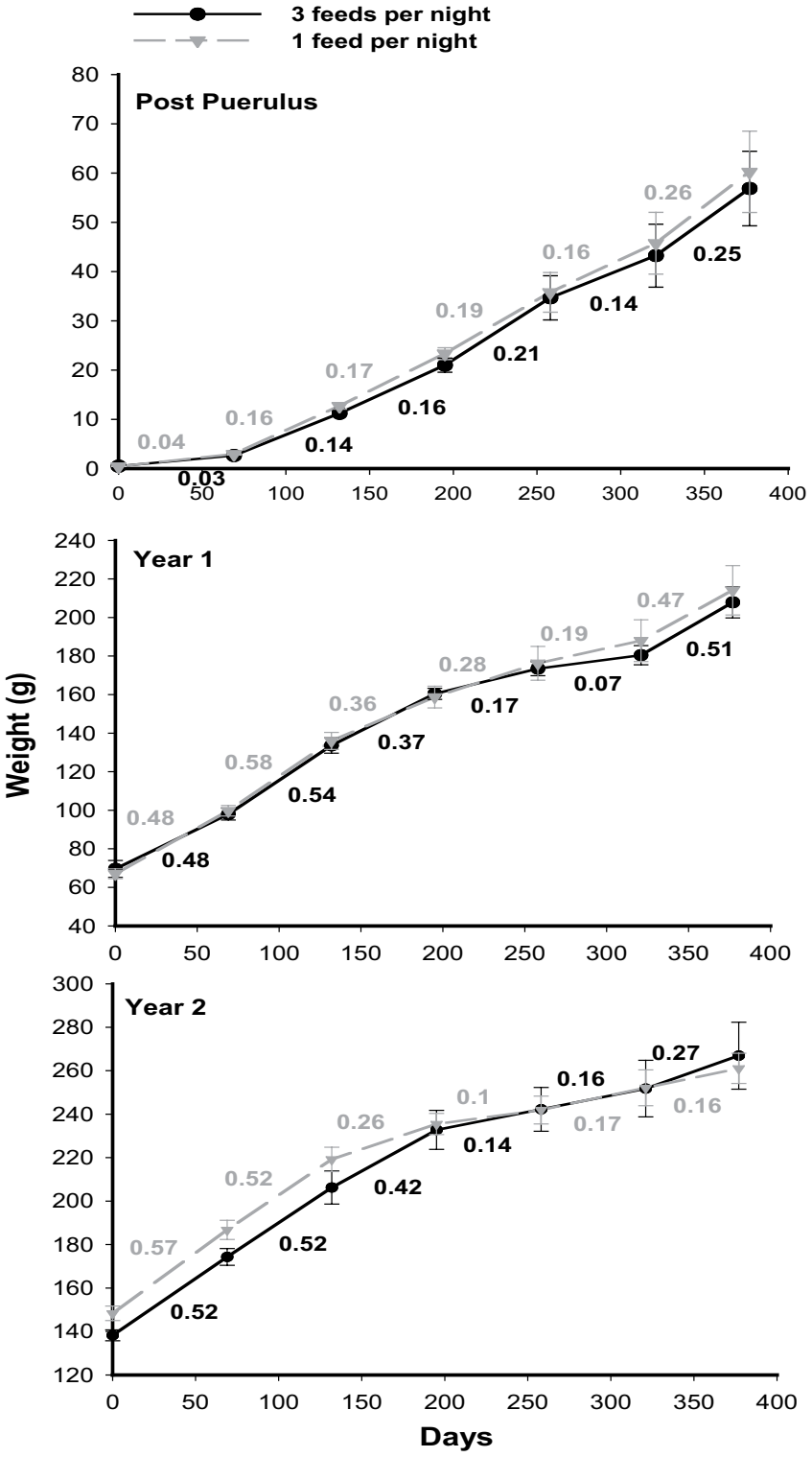

Figure 4. Growth rates of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two feeding frequencies after 12 months (December 04 – December 05). Data are mean and standard error. Weight gain in grams per day between each time period is indicated.

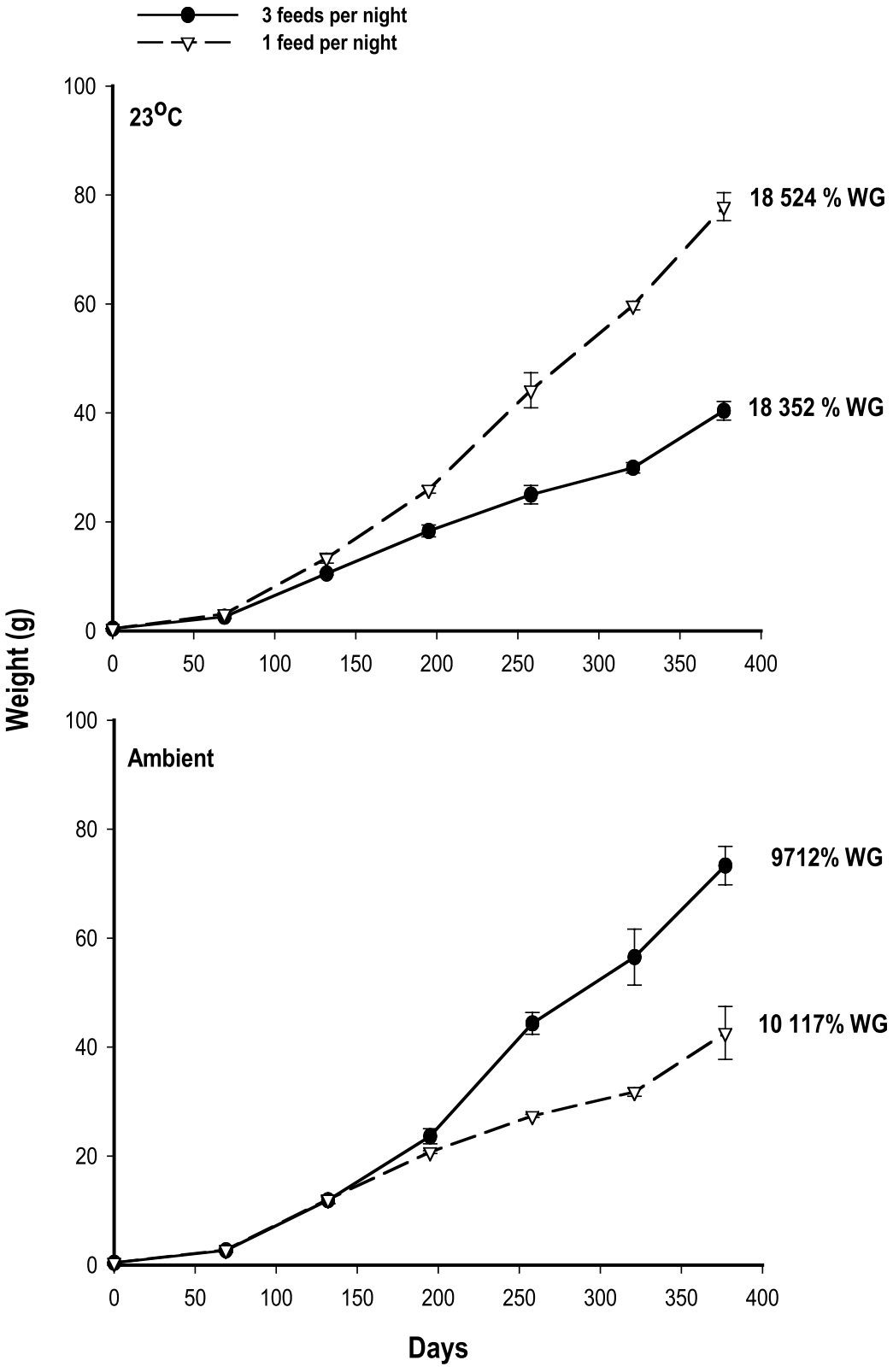

Figure 5. Growth rates of *Panulirus cygnus* post-pueruli demonstrating the interaction effect between temperature and feeding frequencies. Data are mean and standard error. Percentage weight gain over the 12 month period between December 04 and December 05 is indicated.

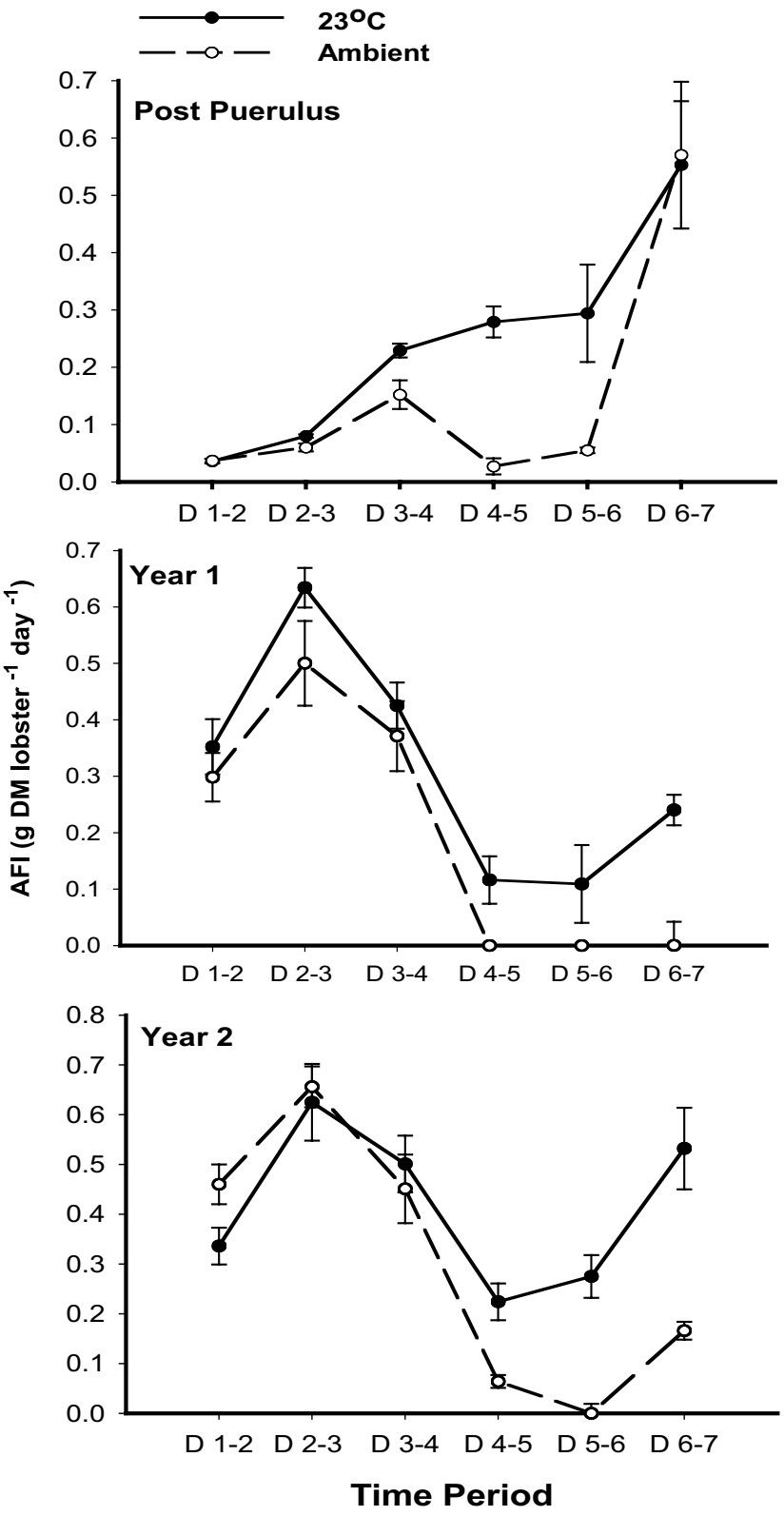

Figure 6. Apparent feed intake of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two temperature regimes after 12 months (December 04 – December 05). Data are mean and standard error. Time period: D1-2, 8 week period between consecutive tank drains and measurements. Ambient temperatures ranged between 15.6 and 23.1°C, mean 19.0 ± 0.07°C.

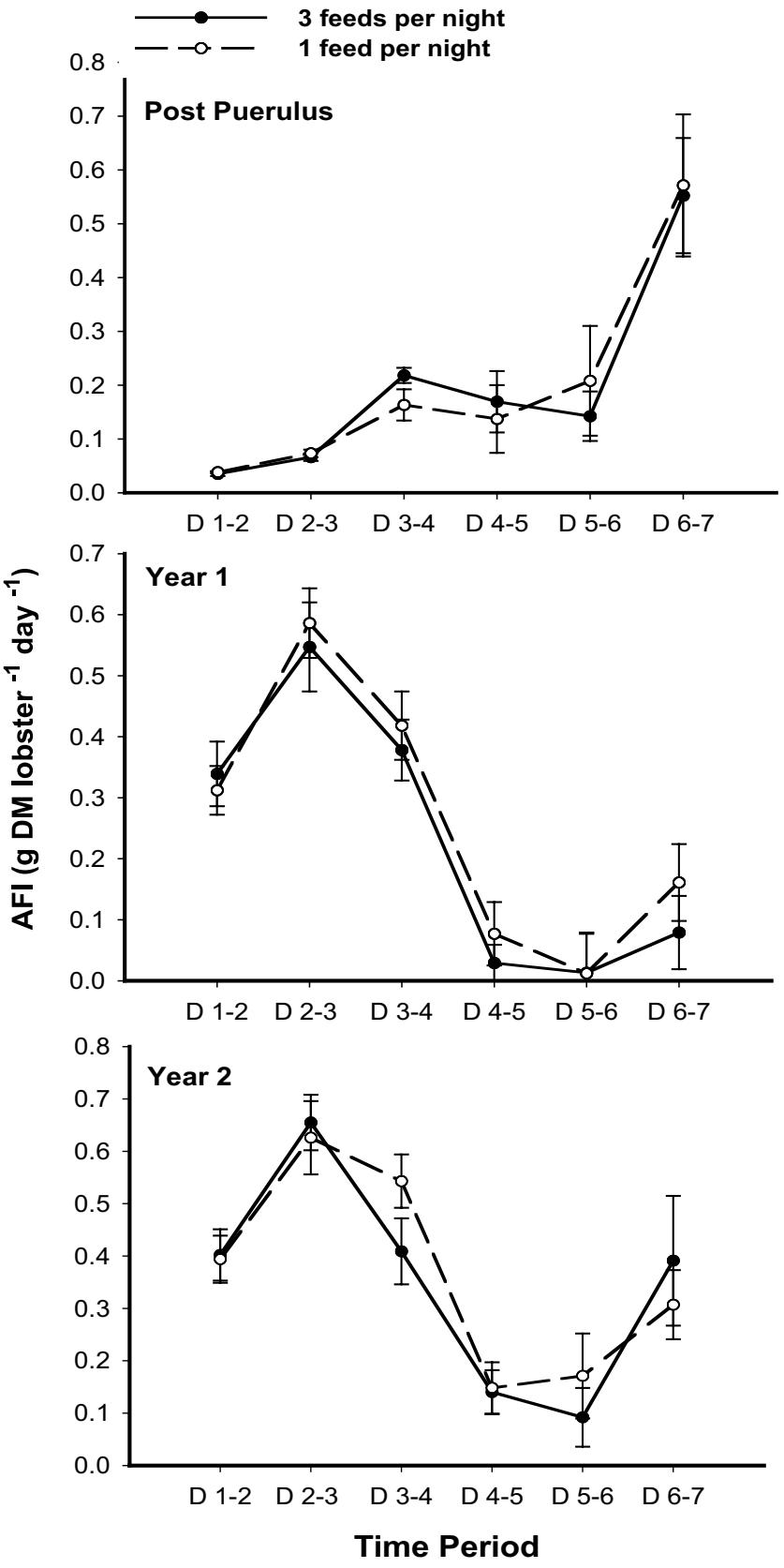

Figure 7. Apparent feed intake of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two feeding frequencies after 12 months. Data are mean and standard error. Time period: D1-2, 8 week period between consecutive tank drains and measurements.

4.5 Discussion

Temperature

For *Panulirus cygnus* culture to be economically viable, it is essential to reach market size in a cost effective time. Elevated temperatures can increase the growth rate of lobsters (Chittleborough, 1975; Serfling and Ford, 1975; Phillips et al., 1977; Lellis and Russell, 1990; Crear et al., 2000; Hazell et al., 2001), potentially reducing the length required for growout. However, mortalities can also increase at elevated temperatures with reduced growth and survival of *P. cygnus* near their upper thermal limit of 25°C - 26°C (Chittleborough, 1975). In this study, *P. cygnus* post-pueruli, year 1 and 2 juveniles held at 23°C grew significantly faster than those held at ambient temperatures, with no significant reduction in the survival of post-pueruli, but with some reduction in survival of year 1 and 2 (11% and 14%, respectively). The increased growth of all juveniles, without large reductions in survival at these elevated temperatures, suggests that market size will be attained much faster than at ambient temperatures, whilst maintaining biomass. It is therefore recommended that *P. cygnus* juveniles are either cultured at ambient temperatures close to 23°C or in water heated to a similar temperature, to ensure that growth is optimised.

The marked increase in growth of post-pueruli, year 1 and year 2 juveniles held at 23°C is most likely attributed to reduced intermoult period. This would be particularly prevalent for post-pueruli where growth was double that of their counterparts held at ambient temperatures. Chittleborough (1974b; 1975) and Phillips et al. (1977) found that frequency of moulting of *P. cygnus* juveniles increased at elevated temperatures (ie. reduced intermoult period), but that growth per moult (moult increment) was unaffected. Accelerated growth of juvenile *Panulirus interruptus* and *Jasus lalandii* at elevated temperatures have also been associated with increased moulting rates (reduced intermoult period) rather than with greater increments per moult (Serfling and Ford, 1975; Hazell et al., 2001). In the case of *Jasus edwardsii* elevated temperature led to a reduction in the intermoult period, but in contrast to the other species mentioned, growth per moult was also reduced (Thomas et al., 2000). The likelihood that increased growth at 23°C may be attributed to reduced densities of year 1 and 2 lobsters on account of higher mortalities at elevated temperatures is unlikely as densities between 10 - 23 m^{-2} were not found to affect growth rates of *P. cygnus* juveniles (see Johnston et al., 2006). Hence reductions in density of year 1 and 2 juveniles, which were stocked at 23 and 19 $m^{-2,}$ respectively, due to increased cannibalism at 23°C would not contribute significantly to their increased growth at elevated temperatures in this study.

Increases in feed consumption by lobsters held at elevated temperature have been reported for *J. edwardsii* (Crear et al., 2000). In this study feed conversion ratios were similar between ambient and 23°C treatments in each size class, suggesting that the efficiency of utilisation of diets was not affected by temperature. Crear et al. (2000) also found FCR for *J. edwardsii* to be unaffected by water temperature, despite an increased feed intake. This however, may not be the case for other species where the efficiency of utilisation of diets decreased at higher temperatures (Lellis and Russell, 1990; Thomas et al., 2000).

Early sexual maturity of lobsters held at elevated temperatures may reduce growth rates due to the progressive diversion of energy from somatic growth into reproductive development. Several ovigerous females were observed in 23°C tanks of year 2+ lobsters in this study, indicating precocious development of these individuals. Growth rates of these year 2+ females were lower than those held at ambient temperatures (Johnston, unpublished data), suggesting

that market size of lobsters cultured at elevated temperatures may need to be lowered to ensure maximum growth efficiency for the entire culture period. Similar findings were reported by Serfling and Ford (1975) and consequently they predicted growth rates of *P. interruptus* to legal size allowing for a 50% reduction in growth rate upon reaching sexual maturity. The market size of cultured *P. cygnus* is currently set at 76 mm CL, the minimum legal size for the wild capture (Ministerial Policy Guideline 20, 2004). The necessity for retaining this restriction in the future has been highlighted for consideration (Department of Fisheries, 2006) when new policy surrounding rock lobster aquaculture is considered.

Feeding Frequency

Post-pueruli and year 1 lobsters fed three times per night achieved 5% and 9% higher survival, respectively, probably due to reduced competition for food between lobsters in these smaller size classes. The higher moult frequency of juvenile lobsters exposes them to a higher incidence of moult related cannibalism and providing a readily available food source throughout the nocturnal foraging period may reduce the likelihood of recently moulted individuals being cannibalised. It is surprising that improvement in survival of post-pueruli fed multiple times during the night was not greater than the reported 5%. It is possible that the pellet diet was not sufficiently palatable to allow a significant reduction in moult-related cannibalism of post-pueruli to occur. This is supported by the fact that feed intake by post-pueruli did not increase despite multiple feeding. Furthermore, feed consumption by year 1 and 2 lobsters fed multiple times per night did not differ significantly to those animals fed once a night, potentially explaining the minimal effect on survival. Similar findings have been reported for *J. edwardsii* and *P. argus* where multiple feeds did not significantly improve survival rates (Thomas et al., 2003; Cox and Davis, 2006). Therefore, although feeding a high ration diet more than once daily reduces feed competition and the incidence of agonsistic behaviour (Thomas et al., 2003), it does not appear to be beneficial in terms of significantly improving the survival of *P. cygnus* juveniles. Whether this is the case with the provision of more palatable pellet diets needs to be verified.

Feeding *P. cygnus* post-pueruli, year 1 and year 2 juveniles three times per night did not significantly improve growth rates. This finding is consistent with Thomas et al. (2003) who reported no significant improvements in the growth of *J. edwardsii* juveniles fed two or four meals a day versus once a day. In both studies feed intake of pellets did not increase with increasing feed frequency, suggesting that pellet palatability and nutritional value were not sufficient to generate an increase in consumption and growth. This is confirmed by Smith et al., (2005) who concluded that increasing feed frequency from two times per day to four times per day was a factor responsible for the improved growth rates and success of this formulated diet for *P. ornatus*. The fact that similar results were not evident in this study, despite the use of the *P. ornatus* diet, confirms that species-specific dietary formulations are required for rock lobster culture. More frequent feeds should theoretically minimise leaching of essential nutrients improving palatability of the pellet resulting in improved feed consumption and growth, which clearly did not occur for *P. cygnus*.

In contrast to this study, and that of Thomas et al., (2003), growth rates were significantly higher for *P. argus* juveniles fed once daily versus twice daily (Cox and Davis, 2006). It is possible that improved growth rates of *P. argus* fed once daily may be due species specific sensitivity to disturbance or to the feeding of a high protein fresh diet (clams, shrimp, squid, oysters), rather than pellets, and a subsequent potential improvement in feed consumption. Unfortunately, feed intake measurements were not reported by Cox and Davis (2006), but it

is clear that feeding fresh, as opposed to formulated diets, changes the response of lobsters to feed frequency. Whether feeding regimes should be tailored according to diet type (fresh versus pellet) will need further clarification in future studies. Nevertheless, feeding trash fish fresh diets are unlikely to be suitable for large-scale aquaculture, so the results of this study with respect to feeding frequency of pelleted diets may be of more practical significance. It should also be noted that feeding once in the morning versus once at night resulted in significantly better growth for *J. edwardsii* (Radford and Marsden, 2005). Although this has not been reported elsewhere, Radford and Marsden (2005) believe that feeding lobsters in the morning maximizes energy utilisation and leading to improved growth rates.

There was a significant interaction between temperature and feeding frequency for post-pueruli, where growth was faster at ambient temperatures when fed three times a day, whereas at 23°C growth was faster when fed once per day. This trend is difficult to explain as feed intake was higher at 23°C, explaining the faster growth rates at elevated temperatures, but was similar between feeding frequencies. In the absence of a greater understanding of the complex physiological processes involved, it is recommended that post-pueruli should be fed three times a night at ambient temperatures, or once a night at 23°C to maximise growth. This latter recommendation is consistent with Cox and Davis (2006).

4.6 Conclusions

This study demonstrated that a constant temperature of 23°C dramatically improves the growth rate of *P. cygnus* juveniles, without significant impact on survival of post-pueruli, but with some impact on survival of older juveniles. Therefore it is recommended that ambient temperatures should be close to 23°C to achieve the shortest culture period for this species, without the risks of marked declines in growth or survival that occur near their upper thermal limit of 25-26°C (Chittleborough, 1974b; 1975). The faster growth rates exhibited by year 1 and 2 juveniles at 23°C may potentially offset their lower survival and warrants a cost benefit analysis for commercial production. There are no obvious benefits to feeding *P. cygnus* juveniles multiple times per night in terms of growth or survival. However, it is possible that trends may have been different if palatability of the *P. ornatus* pellet was improved to more accurately test the possibility that feeding smaller rations more frequently improved feed intake of the pellet by *P. cygnus*. Feeding the *P. ornatus* pellet diet once daily to excess just prior to dusk to coincide with nocturnal feeding behaviour of *P. cygnus* is therefore recommended.

4.7 Health Monitoring Report

Health Monitoring of Western Rock Lobster During the 12-Month Temperature Feeding Frequency Trial

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4.7.1 Abstract

Post-puerulus, year 1 and 2 juvenile western rock lobsters kept under aquaculture conditions for 12 months were monitored regularly for signs of disease. Animals were sampled for histology and the prophenoloxidase and serum protein levels in the haemolymph were measured. A

trend for increasing serum protein levels with size was noted. There was no evidence that the test animals were diseased or were suffering from undue stress. The high level of reserve cells seen in the test animals was surprising. They are believed to have a storage role but their significance, in the context of aquaculture, is unknown.

4.7.2 Introduction

Invertebrates, including lobsters have a range of responses to "stress" that have been the subject of much research both in Australia and overseas. Lobsters are stressed when an external stressor causes the internal physiology to deviate from normal. Environmental changes are known to affect the immune response of lobsters, leading to an enhanced susceptibility to infectious disease agents. These immune responses are mainly initiated by haemocytes (white blood cells) in the haemolymph (blood). Lobsters, like other invertebrates, do not have red blood cells since all of the oxygen transport is via gas either bound to the protein haemocyanin in the haemolymph, or simply dissolved in the haemolymph.

Immunorecognition is thought to be mediated in the haemolymph through recognition molecules including a β-1,3-glucan-binding protein and lipopolysaccharide-binding proteins. When activated, these trigger activation of the prophenoloxidase system – a cascade of serine proteases and prophenoloxidase which in-turn initiates melanization (Söderhäll & Smith, 1986; Söderhäll *et al.,* 1996). Subsequent host defence responses comprise both cellular and humoral mechanisms involving clotting, phagocytic action mediated by lectins and opsonins, circulating antibacterial factors and release of other immunologically active molecules. Invertebrates have not been shown to exhibit acquired immunity (Roch, 1999) although proteins with domains belonging to the immunoglobulin superfamily have been demonstrated (Lanz Mendoza and Faye, 1996).

Levels of prophenoloxidase activity in lobsters are known to fluctuate in response to environmental changes and stress (Moullac and Haffner 2000) and they, together with the level of protein in the haemolymph, have been used as an indicator of lobster health (Chang, 1995; Floreto et al. 2000; Norton et al*.,* 2001, Ozbay and Riley, 2002).

As part of the project to establish post-pueruli growout data for western rock lobster, periodic health assessment was undertaken using using histology and prophenoloxidase activity as indicators of health. The results of that monitoring are reported here.

4.7.3 Methods

Visual assessments of lobster condition were undertaken weekly over the 12 month trial for post-pueruli, year 1 and year 2 post settlement juveniles. There was no evidence of fouling on the shell and gills, lesions or tail fan necrosis.

To assess the ability of lobsters to fight infection, haemolymph was subjected to prophenoloxidase (proPO) analysis (Norton et al., 2001) as follows: three post-pueruli, three year one and three year two juveniles, kept under identical conditions to experimental animals, were sampled from tanks on the following days during the 12 month trial: 13 January, 16 March, 26 May, 23 June and 14 December 2005. Each lobster was measured (weight and CL), sexed, and 1 ml of haemolymph withdrawn from the 3rd walking leg via a 21 gauge needle into a 5 ml disposable syringe containing 2 volumes of anticoagulant solution at pH 4.6 (13.15 g NaCl, 9.00 g glucose, 3.87 g Sodium citrate, 2.73 g citric acid, 1.86 g EDTA made up to 500 mL with Milli-Q water (MQ water).

The nature of the anticoagulant used is critical to the measurement of protein and the proPO system. Citrate anticoagulant has been used in other crustaceans. The modified coagulant used in the current study was optimised during a separate study us ing proPO to measure stess in lobsters (unpublished data; McKinlay 2002).

Samples were kept chilled until returned to the laboratory where they were 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. Each sample was then made up to 9 ml in a centrifuge tube with fresh buffer and centrifuged at 13,500 rpm (20 000 g) for 20 min at 4°C. Supernatant (50 µL) was pre-incubated with 50 µ1 of inducer (1 mg/mL trypsin made up in MQ water) for 30 mins at 20°C. Fifty µL of enzyme substrate L-DOPA (3 mg/mL) was added with 850 µL of MQ 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.

The sampled lobsters were then euthanased and fixed in 10% formalin in seawater for histology. Additional samples in October and December 2005, for which haemolymph was unavailable, were also examined by histology. Following dissection, the digestive glands, antennal glands, heart and mid-gut of lobsters were embedded in wax, sectioned at 6 µm and stained with haemotoxylin and eosin using standard methods.

4.7.4 Results

Results of prophenoloxidase measurement and serum protein measurement are shown in Table 1. Serum protein, but not prophenoloxidase readings, were obtained in December 2005. Serum protein levels were uniformly low in January 2005. Haemolymph protein levels increase with lobster size (Figure 1) but no such trend is evident in the prophenoloxidase activity (Figure 2). When compared to wild caught animals, the trend for high protein levels in large animals is still evident (Figure 3), but for prophenoloxidase, much higher levels of activity were measured in wild caught lobsters, though large wild caught lobsters may show the same low activity levels as cultured small lobsters (Figure 4).

Table 1. Carapace length, protein levels and prophenoloxidase activity of lobsters sampled throughout the 12-month trial. Prophenoloxidase activity expressed as change in absorbance per minute per ml of sample.

Carapace Length		Protein ug/ml	ProPO per min/ml
23 June 2005	13.8	250	0.00
	16.8	300	0.00
	17.3	250	0.04
	38.7	525	0.01
	50.5	1,225	0.27
	52.4	400	0.07
	62.5	1,550	0.28
	65.7	2,000	0.16
	67.2	1,650	0.15
13 Jan 2005	10.9	20	0.08

Carapace Length		Protein ug/ml	ProPO per min/ml
	13.7	20	0.12
	13.9	19	0.32
	35	200	0.16
	46	140	0.04
	48.2	20	0.16
	51.8	20	0.08
	53.7	80	0.04
	55.4	80	0.16
16 Mar 2005	15.31	375	0.00
	16.4	1,200	0.00
	17.02	400	0.00
	40.3	900	0.02
	49.72	2,325	0.07
	52.59	2,500	0.03
	58.09	2,525	0.19
	60.6	2,500	0.11
	68.91	2,300	0.13
26 May 2005	17.3	150	0.00
	18.5	250	0.03
	25.9	350	0.00
	54.3	3,000	0.06
	56.4	3,000	0.03
	57.4	3,000	0.31
	58.1	2,400	0.09
	58.4	3,000	0.06
	61	2,000	0.09
14-Dec-05	30	2,100	
	34.8	2,200	
	36.2	2,000	
	36.3	1,800	
	42.7	2,000	
	44.6	2,250	
	45.1	1,650	
	53.4	2,300	
	53.6	2,300	
	54.1	2,300	
	56.8	2,350	
	57.3	2,400	
	57.5	2,300	
	58.1	2,300	
	58.5	2,150	
	59.4	1,615	
	61	1,800	
	66.5	2,300	

There were no lesions observed by the histological examination that were consistent with either disease or the effects of stress. However, all of the animals sampled, in all months, showed large numbers of circulating “reserve” cells (Figures 5, 6).

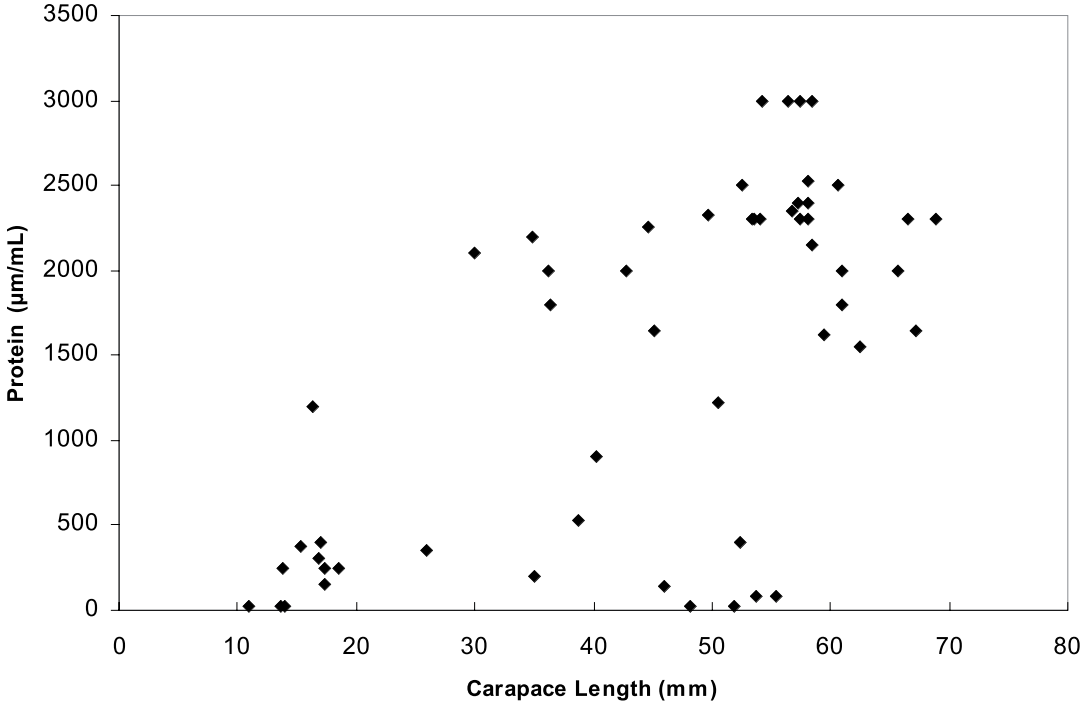

Figure 1. Relationship between lobster size and haemolymph protein levels for *Panulirus cygnus* subsampled over a 12-month growout trial.

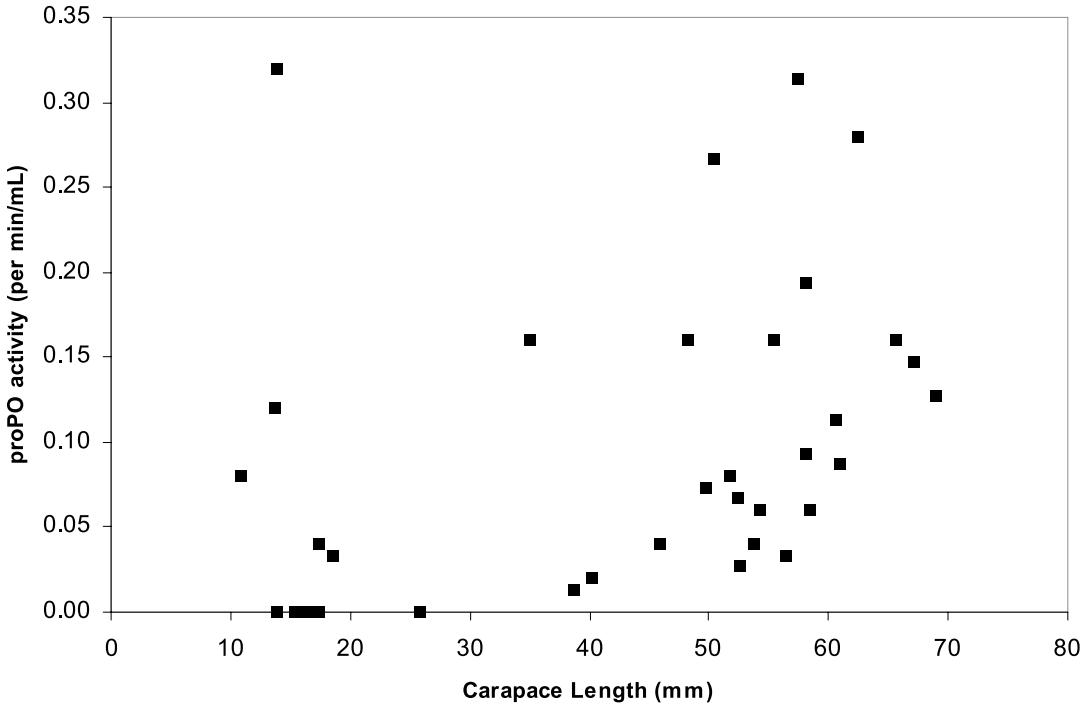

Figure 2. Relationship between lobster size and haemolymph prophenoloxidase activity for *Panulirus cygnus* subsampled over a 12-month growout trial.

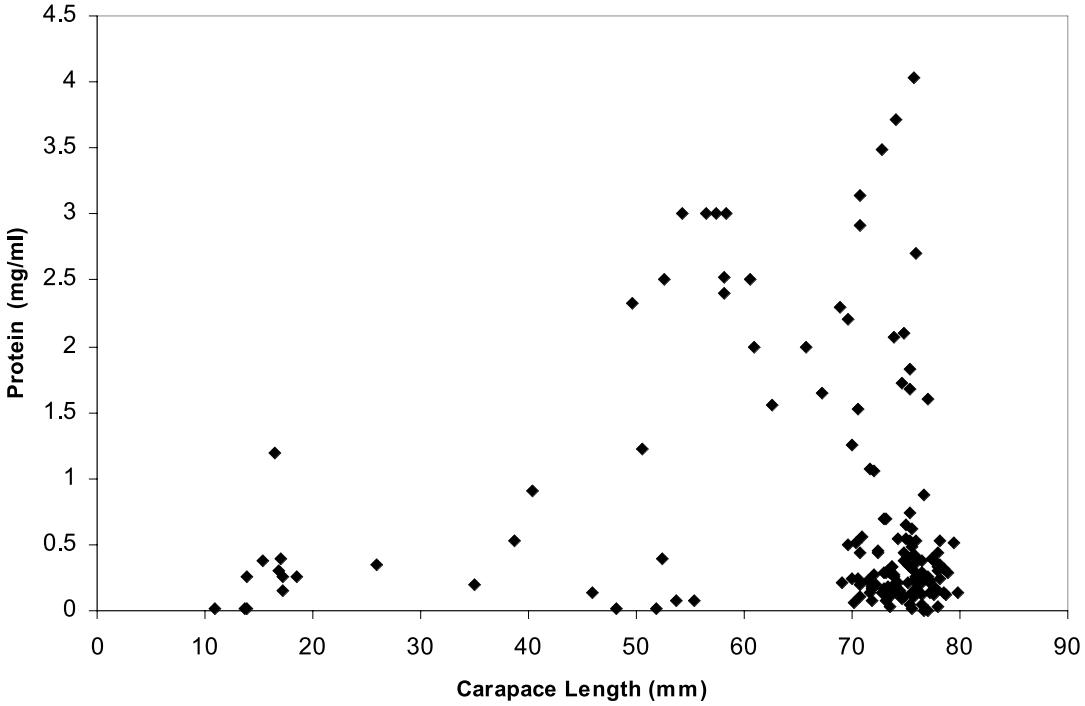

Figure 3. Relationship between lobster size and haemolymph protein levels, comparing *Panulirus cygnus* sampled during the growout trial (all <69 mm), with wild caught lobsters (all>69 mm).

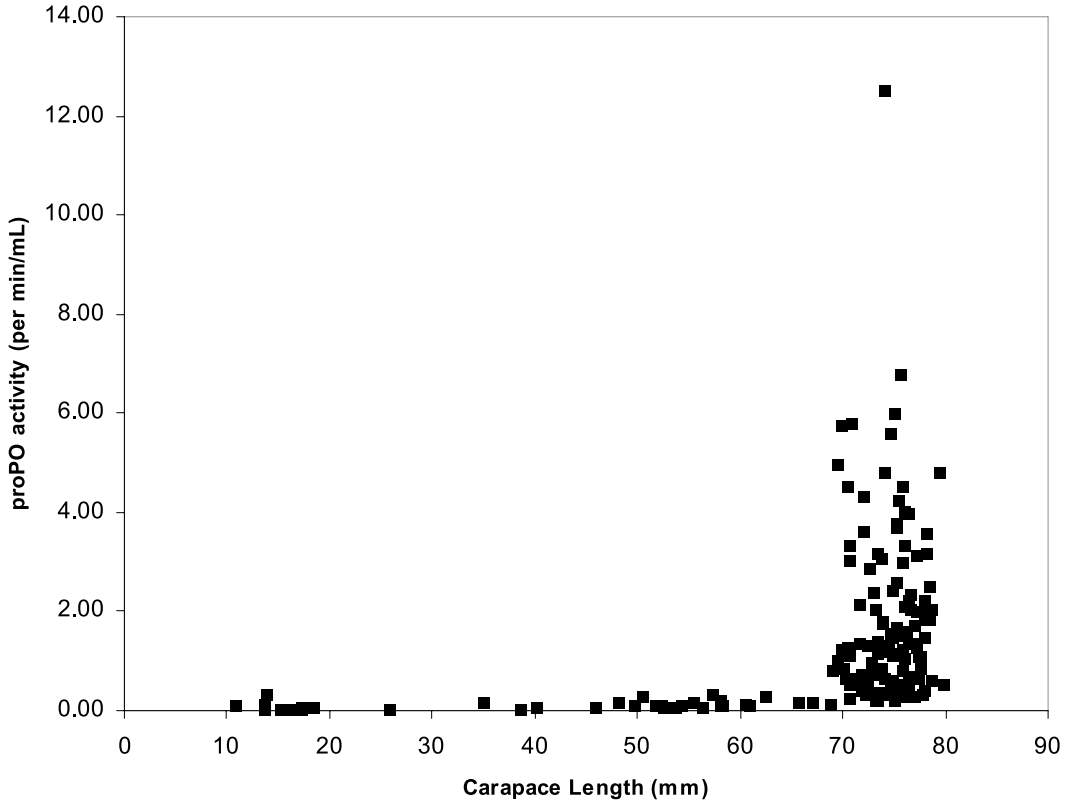

Figure 4. Relationship between lobster size and haemolymph prophenoloxidase levels, comparing *Panulirus cygnus* sampled during the growout trial (all <69 mm) and wild caught lobsters (all>69 mm).

4.7.5 Discussion

Haemolymph protein levels increased with carapace length of lobsters (Figure 1), but this trend was not evident in the proPO activity (Figure 2). This change in haemolymph protein concentration may be due to the change in inter-moult period. Haemolymph protein is known to vary in the period between pre and post molt (Mugnier and Justou, 2004), with protein concentration in the haemolymph being least after moulting due to an uptake of water after the moult, and highest prior to the moult when water is excreted. However, none of the animals in this study were sampled during the moulting phase and this therefore does not explain trends in Figure 1. The different protein levels may be related to seasonal effects but the small sample size and the changes in length over time of the sampled population has precluded this possibility being examined in detail. A similar relationship between protein levels and size of animal has been noted in shrimps (Chen and Cheng, 1993).

Within a size group, an increase in total protein concentration in the haemolymph is an indicator of short term stress (Chang, 1995; Floreto et al., 2000; Norton et al., 2001, Ozbay and Riley, 2002, Mugnier and Justou, 2004). Haemolymph proteins also fall in starved lobsters where the wasting of muscle tissue increases the hemolymph volume (Chang, 1995) and are reduced by up to 40% in animals with shell disease (Floreto et al., 2000). Jussila et al. (1999) showed that wounding also reduced haemolymph protein significantly in western rock lobster.

Prophenoloxidase activity has not been reported as showing seasonal patterns in *Carcinus maenas* (Hauton et al. 1997), but it has been shown to be affected by temperature related stress in shrimp (Vargas-Albores et al., 1998; Moullac and Haffner, 2000). It has also been shown to increase with bacterial abundance in the water column, but be inhibited by ammonia toxicity (Moullac and Haffner, 2000). Cerenius et al. (2003) showed that prophenoloxidase activity was higher in freshwater crayfish that were resistant to infection with the fungus *Aphanomyces astaci* than in those crayfish that were susceptible.

When protein levels and prophenoloxidase activity for *P. cygnus* sampled during this study are compared with laboratory records from 119 lobsters (both wild caught and on grown samples combined) it appears that, for protein, there is a trend for both samples of lobsters to have higher protein levels in the larger sized animals (Figure 3). For the prophenoloxidase activity, all of the growout animals showed low levels of activity, but the wild caught animals (> 69 mm CL) tended to show elevated levels of prophenoloxidase activity (Figure 4). High prophenoloxidase levels in wild caught lobsters may reflect the stress of capture and confinement in pots, subsequent air exposure and temperature changes immediately prior to sampling. Farmed lobsters were not subject to the same treatment.

Histological examination of the growout lobsters revealed no signs of infection or lesions due to stress. The finding of large numbers of reserve cells in all of the captive lobsters is interesting and its significance uncertain. There is little recent literature on reserve cells, or protein cells, which are believed to contain haemocyanin, calcium, iron and other substances (Johnson, 1987). Reserve cells in the numbers seen in this study are usually seen only in moulting animals. During moulting, large oval reserve cells become apparent in the connective tissue between the tubules of the hepatopancreas and contain phosphatase, mucopolysaccaride and calcium (Travis, 1957).

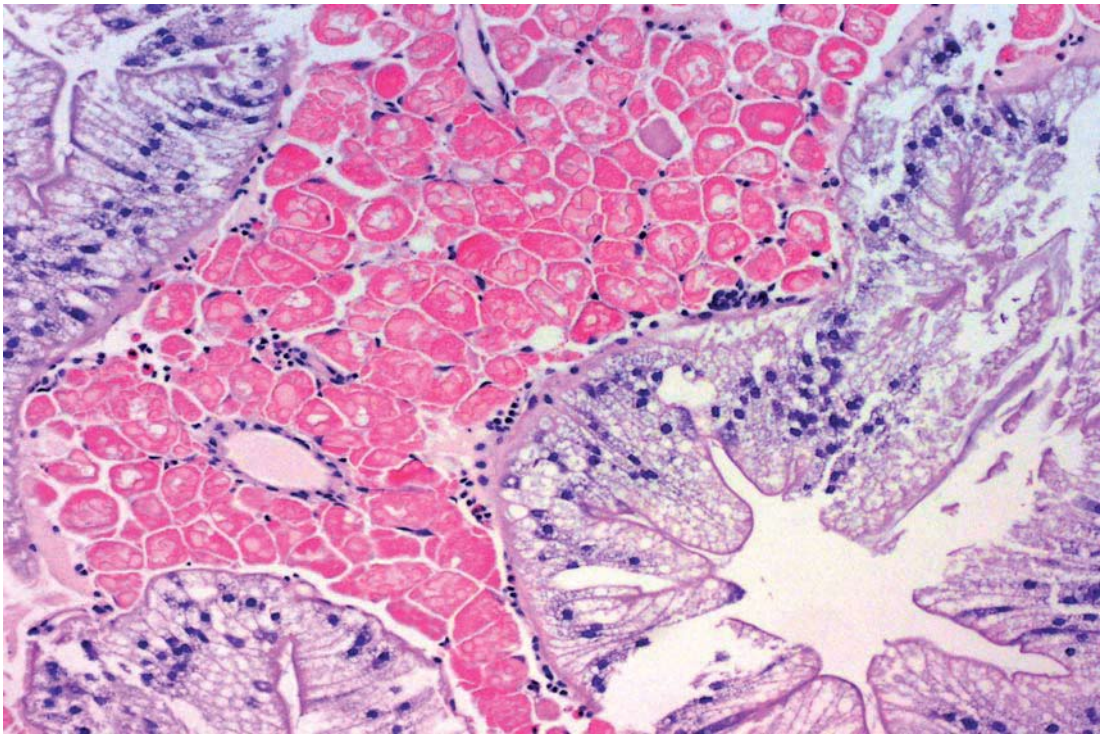

Figure 5. Transverse section through the digestive gland of *Panulirus cygnus* sampled in December 2005 showing extensive infiltration of reserve cells (pink cells) in connective tissue between tubules. Magnification 200 x.

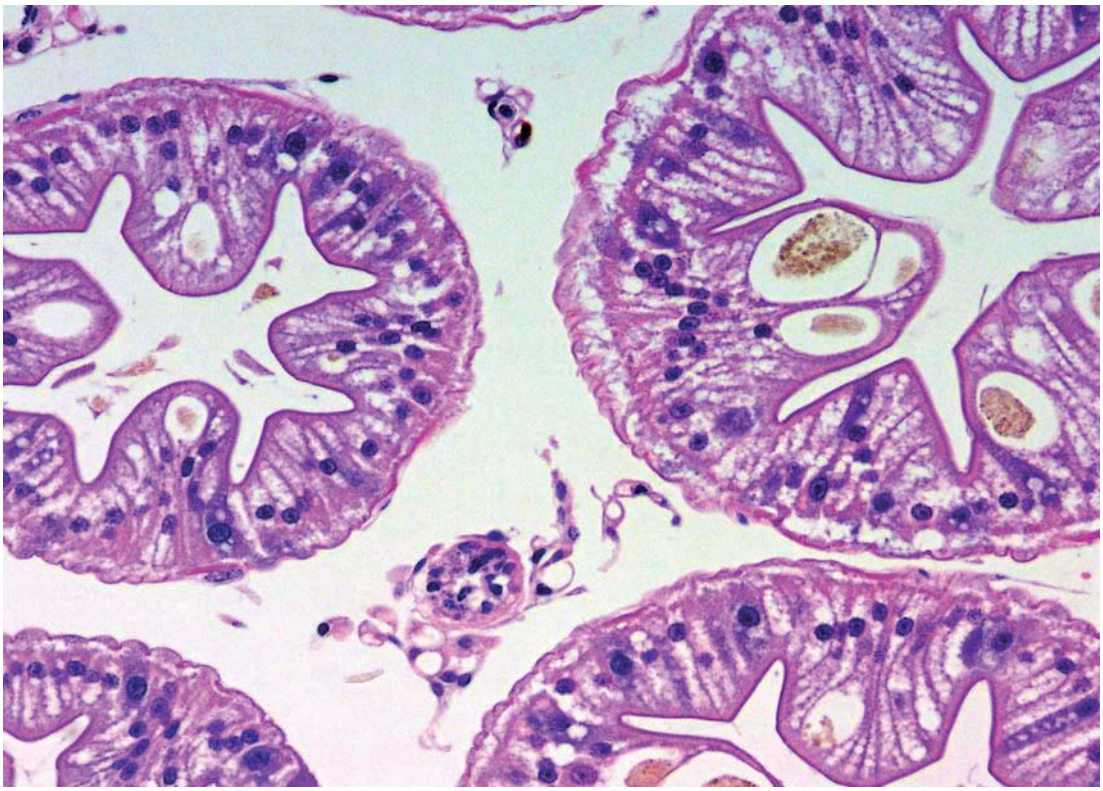

Figure 6. Transverse section through the digestive gland of a wild caught *Panulirus cygnus* showing normal digestive gland structure. Magnification 200 x.

4.7.6 Conclusions

There was no evidence that the test animals were diseased or were suffering from undue stress. The high level of reserve cells seen in the test animals was surprising. They are believed to have a storage role but their significance, in the context of aquaculture, is unknown.

5.0 Objective 3

Estimates of the expected survival rate and time required to produce a marketable sized animal from post-puerulus

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5.1 Abstract

Culturing western rock lobster at 23°C significantly reduced the time taken for both sexes to reach legal size (76 mm CL) by 1 – 2 years compared to those held at ambient Perth temperatures (2.27 – 2.47 compared to 3.57 – 4.80 years). This is a significant achievement and reveals that western rock lobster can attain a marketable size within a significantly shorter period than previously thought, demonstrating their suitability for culture. Males reached legal size faster than females due to the progressive diversion of energy by females from somatic growth into reproductive development. Survival of lobsters from post-pueruli to legal market size was 34 - 36% at 23°C and 35 - 41% at ambient temperatures. These modelled figures account for cumulative mortalities throughout the growout period and are therefore the most realistic survival data for potential operators to assess production. It is expected that experience gained from this study will lead to considerably improved survival in the future.

5.2 Introduction

Fisheries Management Paper 122 (Department of Fisheries, 1998) and Ministerial Policy Guideline 20 (Department of Fisheries, 2004), stipulate that cultured western rock lobsters will be marketed at legal size (76 mm CL). Therefore to accurately estimate expected survival and period required to produce a marketable sized lobster, a continuum of growth and survival data from puerulus settlement through to year 3+ juveniles needed to be modelled. The analysis in this chapter joins the post-pueruli, year 1 and year 2 data sets generated in the temperature feeding frequency trial (objective 2b), and by adjusting the carapace length and weight, the growth of an animal from post-pueruli through to year 3 juveniles, under experimental conditions, was modelled. The model incorporated sex (male and female) and temperature (23°C and ambient) as variables. Similarly, the expected survival rate of post-pueruli, year 1 and year 2 juveniles grown under experimental conditions, at 23°C and ambient temperatures, was modelled using logistic regression.

5.3 Methods

Weight and carapace length modelling

Firstly, the carapace length over time was adjusted for the year 1 and year 2 animals using von Bertalanffy growth curves.

To estimate what the carapace length of an animal would have been if it had lived under experimental conditions for t years, given it commenced as a zero year old (post-puerulus), the following model is used:

$$y_{t,c,m,h} = y_{t,m,h} - (y_{t1} - b_1)X_1 - (y_{t2} - b_2)X_2 \quad (1)$$

where:

- $y_{t,c,m,h}$ is the length of the animal at age *t* and sex *m* given that it started the experiment in age class *c* (PP, YR 1 or YR 2) with experimental conditions of water temperature *h*;
- t_1 (t_2) is the expected age in years of YR 1 (YR 2) animals at the start of the experiment;
- $y_{t,m,h} = a + (L_\infty + L_m\text{Male} + L_h\text{Heated})(1 - \exp(-t(k + k_m\text{Male} + k_h\text{Heated})))$ is the expected carapace length of a lobster at age *t* and sex *m* given that it had undergone experimental conditions of water temperature *h* from age $t = 0$;
- Male is a binary variable indicating the sex of the animal (1 = male, 0 = female);
- Heated is a binary variable indicating the temperature of the tank water (1 = 23°C, 0 = Ambient);
- t_i is the age in years that YR i, (i = 1,2) class animals commenced in the experiment;
- $b_{i,m,h}$ is the average carapace length of YR i animals for the particular treatment, at the start of the experiment;
- X_i , i = 1, 2, is a binary variable indicating that the animal started the experiment in age class *c* = YR 1 (i = 1) or YR 2 (i =2); and
- L_∞, L_m, L_h, k, k_m and k_h are parameters to be estimated.

The mean carapace length of YR 1 and YR 2 animals at the start of the experiment were 40 and 52 mm, respectively. Based on these, the age at which these animals were at the start of the experiment were estimated to be $t_1 = 1.4$ years and $t_2 = 2.1$ years, based on wild capture data (S. de Lestang, pers comm).

Having optimised (1) for the experimental data at hand, we adjusted the carapace length of the experimental animals to a level that might be expected if they had been grown under experimental conditions for the duration of their life i.e. from post-pueruli.

Secondly, using existing data, weight was modelled versus unadjusted carapace length using

$$W_{t,m,h} = (\alpha + \alpha_m\text{Male} + \alpha_h\text{Heated})CL_{t,m,h}^{\beta + \beta m\text{Male} + \beta h\text{Heated}} \quad (2)$$

where:

- $W_{t,m,h}$ is the weight of the animal at age *t* and sex *m* given that it started the experiment under conditions of water temperature *h*;
- $C_{t,m,h}$ is the carapace length of the animal at age *t* and sex *m* given that it started the experiment under conditions of water temperature *h*; and
- α, α_m, α_h, β, β_m, β_h, are parameters to be estimated.

Having solved (2) the adjusted weight was calculated for each animal making use of the

adjusted carapace length. In this way, the growth data of animals as though they had been grown all the way through the experiment, could be simulated.
Modelling the simulated data, the expected weight of a lobster at age t, given the animal has lived under experimental treatment since post-pueruli, is given by:

$$W_{t,m\,h} = \exp\left(a_{m,h}\left(1-e^{-b_{m,h}t}\right)^{d_{m,h}}\right)-1 \tag{3}$$

The inverse of the weight equation is given by:

$$t = -\frac{1}{a_{m,h}}\mathrm{h}\left(1-\sqrt[a_{m,h}]{\frac{\log_e(W_t+1)}{a_{m,h}}}\right) \tag{4}$$

For completeness, the expected carapace length of a lobster, assuming that it has lived under experimental conditions since post-pueruli, is given by:

$$CL_{t,m,h} = a_{m,h} + L_{\infty,m,h}(1-e^{-k_{m,h}t}) \tag{5}$$

The inverse of the carapace length equation is given by:

$$t = -\frac{1}{k_{m,h}}\ln\left(1-\frac{CL_{\infty,m,h}-a_{m,h}}{L_\infty}\right) \tag{6}$$

Survival analysis

Survival of animals at *s* years into the experiment, given the animal started the experiment in age class *c* and was subjected to water heated *h*, were analysed using a logistic regression:

$$S_{c,s,h} = \frac{2e^{a_{c,h}s}}{1+e^{a_{c,h}s}} \tag{7}$$

The standard error for this estimate is given by:

$$s.e.\left(S_{c,s,h}\right) = \frac{1}{1.96}\left(\frac{2e^{b_{c,h}s}}{1+e^{b_{c,h}s}} - \frac{2e^{a_{c,h}s}}{1+e^{a_{c,h}s}}\right) \tag{8}$$

5.4 Results

Weight and Carapace Length Modelling

The simulated weight and age data for the different treatments are presented in Figures 1-4.

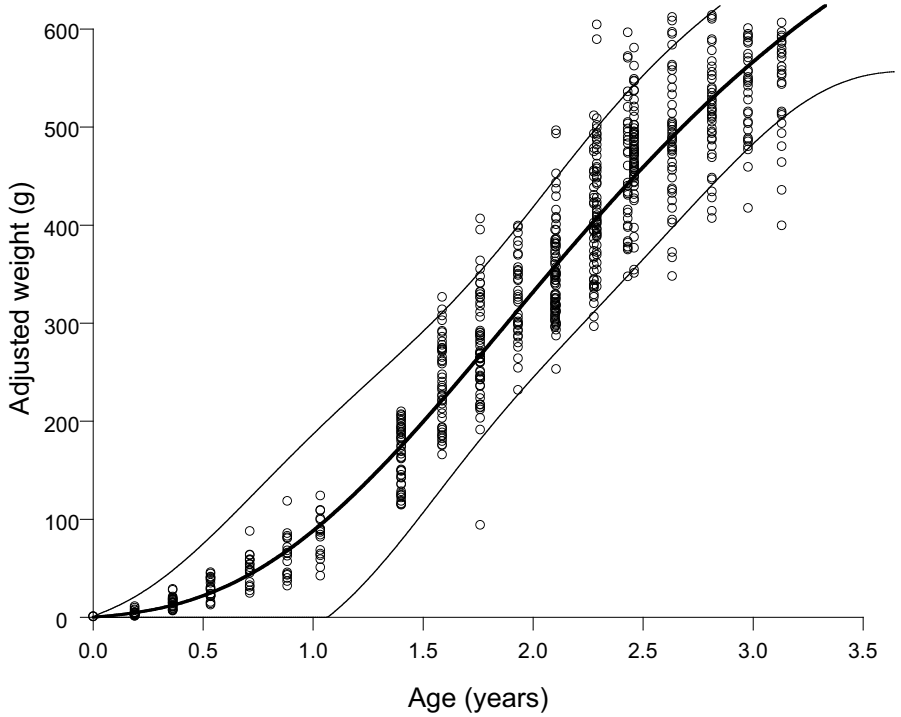

Figure 1. The adjusted weight (grams) of *P. cygnus* assumed to have been in the trial for the duration of their life, given the animals were male and kept in heated water at 23°C. 95% confidence bounds are also included.

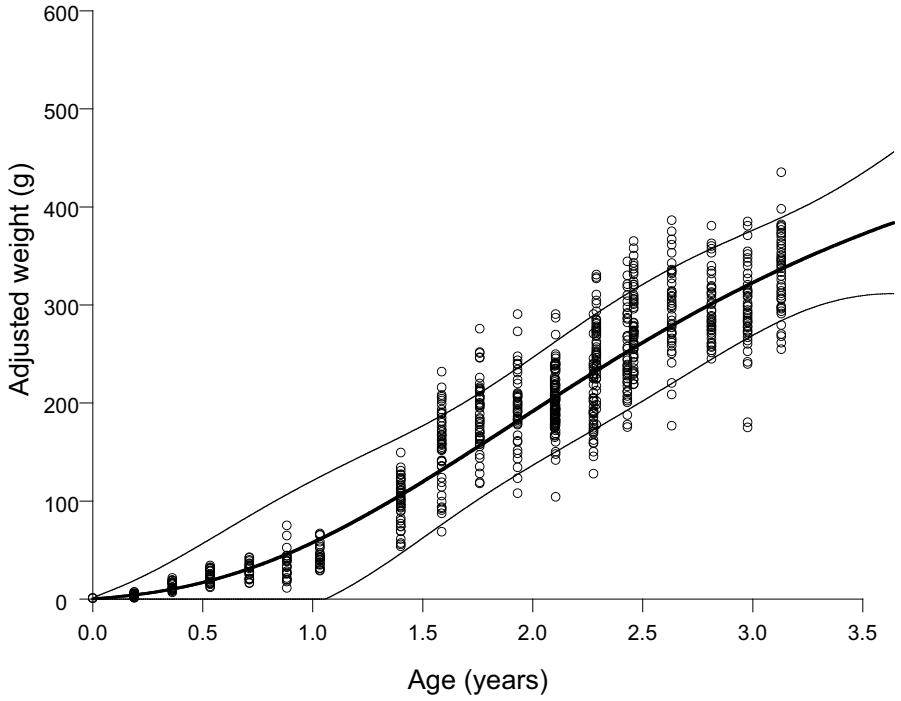

Figure 2. The adjusted weight (grams) for *P. cygnus* assumed to have been in the trial for the duration of their life, given the animals were male and kept in ambient water. 95% confidence bounds are also included.

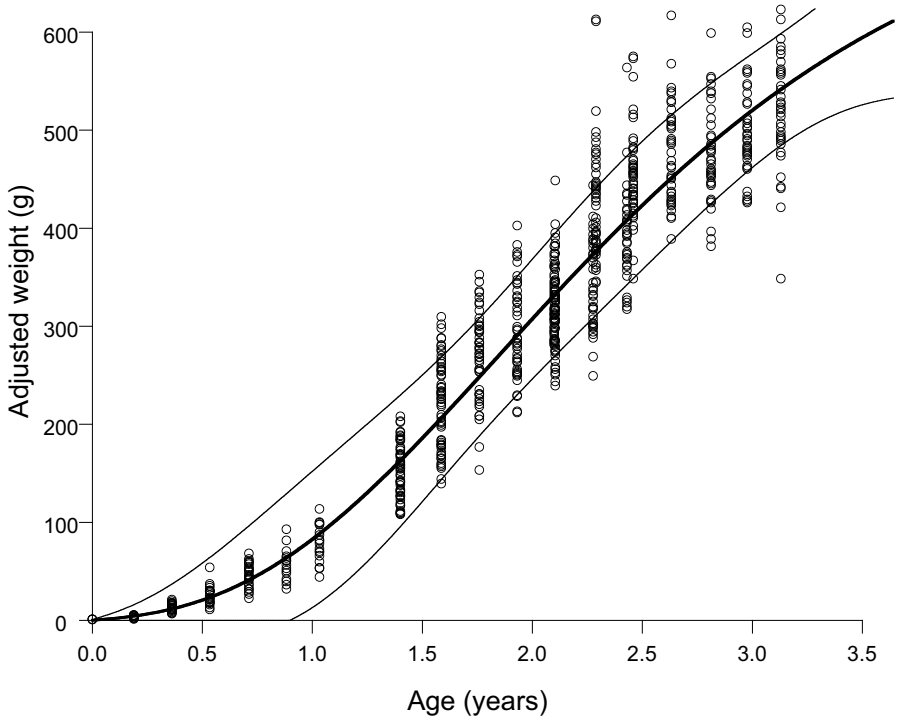

Figure 3. The adjusted weight (grams) for *P. cygnus* assumed to have been in the trial for the duration of their life, given the animals were female and kept in heated water at 23°C. 95% confidence bounds are also included.

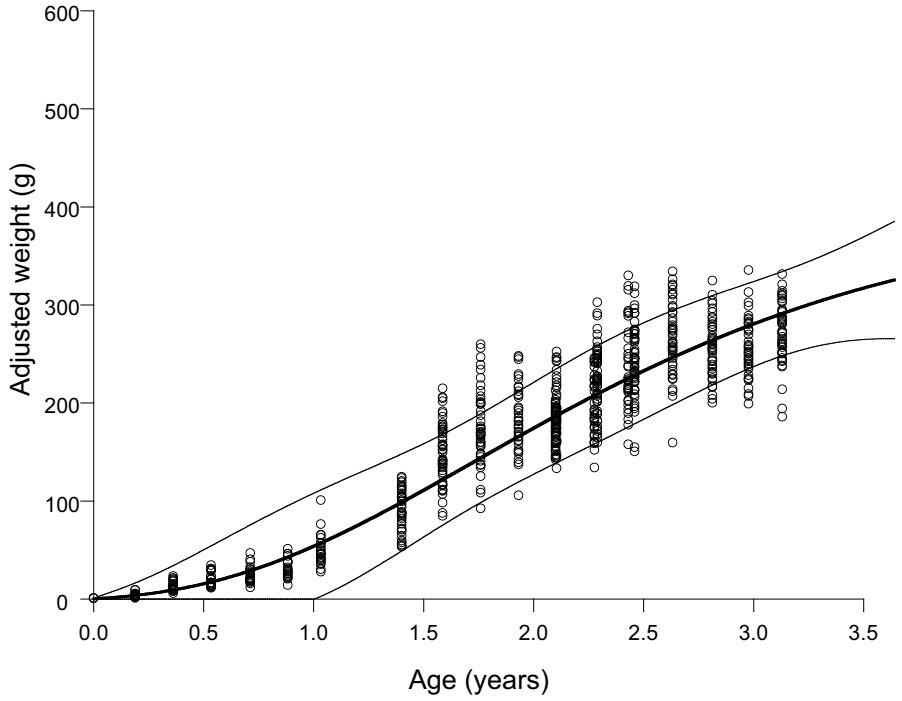

Figure 4. The adjusted weight (grams) for *P. cygnus* assumed to have been in the trial for the duration of their life, given the animals were female and kept in ambient water. 95% confidence bounds are also included.

The coefficients for modelling the weight of a lobster that has lived under experimental conditions since post-pueruli, are given in Table 1.

Table 1. Coefficients for the expected weight of a lobster grown under experimental conditions over time. The standard error for each coefficient is included in brackets.

Sex	Temperature	Coefficient		
		$a_{m,h}$	$b_{m,h}$	$d_{m,h}$
Male	23°C	6.74 (0.05)	0.83 (0.04)	0.71 (0.02)
Male	Ambient	6.23 (0.06)	0.74 (0.04)	0.66 (0.02)
Female	23°C	6.64 (0.05)	0.85 (0.03)	0.73 (0.02)
Female	Ambient	6.00 (0.05)	0.83 (0.04)	0.70 (0.02)

The coefficients for modelling the carapace length of a lobster that has lived under experimental conditions since post-pueruli, are given in Table 2.

Table 2. Coefficients for the expected carapace length of a lobster grown under experimental conditions over time. The standard error for each coefficient is included in brackets.

Sex	Temperature	Coefficient		
		$a_{m,h}$	$L_{oo,m,h}$	$k_{m,h}$
Male	23°C	8.74 (0.42)	113.4 (1.96)	0.39 (0.01)
Male	Ambient	8.74 (0.42)	84.3 (1.49)	0.45 (0.01)
Female	23°C	8.74 (0.42)	103.0 (1.59)	0.43 (0.01)
Female	Ambient	8.74 (0.42)	73.4 (1.12)	0.52 (0.01)

Survival analysis

The coefficients for each of the year classes, for different tank temperatures, are presented in Table 3 and the results are presented graphically in Figures 5 - 12.

Table 3. Coefficients for the mean survival curve and the standard error for the estimate, for each year class and temperature. The standard error for each parameter estimate is included in brackets.

Year Class (c)	Heated (h)	Survival parameter a	Standard error parameter b
Post-pueruli	Ambient	-0.0014 (0.0002)	-0.0010 (2.19E-8)
	23°C	-0.0021 (0.0002)	-0.0017 (1.42E-7)
Year 1	Ambient	-0.0019 (0.0001)	-0.0017 (4.08E-8)
	23°C	-0.0023 (0.0001)	-0.0020 (7.92E-8)
Year 2	Ambient	-0.0006 (0.0001)	-0.0004 (1.04E-8)
	23°C	-0.0013 (0.0001)	-0.0011 (3.31E-8)

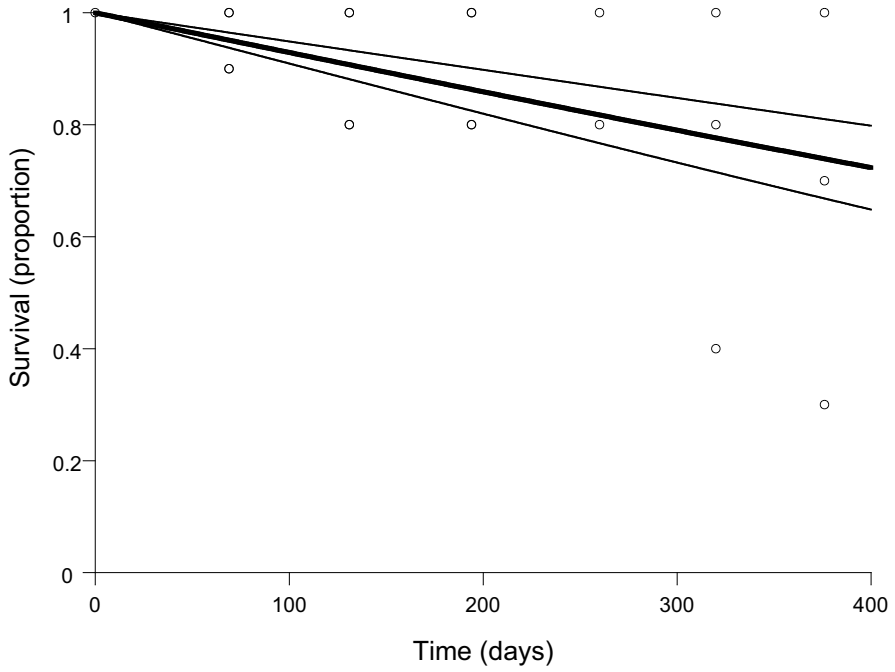

Figure 5. Survival rate of *P. cygnus* post-pueruli over time when held at ambient temperatures. A 95% confidence interval is also included.

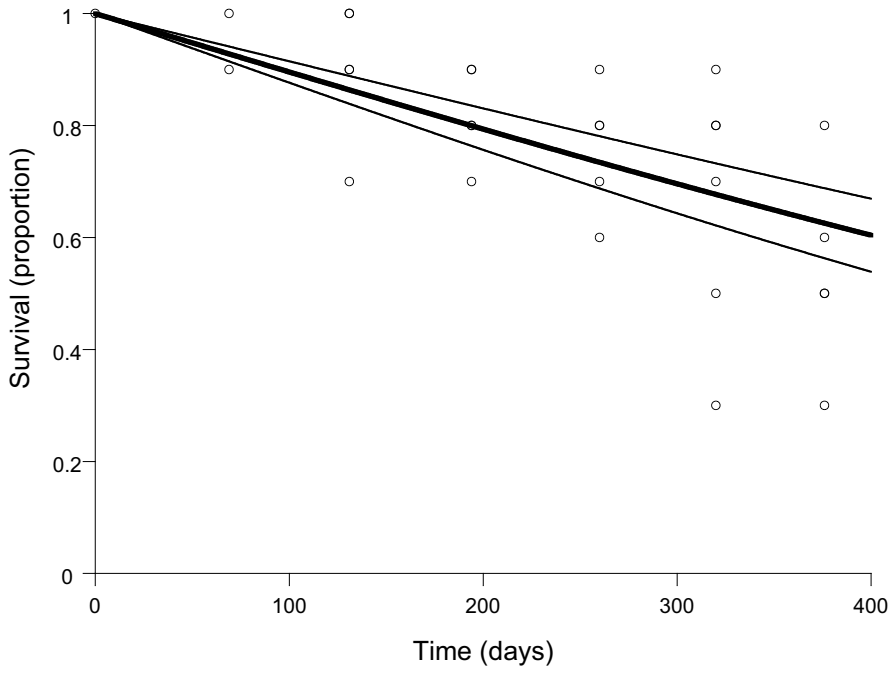

Figure 6. Survival rate of *P. cygnus* post-pueruli over time when held at 23°C. A 95% confidence interval is also included.

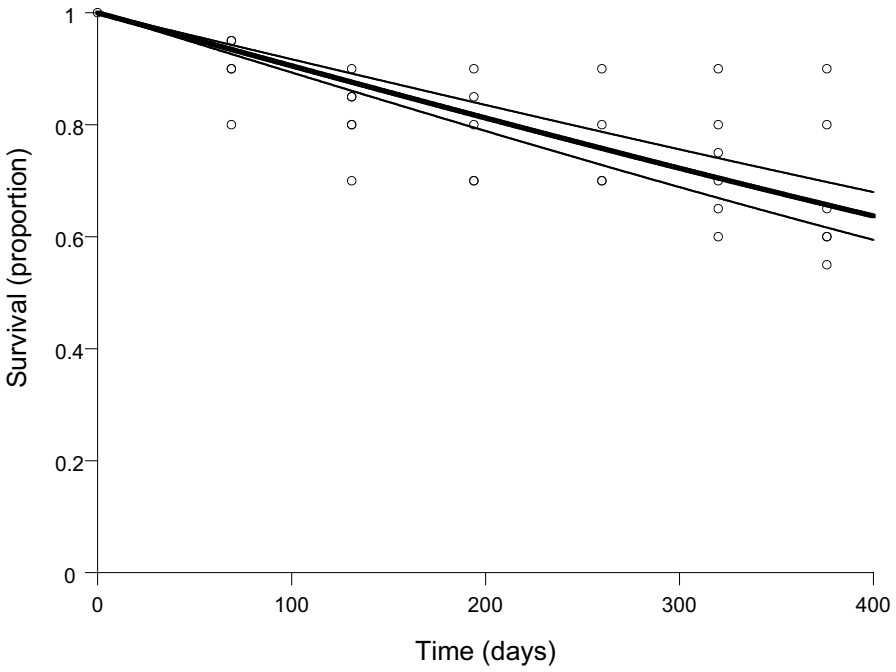

Figure 7. Survival rate of *P. cygnus* year 1 juveniles over time when held at ambient temperatures. A 95% confidence interval is also included.

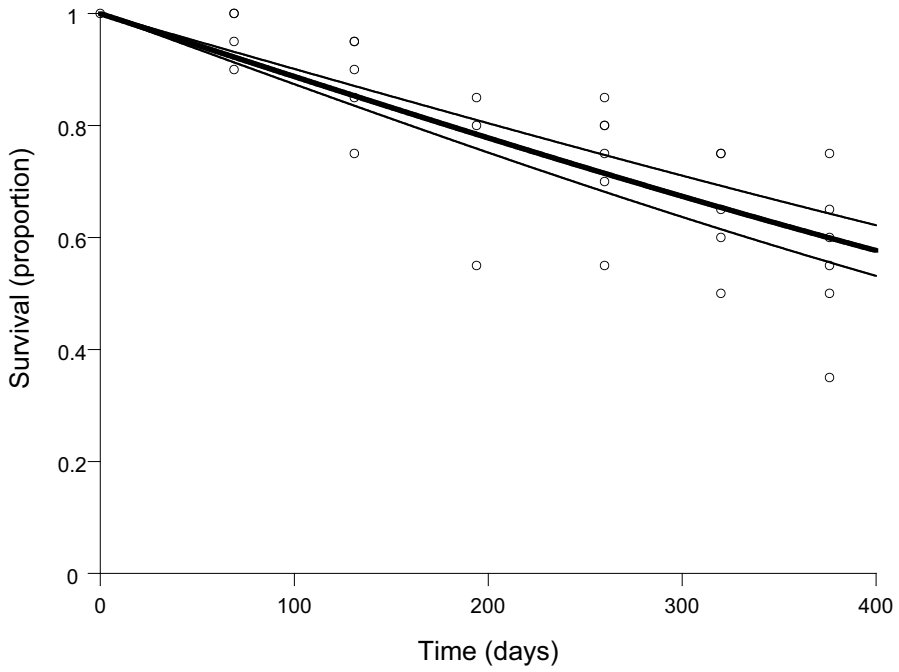

Figure 8. Survival rate of *P. cygnus* year 1 juveniles over time when held at 23°C. A 95% confidence interval is also included.

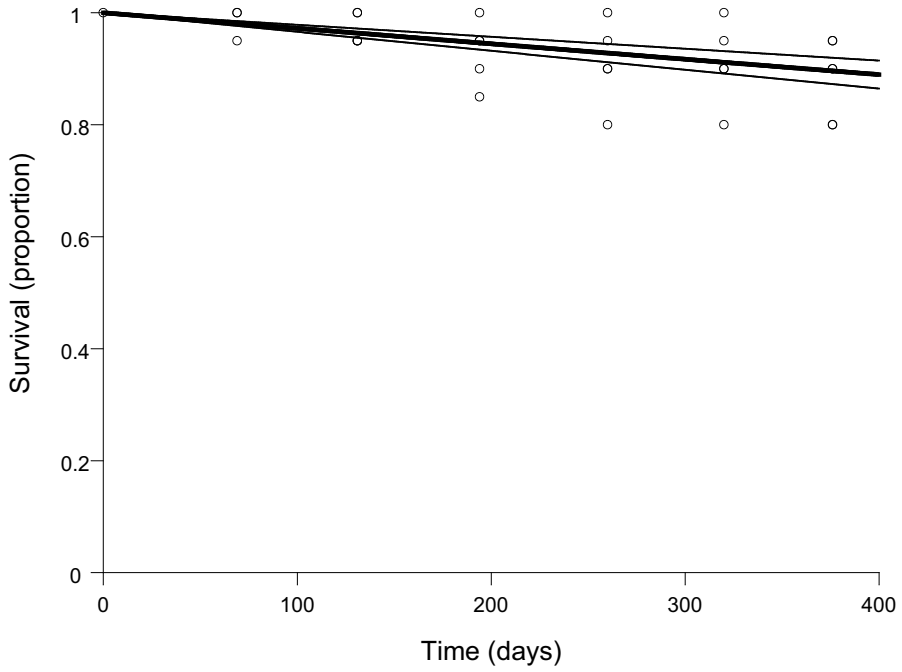

Figure 9. Survival rate of *P. cygnus* year 2 juveniles over time when held at ambient temperatures. A 95% confidence interval is also included.

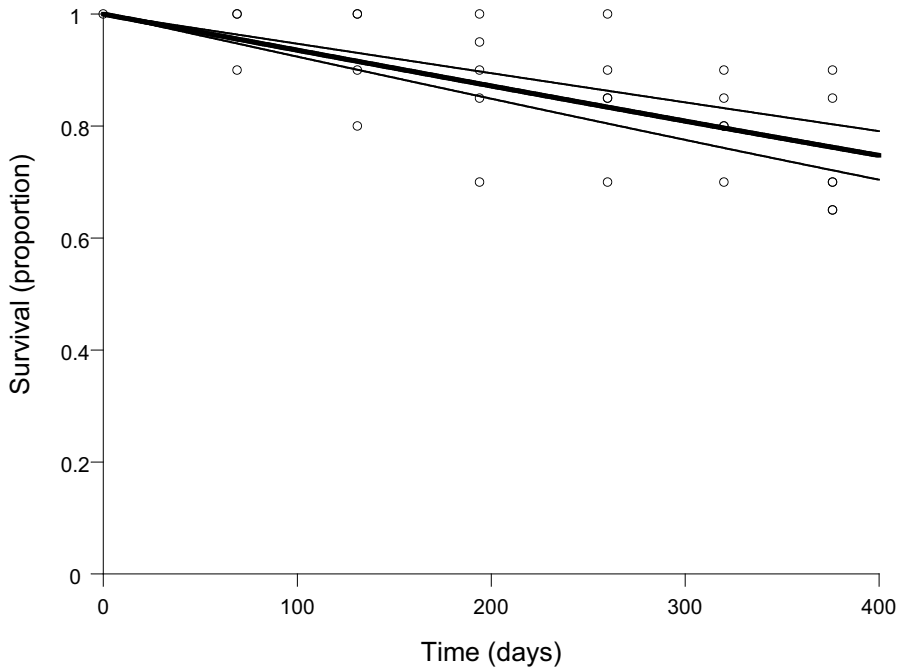

Figure 10. Survival rate of *P. cygnus* year 2 juveniles over time when held at 23°C. A 95% confidence interval is also included.

Estimation of Lobster Weight and Survival at Market (Legal) Size

Given the presented relationships of carapace length, weight and survival of animals to age, the age to expected market (legal) size and hence growout period of *P. cygnus* held at ambient and 23°C temperatures is presented in Table 4, as well as the expected weight of the animal and the probability of surviving to this stage.

Table 4. The age to expected legal size (76mm CL) under different water temperature conditions, by sex, for animals reared under experimental conditions from post-pueruli. The expected weight of these legal animals is also presented as well as the probability of an animal surviving to legal size.

Sex	Temperature	Age to legal size (years)	Weight at legal size (grams)	Probability of surviving to legal size
Male	23°C	2.27	402.2	0.36
Male	Ambient	3.57	378.1	0.41
Female	23°C	2.47	416.8	0.34
Female	Ambient	4.80	370.6	0.35

5.5 Discussion and conclusions

Culturing western rock lobster at 23°C significantly reduces the time it takes for males and females to reach legal size (2.27 – 2.47 years), which is 1-2 years less than when held at ambient Perth temperatures (3.57 – 4.8 years). As expected, males reached legal size faster than females due to the progressive diversion of energy by females from somatic growth into reproductive development. As the economic viability of culture operations depends on achieving market size in the shortest time possible, it is clear that western rock lobster will need to be cultured at average water temperatures of 23°C. Achieving legal size within 2.27 years (males) and 2.47 years (females) indicates that growth rates achieved in tanks at 23°C are substantially faster than in the wild, where legal size is achieved around 4 years post settlement (Chittleborough, 1974a). This is a significant achievement and reveals that western rock lobster can attain legal (market) size within a significantly shorter period than previously thought; further demonstrating their suitability for culture. Nevertheless, a growout period of between 2.27 years (males) and 2.47 years (females) is long compared with other cultured crustacean species such as the tropical rock lobster *Panulirus ornatus* (1 kg in 18 months, Williams 2006) and marine prawns *Penaeus monodon* (5-8 months). It remains to be seen whether this comparatively lengthy growout period will be profitable, or whether cultured western rock lobster will need to be marketed below legal size (< 76 mm CL) to be economically viable, noting that this is not currently permitted (Department of Fisheries, 2006)

Survival of lobsters from post-pueruli to legal market size was 34 - 36% at 23°C and 35 - 41% at ambient temperatures. These modelled figures account for cumulative mortalities throughout the growout period and are therefore much lower than survivals reported for each size class after 12 months (see chapter 4). Consequently, it is difficult to compare these data with other studies, as the majority have reported survival of lobsters during relatively short term culture trials (<6 months). These survival data are the first available for *P. cygnus* over the entire growout period from settlement to legal size and are therefore the most realistic data for potential operators to assess production. It is expected that experience gained from this study will lead to considerably improved survival statistics in the future.

6.0 Objective 4

Provide biological data to assist in assessing the economic potential for growing out western rock lobsters from post-puerulus to marketable size

Data generated for objectives 1, 2a and 2b on the growth, survival, feed intake, food conversion and health of post-pueruli, year 1 and year 2 juveniles under various conditions of density, shelter, temperature, feed frequency and flow rate will be pivotal in assessing the economic potential for growing western rock lobster to market size. Each trial provided crucial biological information that potential investors would need to grow lobsters successfully from recently settled pueruli to 3 year old animals in terms of optimal conditions for maximising growth and survival. These conditions are listed below:

Flow rates: which showed that western rock lobsters are amenable to culture in tanks with reduced flow rates and can tolerate relatively high ammonia levels;

Density: which showed that the species survives well at relatively high stocking densities. Of the two stocking levels examined, maximum survival and production was achieved for post-pueruli at 50 m^{-2} and at 20 to 25 m^{-2} for year 1 and 2 juveniles;

Shelter type: showed significantly better survival and growth of animals held in mesh compared to brick shelters;

Temperature: showed that western rock lobsters grew faster at 23°C than at ambient temperatures;

Feed frequency showed that there is no benefit in feeding western rock lobsters multiple times per night in terms of improving their growth and survival;

Diet: a diet regime of formulated pellet (Smith et al., 2005) supplemented with fresh blue mussels (*Mytilis edulis*) achieved similar growth rates of wild lobsters, but research will be needed to improve the pellet formulation and remove the need for the costly mussel supplement;

Recommendations for key parameters examined in Objectives 1, 2a and 2b have been outlined in this and previous milestone reports, and provide potential investors with a solid foundation upon which to base commercial trials. Results from Objective 3 provide an estimation of the size, survival rate and period required to produce a marketable legal sized lobster under varying conditions of temperature (23°C or ambient), for males and females. This data suggests that *P. cygnus* males held at 23°C can potentially reach legal size within 2.27 years with an estimated 36% survival rate, whereas females can potentially reach legal size within 2.47 years with an estimated 34% survival rate. At ambient temperatures males and females take considerably longer (3.57 - 4.8 years) but have marginally higher rates of survival (~38%). An economic analysis to assess the viability of *P. cygnus* growout was not within the scope of this project. However, this information will be vital in assisting potential investors to assess the economic viability of each scenario (males vs females, at ambient vs 23°C temperatures) under the various culture parameters recommended in this report. It is likely that for *P. cygnus* culture to be economically viable, lobsters will need to be held at mean temperatures of 23°C to ensure that market (legal) size is achieved in a cost effective time.

7.0 Benefits

This project has generated accurate and reliable extended datasets on the growth and survival of western rock lobster from post-pueruli to market sized lobsters under various conditions of culture. Sectors of the industry and community that will benefit from the data generated in this project include aquaculturalists and those in the wild fishery that might consider investing in a western rock lobster aquaculture industry, based on the collection and ongrowing of pueruli. Other beneficiaries would be the national and international research community. This project will make a particularly significant impact on potential investors as it provides a foundation on which to base commercial scale growout which was not previously available for this species.

During the course of this project there have been several discussions with potential investors interested in western rock lobster culture. These investors have seen the significant potential of *P. cygnus* as an aquaculture candidate and are consequently interested in both growout of post-pueruli and seacage culture/ranching of legal sized lobsters for out of season. One applicant has applied for a commercial licence to collect and growout pueruli and their request is currently being processed by the Department of Fisheries. Based on interest that has been received over the life of the project we believe there will be further growout applications. Interest in the potential of this form of aquaculture is to a large extent testimony to the success of this project.

8.0 Further development

This project examined a range of parameters to assess the potential of *P. cygnus* for culture. Although many key biological characteristics indicate that *P. cygnus* is an excellent aquaculture candidate, there are a number of areas that require further research before culture of this species becomes an economic reality:

1. A cost effective formulated diet that is specific for *P. cygnus* needs to be developed. The diet used in these trials was a combination of a formulated pellet developed for *Panulirus ornatus* (Smith et al., 2005) supplemented with fresh mussels two days per week. Although this achieved excellent survival and growth after long periods in captivity (6 – 12 months), mussels are expensive and are unlikely to be cost effective on a commercial basis. Although a cost benefit analysis to assess the frequency with which mussels can be provided without impacting heavily on profitability of commercial operations could be conducted, it is clear that a pellet diet is the only feasible option in the long term. Trials conducted outside this FRDC project revealed that the *P. ornatus* pellet diet significantly reduced growth of *P. cygnus* after six months indicating that its nutritional composition is unsuitable, or that feed intake is unacceptable due to poor palatability. Nutritional studies on formulated diets specific for *P. cygnus* are clearly required using the *P. ornatus* diet as a template to improve the palatability, feed intake and nutritional composition.
2. Commercial scale trials need to be conducted to determine appropriate tank specifications and design for commercial culture. This project used small scale production tanks suited to replicated experimental trials. Although this was appropriate for assessing biological parameters for optimising production (survival and growth) it is clear that commercial scale

trials are required to validate whether large scale *P. cygnus* culture is successful. These trials would also provide the opportunity to develop tanks that are easy to clean, thereby reducing labour costs.

3. There is a need to reduce mortality rates during culture. This may be related to item 1 (above).
4. A comprehensive economic analysis is required to validate whether *P. cygnus* growout will be economically viable. There is a substantial amount of *P. cygnus* growout data dating back to the 1970s which could be utilised to assess the production costs and profitability of a range of potential culture scenarios. Such analyses will be vital in assisting potential investors in determining the most cost effective growout period, market size, feeding regime, tank configuration and product price for achieving the greatest profit margins.
5. Government policy outlining puerulus resource allocation and access is required. There is considerable concern by industry that they will lose access to the puerulus resource and that removal of puerulus may have an impact on recruitment into the fishery. Hence it is essential that a policy is developed to address these issues. Commencement of the process to review the existing policy arrangements (Department of Fisheries, 1998; 2004) that underpin the allocation, harvesting and ongrowing of western rock lobster pueruli has been initiated by the Aquaculture and Pearling Branch of the Department of Fisheries through the release of a scoping paper (Department of Fisheries, 2006).
6. There is no research effort going into the propagation of western rock lobsters. This is because if commercial scale propagation of rock lobsters eventuates, then the future of growing out lobsters would be likely to be economically more viable with a fast growing tropical species such as *Panulirus ornatus*, than with western rock lobsters.

9.0 Planned outcomes

This research project was a preliminary investigation into the feasibility of producing western rock lobster in onshore flow through systems. As has already been noted, there is commercial interest in ongrowing western rock lobster post-pueruli to a marketable size, but basic data on the growth and survival rates of western rock lobster under a range of culture scenarios was not available. This project has addressed this information gap and information generated has directly benefited aquaculturists interested in the development potential of this new industry.

Planned outcomes that have been achieved include data on the following fundamentally important parameters for maximising survival, growth and production of post-pueruli, year 1 juveniles and year 2 juveniles in flow through onshore systems:

- Optimum flow rates
- Tolerance limits for ammonia concentrations
- General water quality conditions
- Optimum stocking densities
- A novel shelter design for maximising survival
- Appropriate water temperatures for maximising growth
- Appropriate feeding frequency
- Appropriate diet and feeding regime
- Expected feed intake and feed conversion ratios
- Tank configurations suited to small scale production
- Biochemical profiles of the experimental animals

10.0 Conclusions

This project has provided important biological information (growth rates, survival, feed intake, feed conversion, health) on a range of key parameters (flow rates, densities, shelter type, temperature, feed frequency) to assist potential investors in assessing the economic potential of *P. cygnus* culture. Data has indicated that *P. cygnus* appears well suited to culture. This species can tolerate low flow rates and relatively high ammonia concentrations, and exhibits very good survival at high stocking densities (up to 100 m^{-2}) without adverse impacts on growth or captivity related health problems. However, overall survival in the culture period (< years) needs to be improved. Growth can be substantially improved by elevating water temperatures to 23°C, particularly in the first year post-settlement (post-pueruli), without significant reductions in survival or negative impacts on health. Lobsters of both sexes held at 23°C can reach legal size (76 mm CL) one to two years faster than those held at ambient temperatures (2.27 – 2.47 versus 3.57 – 4.8 years). Feed conversion of formulated pellet diets is acceptable and comparable with other rock lobster species.

These results indicate that there may be significant potential for commercial growout of *P. cygnus*. However, a number of biological, economic and political hurdles must be overcome for this to become a reality. Research is required to develop a cost effective nutritionally complete formulated diet for this species and commercial scale trials are needed to streamline production. This may also contribute to improving overall survival rates. An economic analysis of various culture scenarios is needed and Government policy on puerulus allocation and collection need to be completed. If these issues can be resolved it is likely that commercial culture of *P. cygnus* will be embraced and developed similar to that of other species such as *Panulirus ornatus* and *Panulirus argus,* where industries are either developing or are established (e.g. Malaysia, Vietnam and the British Virgin Islands). The significant potential of *P. cygnus* demonstrated here suggests that this species will be keenly observed by interested investors in the future.

11.0 References

Allan, G.L, Maguire, G.B. and Hopkins, S.J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved-oxygen levels. Aquaculture **91**, 265-280.

Atema, J.and Cobb, J.S., 1980. Social behaviour. In: Cobb, J.S., Phillips, B.F. (Eds.), The Biology and Management of Lobsters Vol. 1. Academic Press, New York. pp. 409-450.

Barclay, M.C., Irvin, S.J., Williams, K.C. and Smith, D.M., 2006. Comparison of diets for the tropical spiny lobster *Panulirus ornatus*: astaxanthin-supplemented feeds and mussel flesh. Aquacult. Nutr. **12**, 117-125.

Beamish, F.W.H. and Tandler, A., 1990. Ambient ammonia, diet and growth in lake trout. Aquat. Toxicol. **17**, 155-166.

Berrill, M., 1976. Aggressive behaviour of post-puerulus larvae of the western rock lobster *Panulirus longipes* (Milne-Edwards). Aust. J. Mar. Freshwater Res. **27**, 83-88.

Booth, J.D. and Kittaka, J., 2000. Spiny lobster growout. In: Phillips, B.F., Kittaka, J. (Eds.), Spiny Lobster Management. Fishing News Books. Oxford, United Kingdom, pp. 556-585.

Bower, C.E.and Bidwell, J.P., 1978. Ionisation of ammonia in seawater: effects of temperature, pH and salinity. J. Fish. Res. Brd. Can. **35**, 1012-1016.

Brunsen, J.F., Romaire, R.P. and Reigh, R.C., 1997. Apparent digestibility of selected ingredients for white shrimp *Penaeus setiferus* L. Aquacult. Nutr. **3**, 9-12.

Butler, M.J. and Herrnkind, W.F., 1997. A test of recruitment limitations and the potential for artifical enhancement of spiny lobster (*Panulirus argus*) populations in Florida. Can. J. Fish. Aquat. Sci. **54**, 452-63.

Cerenius, L. Bangyeekhun, E., Keyser, P., Söderhäll, I. and Söderhäll, K., 2003. Host prophenoloxidase expression in freshwater crayfish is linked to increased resistance to the crayfish plague fungus *Aphanomyces astaci*. Cell. Microbiol. **5**, 353-357.

Chang, E.S., 1995. Physiological and biochemical changes during the moult cycle in decapod crustaceans: an overview. J. Exp. Mar. Biol. Ecol. 193, 1-14.

Chen, J-C. and Cheng, S.-Y., 1993. Studies on haemocyanin and haemolymph protein levels of *Penaeus japonicus* based on sex, size and moulting cycle. Comp. Biochem. Physiol. B. **106**, 293-296.

Chittleborough, R.G., 1970. Studies on recruitment in the Western Australian rock lobster *Panuliris longipes cygnus* George: density and natural mortality of juveniles. Aust. J. Mar. Freshwater Res. **21**, 131-148.

Chittleborough, R.G., 1974a. Western rock lobster reared to maturity. Aust. J. Mar. Freshwater Res. **25**, 221-225.

Chittleborough, R.G., 1974b. Review of prospects for rearing rock lobsters. Aust. Fish. **33**, 1-5.

Chittleborough, R.G., 1975. Environmental factors affecting growth and survival of juvenile western rock lobsters *Panulirus longipes* (Milne-Edwards). Aust. J. Mar. Freshwater Res. **26**, 177-196.

Chittleborough, R.G. and Phillips, B.F., 1975. Fluctuations of year class strength and recruitment in the western rock lobster *Panulirus longipes* (Milne-Edwards). Aust. J. Mar. Freshwater Res. **26**, 317-328.

Chittleborough, R.G., 1976. Growth of juvenile *Panulirus longipes cygnus* George on coastal reefs compared with those reared under optimal environmental conditions. Aust. J. Mar. Freshwater Res. **27**, 279-295.

Cox, S.L. and Davis, M., 2006. The effect of feeding frequency and ration on growth of juvenile spiny lobster *Panulirus argus* (Palinuridae). Applied Aquaculture 18(4), In Press.

Cox, S.L. and Johnston, D.J., 2003. Feeding biology of spiny lobster larvae and implications for culture. Rev. Fish. Sci. **11**, 89-106.

Crear, B.J., Thomas, C.W., Hart, P.R. and Carter, C.G., 2000. Growth of juvenile southern rock lobsters, *Jasus edwardsii*, is influenced by diet and temperature, whilst survival is influenced by diet and tank environment. Aquaculture **190**, 169-182.

Crear, B.J and Forteath, G.N.R., 2001. Flow rate requirements for captive western rock lobsters (*Panuliris cygnus*): effects of body weight, temperature, activity, emersion, daily rhythm, feeding and oxygen tension on oxygen consumption. Mar. Freshwater Res. **52**, 763-771.

Crear, B. and Allen, G., 2002. Optimising water quality – oxygen. Guide for the rock lobster industry. No 1. Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Hobart, Tasmania. pp 28-29.

Crear, B.J and Forteath, G.N.R., 2002. Feeding has the largest effect on the ammonia excretion rate of the southern rock lobster, *Jasus edwardsii*, and the western rock lobster, *Panuliris cygnus*. Aquacult. Engineering **26**, 239 – 250.

Crear, B., Cobcroft, J.and Battaglene, S., 2003. Recirculating Systems NH_3. Guide for the Rock Lobster Industry, No 2. Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Hobart, Tasmania. 17 pp.

Department of Fisheries, 1998. Opportunities for the Holding/Fattening/Processing and Aquaculture of Western Rock Lobster. Fisheries Management Paper 122: 22 pp.

Department of Fisheries, 2004. Assessment of applications for authorizations with regards to rock lobster aquaculture. Ministerial Policy Guideline, April, 2004. Fisheries Department of Western Australia.

Department of Fisheries, 2006. A scoping paper: Matters relevant to the development of a sustainable allocation and growout model for western rock lobster pueruli. Fisheries Management Paper 219: 55 pp.

Edgar, G.J., 1990. Predator-prey interactions in seagrass beds. I. The influence of macrofaunal abundance and size-structure on the diet and growth of the western rock lobster Panulirus cygnus George. J. Exp. Mar. Biol. Ecol. **139**, 1-22.

Eggleston, D.B. and Lipcius, R.N., 1992. Dynamics of shelter selection in the Caribbean spiny lobster. Lobster Newsletter **5**, 7-8.

Fielder, D.R., 1965. The spiny lobster *Jasus lalandei* (H. Milne-Edwards), in South Australia. Aust. J. Mar. Freshwater Res. **16**, 351-367.

Fitzpatrick, J., Jernakoff, P. and Phillips, B.F., 1989. An investigation of the habitat requirements of the post-puerulus stocks of the western rock lobster. Final Report to the Fishing Industry Research and Development Council. FIRDTF Project 86/83. pp. 80.

Floreto, E.A.T., Prince, D.L., Brown, P.B. and Bayer, R.C., 2000. The biochemical profiles of shell-diseased American lobsters, *Homarus americanus* Milne Edwards. Aquaculture **188**, 247-262.

Gardner, C., MacDiarmid, A., Mills, D., Oliver, M. and Stewart, R., 2004. Rock Lobster Enhancement and Aquaculture Subprogram: evaluating the release and survival of juvenile rock lobsters released for enhancement purposes. FRDC Final Report, 92 pp.

Glencross, B., Smith, M., Curnow, J., Smith, D. and Williams, K., 2001. The dietary protein and lipid requirements of post-puerulus western rock lobster, *Panulirus cygnus*. Aquaculture **199**, 119-129.

Hair C., Bell, J.D. and Doherty, P.J., 2003. The use of wild-caught juveniles in coastal aquaculture and its application to coral reef fishes. In: Stickney, R.R., McVey, J.P. (Eds.), Responsible Marine Aquaculture. CAB International. pp. 327-353.

Hartnoll, R.G., 1982. Growth. In, The Biology of Crustacea. Bliss, D.E. (Ed.), Embryology, Morphology and Genetics 2. Academic Press, pp. 111-196.

Hauton, C., William, J.A. and Hawkins, L.E., 1997 In-situ variability in phenoloxidase activity in the shore crab *Carcinus maenas* (L.). Comp. Biochem. Physiol. B. **81**, 833-835.

Hazell, R.W., Cockcroft, A.C., Mayfield, S. and Noffke, M., 2001. Factors influencing the growth rate of juvenile rock lobsters, *Jasus lalandii*. Mar. Freshwat. Res. **52**, 1367-1373.

Hopkins, K.D., 1992. Reporting fish growth: a review of the basics. J. World Aquacult. Soc. **23**, 173-179.

Insightful, 2001. S-PLUS 6 for Windows Guide to Statistics, Volume 1, Insightful Corporation, Seattle, WA.

James, P.J., Tong, L.J. and Paewai, M.P., 2001. Effect of stocking density and shelter on growth and mortality of early juvenile *Jasus edwardsii* held in captivity. Mar. Freshwater Res. **52**, 1413-17.

James, P., Woods, C. and Jeffs, A., 2003. The effects of holding tank design on lobster growth and survival. NIWA Client Report WLG2003-03, NIWA Project LOB01101, Auckland, New Zealand. pp. 1-21.

Jeffs, A. and Davis, M., 2003. An assessment of the aquaculture potential of the Caribbean spiny lobster, *Panulirus argus*. Proceedings of the Gulf and Caribbean Fisheries Institute **54**, 413-426.

Jeffs, A.G., Hooker, S.H., 2000. Economic feasibility of aquaculture of spiny lobsters *Jasus edwardsii* in temperate waters. J. World Aquacult. Soc. **31**, 30-41.

Johnson, P.T., 1987. A review of fixed phagocytic and pinocytotic cells of decapod crustaceans, with remarks on hemocytes. Develop. Comp. Immunol. **11**, 679-704.

Johnston, D., Melville-Smith, R. Hendriks, B., Maguire, G. and Phillips, B., 2006. Stocking density and shelter type for the optimal growth and survival of western rock lobster *Panulirus cygnus* (George). Aquaculture. **260**, 114-127.

Johnston, D., Melville-Smith, R. and Hendriks, B. Survival and growth of western rock lobster *Panulirus cygnus* (George) fed formulated diets with and without fresh mussel supplement. Aquaculture. Submitted.

Joll, L.M. and Phillips, B.F., 1984. Natural diet and growth of juvenile western rock lobsters *Panulirus cygnus* George. J. Exp. Mar. Biol. Ecol. **75**, 145-169.

Jones, C.M., Linton, L., Horton, D. and Bowman, W., 2001. Effect of density on growth and survival of ornate rock lobster, *Panulirus ornatus* (Fabricius, 1798), in a flow-through raceway system. Mar. Freshwater Res. **52**, 1425-1429.

Jussila, J., Jago, J., Tsvetnenko, E. and Evans, L., 1999. Effects of handling or injury disturbance on total haemocyte counts in western rock lobster (*Panulirus cygnus* George). Proc, International Symposium on Lobster Health Management, Adelaide, September 1999.

Kington, S.W., 1999. Factors influencing the on growing and restocking of *Jasus edwardsii*. MSc Thesis, University of Auckland, New Zealand.

Kittaka, J. and Booth, J.D., 2000. Prospectus for aquaculture. In: Phillips, B.F., Kittaka, J. (Eds.), Spiny Lobsters: Fisheries and Culture, Blackwell Science, Oxford, pp. 465-473.

Kittaka, J., 2000. Culture of the spiny lobsters. In: Phillips, B.F., Kittaka, J. (Eds.), Spiny Lobsters: Fisheries and Culture, Blackwell Science, Oxford, pp 508-532.

Lanz Mendoza, H. and Faye, I., 1996. Immunoglobulin superfamily proteins in invertebrates. In: Söderhäll, K., Iwanaga, S., Vasta, G.R. (Eds.), New Directions in Invertebrate Immunology. SOS Publications, Fair Haven, USA, pp. 285-302.

Lellis, W.A. and Russell, J.A., 1990. Effect of temperature on survival, growth and feed intake of postlarval spiny lobsters, *Panulirus argus*. Aquaculture **90**, 1-9.

Losada-Tosteson, V. and Posada, J.M., 2001. Using tyres as shelters for the protection of juvenile spiny lobsters, *Panulirus argus*, or as fishing gear for adults. Mar. Freshwater Res. **52**, 1445-1450.

MacDiarmid, A., Butler, M. and Booth, J., 1998. Why do juvenile red rock lobsters aggregate? Seafood New Zealand, September 1998, 41-43.

McKinlay, J., 2002. Optimising the Efficiency and Effectiveness of Enforcement to Achieve Compliance in the Western Rock Lobster Fishery FRDC report 1998/156.

Morrissy, N.M., 1992. Density-dependent pond growout of single year class cohorts of a freshwater crayfish *Cherax tenuimanus* (Smith) to two years of age. J. World Aquacult. Soc. **23**, 154-168.

Morrissy, N.M., Walker, P. and Moore, W., 1995a. Predictive equations for managing semi-intensive growout of a freshwater crayfish (marron) *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae), on a commercial farm. Aquacult. Res. **26**, 71-80.

Morrissy, N.M., Bird, C. and Cassells, G., 1995b. Density dependent growth of cultured marron, *Cherax tenuimanus* (Smith 1912). Freshwater Crayfish **10**, 560-568.

Moullac, G.Le. and Haffner, P., 2000. Environmental factors affecting immune responses in Crustacea. Aquaculture **191**, 121-131.

Moyle, K., 2005. Effect of stocking density on the growth, survival and behaviour of *Panulirus cygnus* post-puerulus. Honours Thesis. University of Western Australia.

Mugnier, C. and Justou, C., 2004. Combined effect of external ammonia and molt stage on the blue shrimp *Litopenaeus stylirostris* physiological response. J. Exp. Mar. Biol. Ecol. **309**, 35-46.

Norton, J.H., Levy, N. and Field, K., 2001. A preliminary evaluation of three haemolymph tests to assess health status in tropical rock lobsters *Panulirus ornatus.* Curtin University, Muresk. Proceedings, International Symposium on Lobster Health Management, Adelaide, September 1999, pp. 116-120. http://www.muresk.curtin.edu.au/research/otherpublications/lhm/15_norton.pdf

Ozbay, G. and Riley, J.G., 2002. An analysis of refractometry as a method of determining blood total protein concentration in the American lobster *Homarus* (Milne-Edwards). Aquacult. Res. **33**, 557-562.

Person-Le Ryet, J., Delbard, C., Chartois, H. and Le Delliou, H., 1997. Toxicity of ammonia to turbot juveniles 1. Effects on survival, growth and food utilisation. Aquatic Living Resources **10:** 307-314.

Phillips, B.F., Campbell, N.A. and Rea, W.A., 1977. Laboratory growth of early juveniles of the western rock lobster *Panulirus longipes cygnus*. Mar. Biol. **39**, 31-39.

Phillips, B.F., Melville-Smith, R., Cheng, Y.W. and Rossbach, M., 2001. Testing collector designs for commercial harvesting or western rock lobster (*Panulirus cygnus*) puerulus. Mar. Freshwater Res. **52**, 1465-73.

Phillips, B.F. and Liddy G. C., 2003. Recent developments in spiny lobster aquaculture. In: Phillips, B.F., Magrey, B., Zhou, Y. (Eds.), Proceedings of the Third World Fisheries Congress, Am. Fish. Soc. **38**, 43-57.

Phillips, B.F., Melville-Smith, R., Rossbach, M., Cheng, Y.W., Caputi, N., Thomas, A.W., Mills, D. and Crear, B., 2003a. Towards establishing techniques for large scale harvesting of pueruli and obtaining better understanding of mortality rates. Fisheries Research Report, Western Australian Marine Research Laboratories 144, 138 pp.

Phillips, B.F., Melville-Smith, R. and Cheng, Y.W., 2003b. Estimating the effects of removing *Panulirus cygnus* pueruli on the fishery stock. Fish. Res. **52**, 1465-73.

Power, R., Munro, J.L., Diffenthal, M. and Lane, G., 2005. Preliminary investigations into the feasibility of small scale, commercial aquaculture of *Panulirus argus*, based on collection of pueruli from the wild. Proc. 56th Gulf and Carib. Fish Inst. pp. 233-248.

Radford, C.A. and Marsden, I.D., 2005. Does morning as opposed to night-time feeding affect growth in juvenile spiny lobsters, *Jasus edwardsii*? J. World Aquacult. Soc. **36**, 480-488.

Rayns, N.D., 1991. The growth and survival of juvenile rock lobster *Jasus edwardsii* held in captivity. Ph.D. Thesis, University of Otago, Dunedin, New Zealand.

Robertson, L., Lawrence, A.L. and Castille, F.L., 1993. Effect of feeding frequency and feeding time on growth of *Penaeus vannamei* (Boone). Aquaculture and Fisheries Management **24**, 1-6.

Roch, P., 1999. Defence mechanisms and disease prevention in farmed marine invertebrates. Aquaculture **172**, 125-146.

Sedgwick, R.W., 1979. Effect of ration size and feeding frequency on the growth and food conversion of juvenile *Penaeus merguiensis* De Man. Aquaculture **16**, 279-298.

Serfling, S.A. and Ford, R.F., 1975. Laboratory culture of juvenile stages of the California spiny lobster Panulirus interruptus (Randall) at elevated temperatures. Aquaculture **6**, 377-387.

Smith, D.M, Williams, K.C. and Irvin, S.J., 2005. Response of the tropical spiny lobster Panulirus ornatus to protein content of pelleted feed and to a diet of mussel flesh. Aquacult. Nutr. **11**, 209-217.

Söderhäll, K. and Smith, V.J., 1986. The prophenoloxidase activating system: the biochemistry of its activation and role in arthropod cellular immunity with special reference to crustaceans. In: Brehelin, M. (Ed.), Immunity in Invertebrates, Springer-Verlag, Berlin, pp. 208-23.

Söderhäll, K., Cerenius, L. and Johansson, M.W., 1996. The prophenoloxidase activating system in Invertebrate Immunology. In: Söderhäll, K., Iwanaga, S., Vasta, G.R. (Eds.), Invertebrates In New Directions. SOS Publications, Fair Haven, USA., pp. 229-253.

Spanier, E., Zimmer-Faust, R.K., 1988. Some physical properties of shelter that influence den preference in spiny lobsters. J. Exp. Mar. Biol. Ecol. **121**, 137-49.

Sprague, J. B., 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Res. **3**, 793-821.

Thomas, C.W., Crear, B.J. and Hart, P.R., 2000. The effect of temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, Jasus edwardsii. Aquaculture **185**, 73-84.

Thomas, C.W., Carter, C.G. and Crear, B.J., 2003. Feed availability and its relationship to survival, growth, dominance and the agonistic behaviour of the southern rock lobster, *Jasus edwardsii*. Aquaculture **215**, 45-65.

Tong, L., 1993. Progress toward spiny lobster farming in New Zealand. Lobster Newsletter 6, 14.

Travis, D.F., 1957. The moulting cycle of the spiny lobster, *Panulirus argus* Latreille. IV. Post-ecdysal histological and histochemical changes in the hepatopancreas and integumental tissues. Biol. Bull. **113**, 451-478.

Tuan, L.A., Nho, N.T., Hambrey, J., 2000. Status of cage mariculture in Vietnam, In: Liao, I.C., Lin, C.K., (Eds.), Cage culture in Asia. Proceedings of the First International Symposium on Cage Culture in Asia. Asian Fisheries Society and World Aquaculture Society – South-east Asia Chapter, Manila and Bangkok, pp. 111-123.

Vargas-Albores, F., Baltazar, P.H., Clark, G.P. and Barajas, F.M., 1998. Influence of temperature and salinity on the yellowlegs shrimp, Penaeus californiensis Holmes, prophenoloxidase system. Aquacult. Res. **29**, 549-553.

Ward, L.R., Carter, C.G., Crear, B.J. and Smith, D.M., 2003. Optimal dietary protein level for juvenile southern rock lobster, Jasus edwardsii, at two lipid levels. Aquaculture **217**, 483-500.

Williams, K.C., 2006. Progress in tropical rock lobster Panulirus ornatus grow out in Vietnam. In Developments in Rock Lobster Enhancement and Aquaculture (Ed. by Robert van Barneveld). Summary Proceedings of the Eighth Annual Rock Lobster Enhancement and Aquaculture Subprogram Workshop, Adelaide, Australia, 27 August 2006. RLEAS Publication 11: 22.

Wyban, J.A. and Sweeney, J.N., 1989. Intensive shrimp growout trials in a round pond. Aquaculture **76**, 215-225.

Zimmer-Faust, R.K. and Spanier, E., 1987. Gregariousness and sociality of spiny lobsters: implications for den habitation. J. Exp. Mar. Biol. Ecol. **105**, 57-71.

12.0 Appendices

Appendix I

Intellectual Property:

There is no identifiable intellectual property arising from the project.

Appendix II

Staff:

Dr Roy Melville-Smith	Principal Investigator
Dr Danielle Johnston	*Co-Investigator
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Appendix III

Biochemical profiles of western rock lobsters held under aquaculture conditions compared to animals in the wild

Melville-Smith, R., Johnston, D., Schenk, T., Glencross, B., Thomson, A., Miller, M. and Fisher, S.

1.0 Abstract

Western rock lobsters captured in the wild as pueruli, year 1 and year 2 post settlement, were held for one year under aquaculture conditions. Treatments included two holding temperatures (ambient and 23°C); two feeding regimes (once nightly and three times per night) and two diets (pellet with mussel supplement and pellet only). At the end of the experiment, the biochemical profiles of hepatopancreas and muscle tissue from the three aquaculture-held year classes, were compared with that of wild-caught animals corresponding to those cohorts. The major lipid classes were triacylglycerols (TAG), polar lipids (PL) and sterols (ST). There were elevated levels of TAG in the tissues of the aquacultured animals compared to those caught in the wild. There were also significant differences in the fatty acid composition of lobsters held in tanks compared to those in the wild. Those held under aquaculture conditions showed significant differences in fatty acid composition in both muscle tissue and hepatopancreas in relation to lobster age and diet, but not frequency of feed delivery or temperature of culture conditions. The biochemical composition of the aquacultured and wild caught lobsters are discussed in relation to their size and aquaculture holding treatments.

2.0 Introduction

There has been interest in Western Australia for the possibility of growing out western rock lobsters, *Panulirus cygnus*, from pueruli to marketable sized animals. Enthusiasm to pursue this possibility has led to one aquaculture company being granted an exemption to harvest pueruli for ongrowing (Anon. 2008).

There is a wide range of literature on the effect of diets on composition of aquacultured animals, and indeed this is generally regarded as a mandatory element of most nutritional studies in aquaculture (Glencross et al. 2007). Of those dietary nutrients fed to aquatic animals, none has a more direct effect on animal tissue composition than that of the fatty acids (Sargent et al. 1999). Although the effects of such nutrients (fatty acids) on tissue composition are well known, a comparison of the composition of aquacultured animals compared to their wild analogues is comparatively rare.

A similar study was completed in 2005 involving southern rock lobster, *Jasus edwardsii*. That study compared small and large wild lobsters with rock lobster fed for 120 days on fresh squid and mussels or a formulated prawn pellet diet. Results indicated that *J. edwardsii* could be cultured to provide a fatty acid composition both in the muscles and digestive glands comparable to that of wild caught lobster. Furthermore, taste tests indicated that untrained tasters could not statistically separate samples from either cultured or wild animals, although colour and texture effects needed further investigation (Nelson et al. 2005).

This study was initiated to examine the biochemical differences between *P. cygnus* held for extended time under aquaculture conditions compared to wild caught animals. The key objective was to determine whether there were significant differences in the lipid and fatty acid composition between the different groups of animals, which in turn might indicate deficiencies

in the diet of the aquacultured animals and therefore the cultured product. A separate study, to be reported elsewhere, has compared taste and texture of the two products.

3.0 Methods

The way that the western rock lobsters used in this investigation were collected and stocked has been described in Section 4.0 Objective 2B.

In brief, *P. cygnus* lobsters were maintained in tanks receiving flow through seawater. Six tanks for each of three size classes (puerulus/early post pueruli, year 1 and year 2 post settlement) were held at ambient temperatures (16 to 24°C) and six were heated to 23°C ± 1°C. The lobsters were randomly stocked at densities of 50 m^{-2} for puerulus/early post pueruli (10 per tank), 23 m^{-2} for year 1 (20 per tank) and 19 m^{-2} for year 2 post settlement juveniles (20 per tank). The animals fed on two diet treatments; three tanks in each size class were given feed frequencies of once per night at 1700h, and three tanks were allocated three feeds per night at 1800h, 0000h, and 0500h. The same daily ration of pellet diet was provided to tanks within each size class irrespective of feeding regime. In addition to these tanks, six other tanks at ambient temperature were stocked with post pueruli (3 tanks) and year 1 animals (3 tanks). These animals were fed to saturation once daily at 1700h on an exclusive diet of fresh blue mussels (*Mytilus edulis)*. Refer to Johnston et al. (2008) for more detail of the experimental design.

The lobster pellet diet was made in two-monthly batches at the Western Australian Marine and Fisheries Research Laboratory's nutrition lab using a pasta maker and was oven dried at 70°C. Automated feeders were used to deliver pellets into the experimental tanks at the prearranged feeding times. All tanks were supplemented with fresh blue mussels (*Mytilus edulis*) on the weekends to address possible nutritional deficiencies in the pellet diet and maximise growth and survival. Also, to minimise cannibalism in recently settled post-pueruli, this size group was fed mussels for the first two months of the trial before being weaned onto the pellet diet. Automated feeders were not used when feeding mussels.

Animals were maintained under the cultured conditions for 12 months, after which all were measured and weighed and one lobster from each replicate tank for each year class was randomly chosen and euthanized in iced seawater. These lobsters provided three samples (one from each replicate tank) of hepatopancreas and muscle tissue for each treatment. The wet weight of these lobsters was measured using an electronic balance to the nearest 0.1 g after blotting dry with absorbent towel and their carapace length was measured using vernier callipers to the nearest 0.1mm. Results of these samples were compared with wild caught Western Rock Lobsters of each year class collected from waters off Lancelin (31°01´S, 115°20´E), Western Australia. The wild caught animals (n=19 post pueruli and year 1; n=10 year 2) were ascribed to particular cohorts (post pueruli, year 1 and year 2) based on length frequency modes, so that they could be compared with the same year classes as the aquaculture-held animals.

A small sample of hepatopancreas (~5 g) and abdominal muscle tissue (~5 g) were dissected and placed into pre-weighed plastic vials. Samples of *M. edulis* mussel tissue (whole mussel dissected from shell n=10) and pellet diet (n=3) were also placed in pre-weighed vials to determine the lipid content of the diet fed to lobsters throughout the trial. Vials were stored at -80°C until lipid analysis. Prior to analysis the muscle and hepatopancreas tissue were freeze-dried.

Lipid and fatty acid analysis

Samples were extracted via a modified Bligh Dyer extraction (Bligh and Dyer 1959); a single

phase extraction, followed by phase separation with a final ratio of cholorform:methanol:water (1:1:0.9 v:v:v), to yield a total lipid extract (TLE). A 1 µl aliquot of the TLE was analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyzer (Tokyo, Japan) to quantify individual lipid classes (Ackman 1981; Volkman and Nichols 1991). Samples were spotted onto silica gel SIII Chromarods (5 µm particle size) and developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane: diethyl ether: acetic acid (60:17:0.1, v/v/v) (Volkman and Nichols 1991). After development for 25 min, the chromarods were oven-dried and analysed immediately to minimise adsorption of atmospheric contaminants. Lipid classes were quantified by DAPA software (Kalamunda, WA, Australia). The FID was calibrated for each compound class: phosphatidylcholine, wax ester (derived from fish oil), cholesterol, oleic acid; hydrocarbon (squalene), triacylglycerol (derived from fish oil), and diacylglycerol esters (DAGE) (purified from shark liver oil). TLC-FID results are generally reproducible to 5-10% of individual class abundances (Volkman and Nichols 1991).

An aliquot of the TLE was trans-methylated in methanol: chloroform: hydrochloric acid (10:1:1, v/v/v) for 1 h at 100°C. After addition of water, the mixture was extracted three times with hexane: chloroform (4:1, v/v) to obtain fatty acid methyl esters (FAME). Samples were made up to 1 ml and analysed by gas chromatography (GC) using an Agilent Technologies 6890N GC (Palo Alto, California, USA) equipped with an Equity™-1 fused silica capillary column (15 m × 0.1 mm i.d., 0.1µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683 Series auto sampler and injector. Helium was used as the carrier gas. Samples were injected in splitless mode at an oven temperature of 120°C. After injection, the oven temperature was raised to 250°C at 10°C per min and finally to 270°C at 3°C per min. Peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, California, USA).

Individual components were identified by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are typically subject to an error of up to ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, Texas, USA). The GC was equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m×0.32 mm i.d.). Helium was used as the carrier gas, with operating conditions previously described (Miller et al. 2006).

Both lipid class and fatty acid content were calculated as a percentage and an absolute (mg/g wet weight (WW)).

All abbreviations and lipid nomenclature used in this report are contained in Appendix IV.

Other minor components include:

- Other SFA include i15:0 and i17:0
- Other MUFA include 16:1n-5, 16:1n-9, 18:1n-5, 20:1n-5, 22:1n-11+n-13, 24:1n-11+13, 24:1n-9 and 24:1n-7
- Other omega 3 PUFA include 21:5n-3 and 22:4n-3 (Which co-elutes with 22:2 NMI)
- Other omega 6 PUFA include 22:3n-6 and 18:3n-6

The fatty acid 20:1n-9+n-11 and 20:3n-3 co-elute. This is unavoidable with the column used, however the result provided is predominately 20:1n-9+n-11 with only 20:3n-3 a minor component.

Statistical analysis

The effect of temperature (23°C vs ambient), diet (pellet vs mussel) and feed frequency (once vs three times daily) were identified with non-metric multidimensional scaling (MDS) and using ANOSIM.

Fatty acid profiles for both hepatopancreas and abdominal muscle were compared using MDS in two dimensions using the Kruskal Loss Function. The raw data was first transformed using a square-root transformation before creating a resemblance matrix using Pearson's correlation coefficient. The resemblance matrix is the required input for the MDS.

Having constructed MDS plots, the significance of various factors were quantified using ANOSIM. When testing the effect of heat and diet on the aquacultured animals and comparing these with wild lobsters, these effects were nested within the condition level (wild or cultured).

4.0 Results

Quantity and size range of lobsters sampled

The total numbers of wild and aquaculture-held lobsters sampled in this biochemical analysis as well as their mean weight and carapace lengths are provided in Table 1.

Table 1. Numbers of aquaculture-held and wild caught western rock lobsters in three different cohorts that were biochemically sampled in the course of this study.

	Aquaculture-held			Wild caught		
	Numbers sampled (N)	Mean weight (g)	Mean size (mm CL)	Numbers sampled (N)	Mean weight (g)	Mean size (mm CL)
Post pueruli	15	53.44±5.54	37.51±1.36	4	61.43±5.60	39.23±1.56
Year 1	15	197.83±9.73	93.16±33.53	15	178.30±18.9	56.31±1.98
Year 2	12	252.51±20.40	66.34±1.02	10	397.22±5.12	76.72±0.71

Lipid class composition

Lipid classes found in wild and aquaculture-held lobster hepatopancreas and muscle tissue samples, as well as in the feed samples, are presented according to composition in Tables 2, 3 and 4.

The major lipid classes found in the hepatopancreas samples were triacylglycerol (TAG), polar lipid (PL) and minor amounts of sterol (ST). The major lipid classes found in the muscle tissue were PL and ST with minor amounts of TAG, free fatty acids (FFA) and wax esters (WE).

Fatty acid analysis

Fatty acids of the wild and aquaculture-held lobster hepatopancreas and muscle tissue samples, as well as in the feed samples, are presented according to percentage composition (Tables 5 and 6) and wet weight (mg/g) (Tables 7 and 8; Figure 1 and 2). The major (on average >10%) fatty acids (FA) in the samples were 16:0, 18:1n-9 (Oleic acid, OLA), 20:5n-3 (eicosapentaenoic acid, EPA), 22:6n-6 (docosahexaenoic, DHA) and 20:4n-6 (arachidonic acid ARA). Other fatty acids 18:0, 16:1n-7, 18:1n-7 and 18:2n-6 (linolenic acid, LOA) occurred in moderate proportions in the region of >5% but >10%.

Tail muscle tissue

The concentration of the fatty acids ARA, DHA and EPA in tail muscle tissue of specimens from each of the year classes and for the various diet treatments are shown in Figure 1. These three fatty acids were selected for examination since they are biologically important long-chain polyunsaturates that are unlikely to be able to be produced by the western rock lobsters (or humans) in sufficient quantities to allow normal metabolic requirements, and must therefore be obtained from the diet. In western rock lobster, these fatty acids are important in the development of the nervous system and also play an important role in regulation of hormone (eicosanoid) production (Sargent et al. 1995).

There was no apparent difference between each of the treatments in the relative content of each of ARA, DHA and EPA in the tail muscle of the post puerulus specimens. Average concentrations of ARA ranged from 0.23 mg/g for specimens fed a pelletised diet manually at ambient temperatures once per day to 0.43 mg/g for those fed mussel flesh only (Table 8). Average concentrations of DHA ranged from 0.69 mg/g for those fed a pelletised diet manually at a constant water temperature of 23°C to 0.84 mg/g for those fed mussel flesh (Table 8). EPA average concentrations ranged from 0.78 mg/g (manual feeding once per day at ambient temperature) to 1.13 mg/g for those fed thrice daily at a constant water temperature of 23 °C (Table 8).

The results for the Year 1 cultured specimens were more variable, with the highest concentrations of AA (0.67 mg/g), DHA (1.30 mg/g) and EPA (1.87 mg/g) measured in the specimens fed the pelletised diet once daily. Concentrations in the other cultured samples ranged from 0.34 mg/g (fed once daily, 23 °C) to 0.57 mg/g (mussel flesh) for ARA, 0.76 mg/g (mussel flesh) to 0.88 (fed once daily, 23 °C) for DHA and 0.82 mg/g (mussel flesh) to 1.28 mg/g (fed thrice daily, 23 °C) for EPA.

Results for the Year 2 cultured specimens were also variable, but it is apparent that amongst the pellet fed specimens, the frequency of feeding (i.e. thrice daily versus once daily) had little effect on the relative concentrations of the essential fatty acids, while controlling the water temperature decreased the overall concentrations of each of the essential fatty acids. Most notable was the low concentration of the essential fatty acid in the wild caught specimens (average ARA = 0.05 mg/g, DHA = 0.27 mg/g and EPA = 0.51 mg/g) relative to the cultured specimens (ARA ranging from 0.40 mg/g to 0.6 mg/g; DHA 0.75 - 1.03 mg/g and EPA 1.11 – 1.55 mg/g) (Table 8).

There were increased amounts of TAG and omega 3 in the aquaculture reared western rock lobsters. This is a reflection of their diets, which is demonstrated by the increase in EPA, DHA and TAG in the pellet fed compared to wild caught lobsters. The availability of food/oil would be expected to be greater in the aquaculture situation rather in the wild, and of the available oils, there would be greater concentrations of DHA and EPA.

Digestive gland samples

Results are similarly presented for the digestive glands from the western rock lobster specimens in Figure 2. In all instances, the concentration of the three essential fatty acids in the digestive gland was approximately one order of magnitude greater than in the tail muscle tissue.

For the post puerulus cultured samples, concentrations of each were highest in specimens fed thrice daily at a water temperature of 23 °C (ARA = 4.01 mg/g, DHA = 20.70 mg/g and EPA = 17.13 mg/g), compared with the other treatments ranging in concentration from 2.12 – 3.92 mg/g of ARA, 9.96 – 14.47 mg/g of DHA and 7.19 – 10.26 mg/g of EPA (Table 7).

For the year 1 samples, there was no apparent difference in the concentration of ARA in the hepatopancreas, with values ranging from 4.64 mg/g in the wild caught animals to 6.64 mg/g in the specimens fed three times daily in ambient water (Table 7). Concentration of EPA was highest in the specimens held at 23°C and fed three times daily (21.29 mg/g EPA). Other treatments ranged from 5.04 mg/g (wild) to 20.33 mg/g (23°C, fed once daily). Concentrations of DHA ranged from 17.93 mg/g (mussel fed) to 31.52 mg/g (ambient, fed three times) within the aquacultured animals. However, the wild caught animals had a low average concentration of DHA, only 2.47 mg/g (Table 7).

For the year 2 samples, concentrations of ARA remained similar across all of the treatment groups, ranging from 6.36 mg/g (ambient, fed once daily) to 8.83 mg/g (ambient, fed three times) (Table 7). The specimens kept at 23°C and fed once daily had the highest concentrations of both EPA (27.16 mg/g) and DHA (37.64 mg/g). In the other treatments, the concentrations ranged from 23.27 mg/g to 26.75 mg/g EPA and from 33.54 mg/g to 34.66 mg/g DHA (Table 7). As in tail muscle tissue, the hepatopancreas of wild caught specimens presented much lower concentrations of all essential fatty acids than aquacultured specimens (average ARA = 2.13, EPA = 7.97 and DHA = 6.42) (Table 7).

MDS scatterplots were used to determine whether lobsters fed once daily differed in terms of fatty acid composition of muscle tissue (Figure 3) and hepatopancreas (Figure 4) to those fed by auto-feeders three times a day. There was greater variation between the scatterplots for individual animals for hepatopancreas tissue than for muscle tissue. The ANOSIM analysis showed that there were significant differences in fatty acid composition in both the muscle tissue and the hepatopancreas related to age of the lobster, post pueruli compared to year 1 and year 2 ($P < 0.01$), but that frequency of feed delivery, whether once or three times per day, was not significant ($P > 0.05$).

The analysis was repeated to compare fatty acid composition of muscle tissue (Figure 5) and hepatopancreas (Figure 6) of aquacultured animals held in heated and ambient temperatures with those caught in the wild. The ANOSIM analysis showed that there were significant differences in fatty acid composition in both the muscle tissue and the hepatopancreas related to lobster age, post pueruli compared to year 1 and year 2 ($p<0.01$), and wild compared to aquaculture held animals ($P < 0.01$), but that temperature did not significantly influence fatty acid composition ($P > 0.05$).

Finally, MDS scatterplots were used to determine whether lobsters that were fed a diet of mussel, pellet or were wild-caught, differed in terms of fatty acid composition of muscle tissue (Figure 7) and hepatopancreas (Figure 8). The ANOSIM analysis showed that there were significant differences in fatty acid composition in both the muscle tissue and the hepatopancreas related to the diets ($P < 0.01$) of the aquacultured animals, with those held on a mussel diet differing to those on a pellet diet; lobster age, ($P < 0.01$); and whether they were caught in the wild and therefore eating a natural diet compared to being aquaculture held and fed either a pellet or mussel diet ($P < 0.01$).

In all cases the differences in scatter plots in fatty acid composition between year 1 and year 2 tank held animals shown by the MDS scatterplots was less marked than when these two-year classes were compared to the post pueruli year class (Figs. 3-8).

Table 2. Lipid class composition [mean mg/g (standard error)] of hepatopancreas tissue from wild and aquaculture-held *P. cygnus* of three different year classes based on wet weight.

FATTY ACID	WILD CAUGHT		CULTURED													
	Year 1 and Post Pueruli	Year 2	Post Pueruli					Year 1					Year 2			
			Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
			1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
Wax esters	0.46 (0.43)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Triacylglycerols	30.6 (4.04)	93.77 (11.60)	93.42 (20.21)	82.03 (27.99)	106.79 (8.54)	67.81 (3.75)	161.73 (47.66)	179.16 (5.65)	247.74 (83.02)	116.01 (31.98)	227.42 (29.06)	231.19 (22.20)	261.84 (11.38)	261.88 (13.37)	294.09 (10.36)	283.83 (57.36)
Free fatty acids	0.27 (0.07)	0.09 (0.03)	0.37 (0.19)	1.06 (0.29)	0.48 (0.34)	0.06 (0.06)	0.09 (0.09)	0.32 (0.19)	0	0	0.08 (0.08)	0.31 (0.18)	0	0.59 (0.30)	0.19 (0.19)	0.07 (0.07)
Sterols	1.10 (0.08)	0.61 (0.06)	1.38 (0.32)	1.87 (0.38)	0.97 (0.55)	0.56 (0.26)	0.8 (0.21)	0.69 (0.21)	0.57 (0.06)	1.08 (0.39)	0.26 (0.04)	2.01 (0.70)	0.15 (0.15)	1.03 (0.22)	0.84 (0.32)	0.80 (0.23)
Polar Lipids	28.53 (2.31)	13.41 (1.68)	13.18 (0.93)	14.96 (1.79)	8.98 (2.56)	12.47 (0.87)	13.16 (3.60)	13.15 (2.46)	18.49 (7.58)	17.27 (3.97)	11.67 (1.60)	22.92 (3.67)	12.73 (2.51)	16.36 (3.51)	11.03 (1.54)	15.98 (2.85)

Table 3. Lipid class composition [mean mg/g (standard error)] of tail muscle from wild and aquaculture-held (heated/ambient tanks; fed once or thrice daily) *P. cygnus* of three different year classes based on wet weight.

FATTY ACID	WILD CAUGHT		CULTURED													
	Year 1 and Post Pueruli	Year 2	Post Pueruli					Year 1					Year 2			
			Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
			1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
Wax esters	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Triacylglycerols	0.02 (0.01)	0.04 (0.01)	1.00 (0.42)	0.14 (0.14)	0.05 (0.05)	0	0.14 (0.11)	0.09 (0.03)	0.3 (0.13)	0.08 (0.04)	0.3 (0.13)	0.02 (0.00)	0.21 (0.10)	0.51 (0.30)	0.06 (0.02)	0.04 (0.01)
Free fatty acids	0	0	0	0	0	0	0	0.02 (0.02)	0.02 (0.02)	0	0.02 (0.02)	0	0.01 (0.01)	0	0	0
Sterols	0.61 (0.03)	0.41 (0.04)	0.72 (0.14)	0.70 (0.09)	0.45 (0.03)	0.54 (0.05)	0.60 (0.04)	0.62 (0.13)	0.48 (0.09)	0.44 (0.09)	0.48 (0.09)	0.78 (0.17)	0.65 (0.16)	0.54 (0.02)	0.55 (0.18)	0.43 (0.04)
Polar Lipids	5.07 (0.21)	3.87 (0.34)	3.96 (0.89)	5.1 (0.93)	5.18 (0.24)	4.72 (0.23)	5.99 (0.47)	6.75 (0.63)	6.21 (1.36)	5.18 (1.10)	6.21 (1.36)	6.99 (0.64)	9.08 (2.19)	8.16 (1.01)	6.51 (1.31)	6.42 (0.89)

Table 4. Lipid classes [mean mg/g (standard error)] in the diets provided to the aquacultured western rock lobsters based on wet weight.

Fatty Acid	Mussel	Pellet without krill meal	Pellet with krill meal
Wax esters	0	0	0
Triacylglycerols	0.9 (0.33)	89.77 (2.61)	51.96 (5.04)
Free fatty acids	0	3.02 (0.11)	7.18 (0.81)
Sterols	0.81 (0.06)	6.61 (0.22)	13.52 (1.78)
Polar Lipids	5.52 (0.15)	24.33 (1.55)	52.41 (7.53)

Table 5. Relative percentage fatty acid composition of western rock lobster hepatopancreas tissue for wild and aquaculture-held *P. cygnus* of three different year classes held in heated and ambient tanks and for different feed and feed frequencies provided to the aquacultured lobsters.

	Diets			Wild		Post Pueruli Cultured					Year 1 Cultured					Year 2 Cultured			
						Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
	Mussel	Pellet Without Krill	Pellet with Krill	Year 1 & Post Pueruli	Year 2	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
14:0	0.75	2.04	1.37	1.06	1.08	1.19	0.59	0.68	0.79	1.00	0.66	0.57	0.45	0.76	0.78	0.88	0.72	0.89	0.90
16:1n-7c	1.58	2.83	2.54	2.08	2.90	2.79	2.88	4.41	2.63	2.71	3.28	3.20	3.92	2.74	2.92	2.91	3.18	2.42	2.65
16:0	7.04	7.80	8.88	8.49	7.45	7.56	7.38	7.74	7.36	7.31	6.38	6.47	5.78	6.90	6.91	6.45	6.22	6.67	6.87
17:0	0.53	0.21	0.17	0.64	0.44	0.35	0.39	0.47	0.42	0.32	0.30	0.27	0.34	0.35	0.34	0.30	0.23	0.23	0.39
18:2n-6	0.69	5.89	5.08	0.86	0.77	4.21	4.39	0.60	3.40	3.78	2.64	3.12	0.55	3.10	3.36	3.47	2.56	3.78	3.56
18:1n-9	0.63	4.63	4.21	5.10	7.33	4.56	4.54	3.85	4.42	4.91	6.46	7.21	5.06	6.26	6.29	5.84	6.07	5.85	6.19
18:3n-3	0.45	0.73	0.61	0.35	0.15	0.46	0.45	0.27	0.36	0.42	0.34	0.32	0.20	0.35	0.37	0.43	0.37	0.35	0.37
18:1n-7	0.92	1.43	2.07	2.36	2.42	1.75	1.85	1.63	1.80	1.81	2.21	2.22	1.78	1.95	2.00	2.01	1.96	1.97	1.86
18:0	1.95	1.70	1.40	3.99	2.75	3.16	3.37	3.75	3.48	3.14	2.88	2.86	3.40	3.14	2.82	2.74	2.92	2.70	3.06
20:4n-6	2.37	0.34	0.27	3.65	0.95	1.08	1.15	1.67	1.22	1.04	1.24	1.11	2.30	1.07	1.04	1.03	1.35	0.98	1.02
20:5n-3	6.09	4.89	5.41	3.97	3.58	4.16	4.24	4.38	4.15	4.43	3.38	3.35	4.14	3.81	3.73	3.95	3.78	3.90	3.91
20:2n-6	0.26	0.07	0.05	0.55	0.47	0.39	0.28	0.36	0.50	0.59	0.56	0.53	0.28	0.54	0.50	0.46	0.41	0.50	0.46
20:1n-9/n-11+20:3n-3	2.28	0.39	0.31	1.65	1.92	1.50	1.42	2.17	1.61	1.25	1.78	1.49	2.23	1.42	1.45	1.44	1.51	1.34	1.38
20:0	0.01	0.06	0.09	0.40	0.40	0.12	0.14	0.12	0.17	0.25	0.16	0.16	0.14	0.21	0.19	0.19	0.26	0.25	0.32
22:6n-3	7.15	4.12	4.23	1.94	2.88	5.12	5.20	6.18	5.74	5.36	5.37	5.29	6.29	5.15	5.17	5.44	5.49	5.40	5.07
22:5n-3	0.66	0.01	0.48	1.08	0.98	0.55	0.55	0.48	0.56	0.54	0.61	0.66	0.56	0.61	0.57	0.65	0.69	0.91	0.75
Total SFA	10.71	12.33	12.42	17.00	13.92	13.23	12.71	13.64	13.07	12.73	11.35	11.44	11.11	12.34	11.96	11.49	11.47	11.66	12.54
Total MUFA	6.29	9.88	9.62	13.35	16.76	11.95	11.94	14.10	12.01	11.87	15.28	15.47	14.71	13.94	14.14	13.75	14.67	12.71	13.02
Total PUFA	21.19	17.13	16.44	15.54	16.32	17.49	17.81	16.23	17.68	17.88	17.34	16.99	18.00	17.38	17.51	18.03	17.89	18.51	17.61
Total n-3	16.68	10.59	10.98	8.72	9.28	11.23	11.36	12.78	11.78	11.94	11.70	11.10	13.43	11.50	11.52	11.81	12.20	12.27	11.60
Total n-6	4.38	6.54	5.46	6.57	6.82	6.13	6.32	3.28	5.74	5.88	5.51	5.68	4.50	5.81	5.88	6.09	5.59	6.19	5.94
Ratio n-3/n-6	3.68	0.56	0.70	0.30	0.19	0.27	0.28	0.60	0.33	0.23	0.19	0.14	0.40	0.19	0.15	0.17	0.16	0.14	0.15
Ratio EPA/ARA	2.49	4.97	6.95	0.24	0.12	0.56	0.57	0.40	0.55	0.48	0.25	0.22	0.24	0.36	0.28	0.34	0.21	0.29	0.29
Ratio DHA/EPA	1.20	0.29	0.27	0.12	0.11	0.18	0.19	0.22	0.22	0.14	0.14	0.11	0.21	0.13	0.11	0.12	0.10	0.10	0.09

Table 6. Relative percentage fatty acid composition of western rock lobster abdominal muscle tissue for wild and aquaculture-held *P. cygnus* of three different year classes held in heated and ambient tanks and for the different feed and feed frequencies provided to the aquacultured lobsters.

	Wild		Post Pueruli Cultured					Year 1 Cultured					Year 2 Cultured			
	Year 1 & Post Pueruli	Year 2	Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
			1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
14:0	0.35	0.48	0.41	0.39	0.27	0.28	0.36	0.33	0.26	0.21	0.34	0.28	0.42	0.40	0.27	0.32
16:1n-7c	1.12	1.47	1.44	1.19	2.01	1.06	1.24	1.55	1.18	1.85	1.28	1.25	1.59	1.72	1.18	1.23
16:0	5.17	4.89	4.96	4.86	4.55	4.39	4.99	4.81	4.55	4.34	5.13	4.91	5.21	5.11	5.00	5.00
17:0	0.57	0.41	0.34	0.35	0.43	0.34	0.30	0.34	0.27	0.38	0.29	0.31	0.33	0.31	0.32	0.30
18:2n-6	0.89	0.73	3.04	3.08	0.55	2.71	3.00	2.23	3.12	0.63	2.65	2.81	2.84	2.44	3.05	2.79
18:1n-9	3.74	5.47	3.37	3.20	2.97	2.78	3.36	4.33	6.17	3.87	4.17	3.91	4.47	4.34	4.22	4.14
18:3n-3	0.34	0.17	0.27	0.25	0.18	0.22	0.25	0.23	0.41	0.16	0.23	0.24	0.28	0.28	0.25	0.25
18:1n-7	1.32	1.27	1.16	1.17	0.86	0.92	1.09	1.12	1.18	1.02	1.17	1.08	1.32	1.23	1.23	1.07
18:0	3.79	3.95	3.52	3.56	3.71	3.78	3.61	3.82	3.35	3.96	3.63	3.86	3.77	3.59	3.95	3.79
20:4n-6	6.75	0.52	1.80	1.95	3.24	2.07	1.64	2.65	2.16	4.32	1.98	2.22	2.53	2.80	2.41	2.42
20:5n-3	7.15	5.85	5.97	6.52	6.63	7.34	6.97	7.41	6.51	6.23	6.74	7.12	6.72	6.78	6.71	6.78
20:2n-6	0.52	0.44	0.48	0.52	0.31	0.49	0.52	0.39	0.41	0.34	0.48	0.49	0.43	0.41	0.49	0.44
20:1n-9/n-11+20:3n-3	0.58	0.73	0.71	0.59	0.80	0.48	0.58	0.62	0.61	0.90	0.67	0.60	0.81	0.74	0.68	0.57
20:0	0.42	0.58	0.29	0.28	0.26	0.38	0.25	0.31	0.33	0.29	0.28	0.32	0.34	0.31	0.42	0.32
22:6n-3	1.97	3.07	5.44	5.50	6.36	5.49	5.21	5.16	4.66	5.76	4.85	4.90	4.43	4.77	4.56	4.67
22:5n-3	0.87	0.61	0.33	0.29	0.26	0.21	0.26	0.32	0.31	0.34	0.30	0.28	0.34	0.42	0.33	0.27
Total SFA	11.35	11.55	10.11	10.01	9.76	9.75	10.02	10.39	9.39	9.86	10.29	10.34	10.86	10.54	10.71	10.37
Total MUFA	7.73	9.95	7.30	6.69	7.41	5.84	6.74	8.51	9.84	8.45	7.93	7.49	9.04	8.77	8.13	7.65
Total PUFA	20.37	21.20	18.43	19.07	18.76	19.42	18.64	20.00	18.66	19.20	18.43	19.28	18.89	19.37	19.10	18.78
Total n-3	11.01	10.61	12.65	13.12	14.09	13.79	13.17	13.96	12.49	13.21	12.77	13.24	12.55	13.09	12.62	12.68
Total n-6	9.26	10.51	5.70	5.90	0.05	5.60	5.44	6.00	6.13	5.97	5.62	6.01	6.32	6.24	6.43	6.06
Ratio n-3/n-6	2.23	2.49	4.24	4.01	6.04	4.61	4.01	2.19	2.85	4.21	3.79	3.23	2.39	2.52	2.87	3.77
Ratio EPA/ARA	1.98	1.74	0.06	5.97	4.05	6.67	7.10	2.64	4.15	2.76	5.78	4.81	3.28	2.96	4.06	5.10
Ratio DHA/EPA	0.52	1.29	1.79	1.52	1.90	1.40	1.24	0.68	1.00	1.76	1.22	1.00	0.84	0.85	0.99	1.24

Table 7. Fatty acid composition (in mg/g wet mass) of western rock lobster hepatopancreas tissue for wild and aquaculture-held *P. cygnus* of three different year classes held in heated and ambient tanks and for the different feed and feed frequencies provided to the aquacultured lobsters.

	Diets			Wild		Post Pueruli Cultured					Year 1 Cultured					Year 2 Cultured			
						Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
	Mussel	Pellet Without Krill	Pellet With Krill	Year 1 &Post Pueruli	Year 2	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
14:0	0.10	5.84	3.81	1.35	2.41	2.78	1.26	1.60	1.38	3.85	2.83	3.39	1.27	4.04	4.46	5.40	4.41	6.20	6.18
16:1n-7c	0.21	8.11	7.09	2.64	6.46	6.49	6.16	10.31	4.56	10.47	14.03	19.07	11.16	14.62	16.67	17.95	19.59	16.87	18.11
16:0	0.96	22.36	24.76	10.79	16.61	17.58	15.79	18.12	12.76	28.24	27.30	38.56	16.47	36.81	39.41	39.76	38.28	46.49	46.95
17:0	0.07	0.59	0.48	0.82	0.98	0.82	0.83	1.10	0.72	1.25	1.26	1.62	0.97	1.85	1.95	1.88	1.39	1.60	2.66
18:2n-6	0.09	16.90	14.16	1.09	1.71	9.80	9.40	1.41	5.89	14.59	11.27	18.61	1.57	16.54	19.18	21.39	15.72	26.32	24.38
18:1n-9	0.09	13.28	11.74	6.49	16.35	10.61	9.72	9.00	7.65	18.97	27.61	42.92	14.41	33.41	35.89	36.00	37.36	40.79	42.36
18:3n-3	0.06	2.09	1.71	0.44	0.35	1.07	0.97	0.64	0.62	1.63	1.46	1.90	0.58	1.89	2.09	2.66	2.29	2.46	2.50
18:1n-7	0.12	4.11	5.77	3.00	5.40	4.07	3.97	3.80	3.12	6.98	9.46	13.23	5.06	10.40	11.42	12.36	12.06	13.71	12.70
18:0	0.26	4.87	3.90	5.08	6.13	7.34	7.22	8.79	6.04	12.14	12.32	17.01	9.68	16.74	16.12	16.89	17.95	18.82	20.91
20:4n-6	0.32	0.97	0.76	4.64	2.13	2.50	2.45	3.92	2.12	4.01	5.29	6.64	6.55	5.69	5.96	6.36	8.32	6.83	7.01
20:5n-3	0.83	14.04	15.09	5.04	7.97	9.66	9.07	10.26	7.19	17.13	14.45	19.97	11.81	20.33	21.29	24.35	23.27	27.16	26.75
20:2n-6	0.04	0.19	0.14	0.70	1.06	0.90	0.60	0.83	0.87	2.27	2.38	3.18	0.78	2.86	2.88	2.83	2.54	3.47	3.15
20:1n-9/n-11+20:3n-3	0.31	1.11	0.87	2.09	4.28	3.48	3.03	5.08	2.79	4.82	7.61	8.90	6.36	7.55	8.28	8.85	9.26	9.31	9.43
20:0	0.00	0.18	0.24	0.50	0.88	0.29	0.31	0.28	0.29	0.97	0.68	0.93	0.41	1.12	1.10	1.20	1.59	1.73	2.21
22:6n-3	0.97	11.80	11.80	2.47	6.42	11.90	11.13	14.47	9.96	20.70	22.97	31.52	17.93	27.48	29.51	33.54	33.76	37.64	34.66
22:5n-3	0.09	1.73	1.34	1.37	2.19	1.28	1.17	1.11	0.97	2.10	2.59	3.94	1.59	3.25	3.23	4.02	4.24	6.37	5.11
Total SFA	1.46	35.35	34.63	21.60	31.03	30.75	27.20	31.92	22.66	49.20	48.54	68.11	31.65	65.80	68.27	70.83	70.55	81.25	85.78
Total MUFA	0.85	28.34	26.83	16.96	37.36	27.77	25.57	32.98	20.83	45.88	65.36	92.17	41.90	74.36	80.66	84.77	90.27	88.55	89.04
Total PUFA	2.88	49.12	45.87	19.75	36.38	40.65	38.13	37.98	30.65	69.09	74.14	101.21	51.27	92.73	99.90	111.12	110.09	128.97	120.41
Total Fatty Acids	9.73	220.99	210.98	106.80	186.10	189.75	173.96	193.59	141.06	314.28	351.55	492.87	231.40	437.48	468.27	502.15	502.95	564.52	560.29
Total n-3	2.27	30.36	30.63	11.09	20.69	26.11	24.32	29.90	20.42	46.14	50.02	66.09	38.25	61.34	65.74	72.82	75.08	85.52	79.36
Total n-6	0.59	18.76	15.23	8.35	15.21	14.26	13.54	7.67	9.95	22.70	23.57	33.83	12.82	30.97	33.58	37.53	34.40	43.14	40.62
Ratio n-3/n-6	0.50	1.59	1.95	0.38	0.42	0.63	0.60	1.40	0.58	0.88	0.83	0.83	1.13	1.01	0.86	1.08	0.97	0.99	1.03
Ratio EPA/ARA	0.34	14.26	19.39	0.31	0.27	1.31	1.23	0.94	0.95	1.84	1.08	1.31	0.68	1.91	1.58	2.12	1.29	2.00	1.95
Ratio DHA/EPA	0.16	0.83	0.76	0.15	0.25	0.43	0.41	0.51	0.39	0.53	0.62	0.68	0.59	0.69	0.62	0.76	0.64	0.69	0.65

Table 8. Fatty acid composition (in mg/g wet mass) of western rock lobster abdominal muscle tissue for wild and aquaculture-held *P. cygnus* of three different year classes held in heated and ambient tanks and for the different feed and feed frequencies provided to the aquacultured lobsters.

	Wild		Post Puerulus Cultured					Year 1 Cultured					Year 2 Cultured			
	Year 1 & Post Pueruli	Year 2	Ambient			Heated 23°C		Ambient			Heated 23°C		Ambient		Heated 23°C	
			1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	Mussel	1x Fed	3x Fed	1x Fed	3x Fed	1x Fed	3x Fed
14:0	0.04	0.04	0.05	0.05	0.04	0.03	0.06	0.08	0.04	0.03	0.06	0.05	0.10	0.09	0.04	0.05
16:1n-7c	0.14	0.13	0.19	0.16	0.27	0.13	0.20	0.39	0.20	0.24	0.22	0.22	0.37	0.37	0.20	0.21
16:0	0.64	0.43	0.65	0.67	0.61	0.55	0.81	1.21	0.79	0.57	0.87	0.88	1.20	1.10	0.83	0.84
17:0	0.07	0.04	0.04	0.05	0.06	0.04	0.05	0.09	0.05	0.05	0.05	0.06	0.08	0.07	0.05	0.05
18:2n-6	0.11	0.06	0.40	0.42	0.07	0.34	0.48	0.56	0.54	0.08	0.45	0.50	0.65	0.52	0.50	0.47
18:1n-9	0.46	0.48	0.44	0.44	0.39	0.35	0.54	1.09	1.07	0.51	0.71	0.70	1.03	0.93	0.70	0.69
18:3n-3	0.04	0.02	0.04	0.03	0.02	0.03	0.04	0.06	0.07	0.02	0.04	0.04	0.07	0.06	0.04	0.04
18:1n-7	0.16	0.11	0.15	0.16	0.11	0.12	0.18	0.28	0.20	0.13	0.20	0.19	0.30	0.26	0.20	0.18
18:0	0.47	0.35	0.46	0.49	0.49	0.48	0.58	0.96	0.58	0.52	0.62	0.69	0.87	0.77	0.65	0.64
20:4n-6	0.84	0.05	0.23	0.27	0.43	0.26	0.26	0.67	0.38	0.57	0.34	0.40	0.58	0.60	0.40	0.41
20:5n-3	0.89	0.51	0.78	0.90	0.88	0.92	1.13	1.87	1.13	0.82	1.15	1.28	1.55	1.46	1.11	1.14
20:2n-6	0.06	0.04	0.06	0.07	0.04	0.06	0.08	0.10	0.07	0.05	0.08	0.09	0.10	0.09	0.08	0.07
20:1n-9/n-11+20:3n-3	0.07	0.06	0.09	0.08	0.11	0.06	0.09	0.16	0.11	0.12	0.11	0.11	0.19	0.16	0.11	0.10
20:0	0.05	0.05	0.04	0.04	0.03	0.05	0.04	0.08	0.06	0.04	0.05	0.06	0.08	0.07	0.07	0.05
22:6n-3	0.24	0.27	0.71	0.76	0.84	0.69	0.84	1.30	0.81	0.76	0.83	0.88	1.02	1.03	0.75	0.78
22:5n-3	0.11	0.05	0.04	0.04	0.03	0.03	0.04	0.08	0.05	0.04	0.05	0.05	0.08	0.09	0.06	0.04
Total SFA	1.41	1.01	1.32	1.38	1.30	1.23	1.62	2.62	1.63	1.30	1.76	1.85	2.50	2.27	1.77	1.74
Total MUFA	0.96	0.87	0.95	0.92	0.98	0.74	1.09	2.15	1.71	1.11	1.35	1.34	2.08	1.89	1.34	1.28
Total PUFA	2.53	1.86	2.40	2.63	2.49	2.45	3.01	5.05	3.24	2.53	3.14	3.45	4.35	4.17	3.15	3.15
Total Fatty Acids	9.31	6.45	9.04	9.58	9.21	8.56	11.14	18.81	12.74	9.50	12.08	12.84	17.18	15.98	12.06	11.92
Total n-3	1.37	0.93	1.65	1.81	1.87	1.74	2.13	3.52	2.17	1.74	2.18	2.37	2.89	2.82	2.08	2.13
Total n-6	1.15	0.92	0.74	0.81	0.61	0.71	0.88	1.52	1.07	0.79	0.96	1.08	1.46	1.34	1.06	1.02
Ratio n-3/n-6	0.28	0.22	0.55	0.55	0.80	0.58	0.65	0.55	0.50	0.55	0.65	0.58	0.55	0.54	0.47	0.63
Ratio EPA/ARA	0.25	0.15	0.82	0.82	0.54	0.84	1.15	0.67	0.72	0.36	0.99	0.86	0.75	0.64	0.67	0.85
Ratio DHA/EPA	0.06	0.11	0.23	0.21	0.25	0.18	0.20	0.17	0.17	0.23	0.21	0.18	0.19	0.18	0.16	0.21

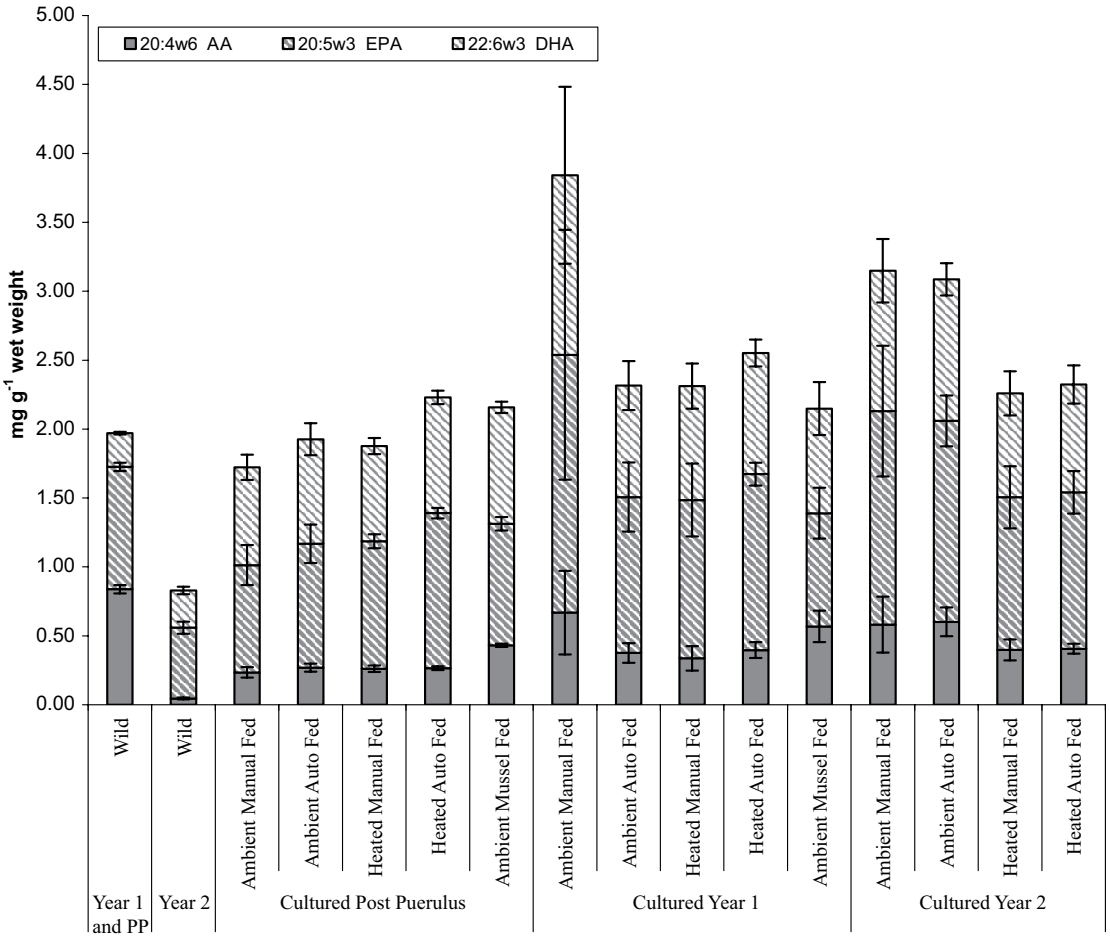

Figure 1. Arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the tail muscle of western rock lobster, *Panulirus cygnus*, presented as mean ± se mg/g wet weight. Manual fed refers to being fed once per night; auto fed refers to being fed three times nightly.

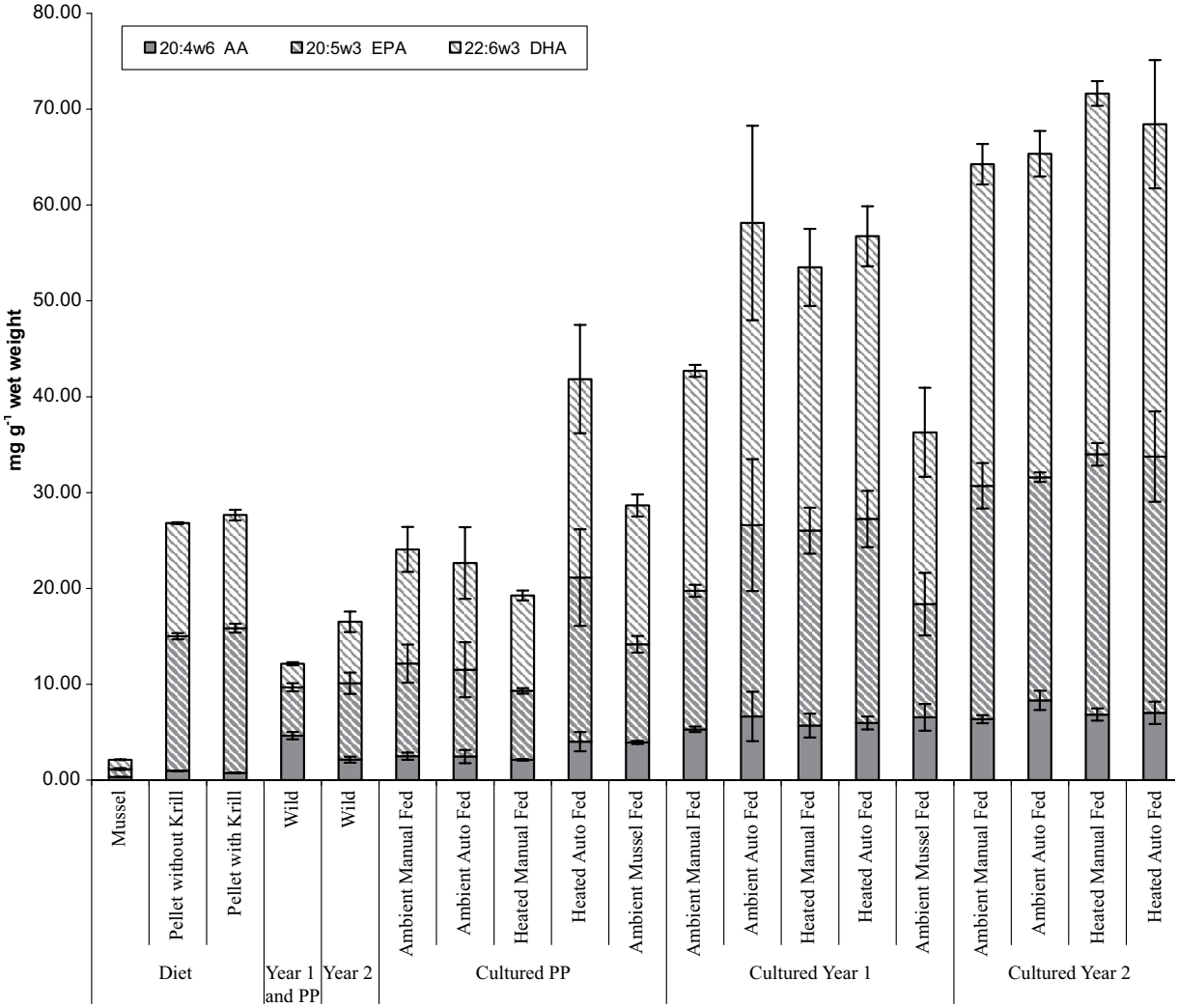

Figure 2. Arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in diets and hepatopancreas of western rock lobster, *Panulirus cygnus*, presented as mean ± se mg/g wet weight. Manual fed refers to being fed once per night; auto fed refers to being fed three times nightly.

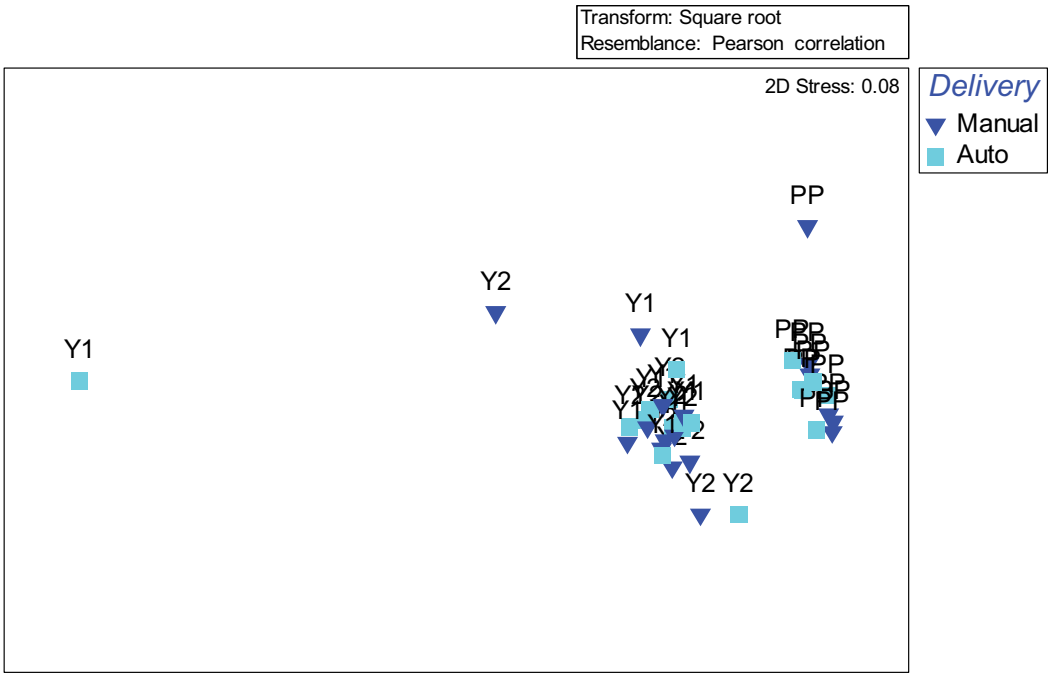

Figure 3. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* muscle tissue for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured animals which were fed once (manual) and three times (auto) per night. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

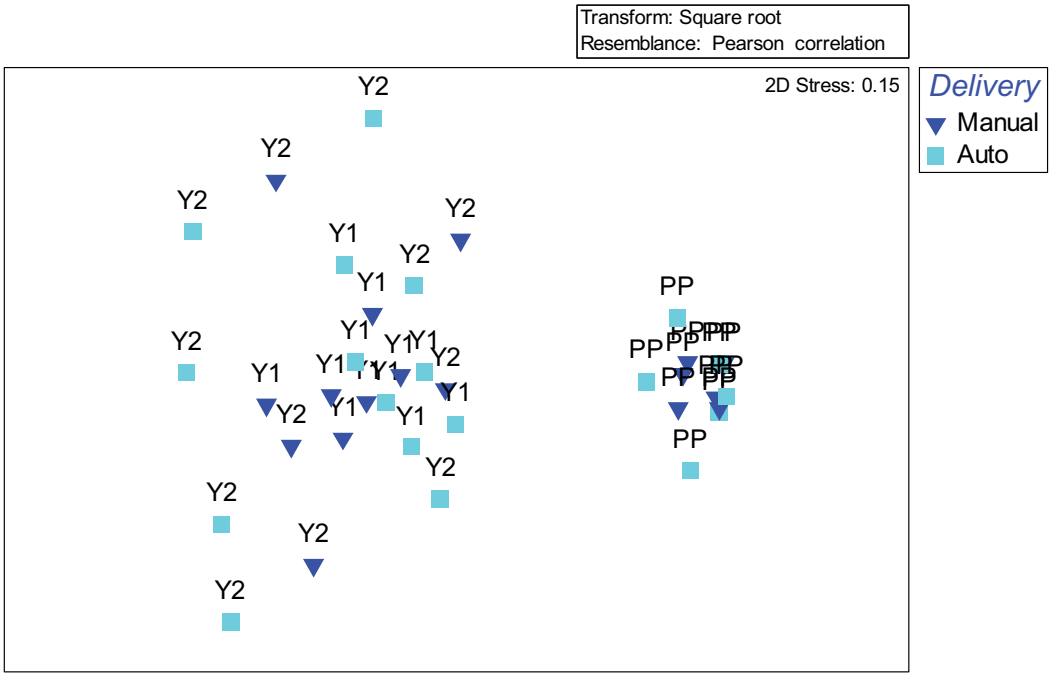

Figure 4. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* hepatopancreas for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured animals which were fed once (manual) and three times (auto) per night. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

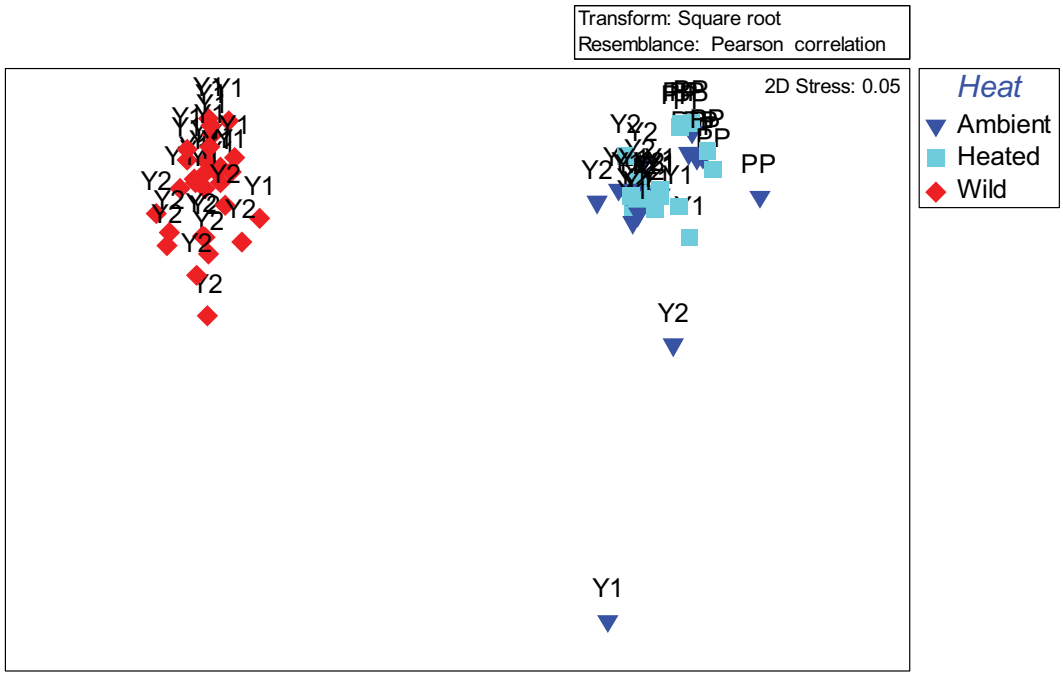

Figure 5. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* muscle tissue for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured animals which were held at ambient temperature and at 23°C. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

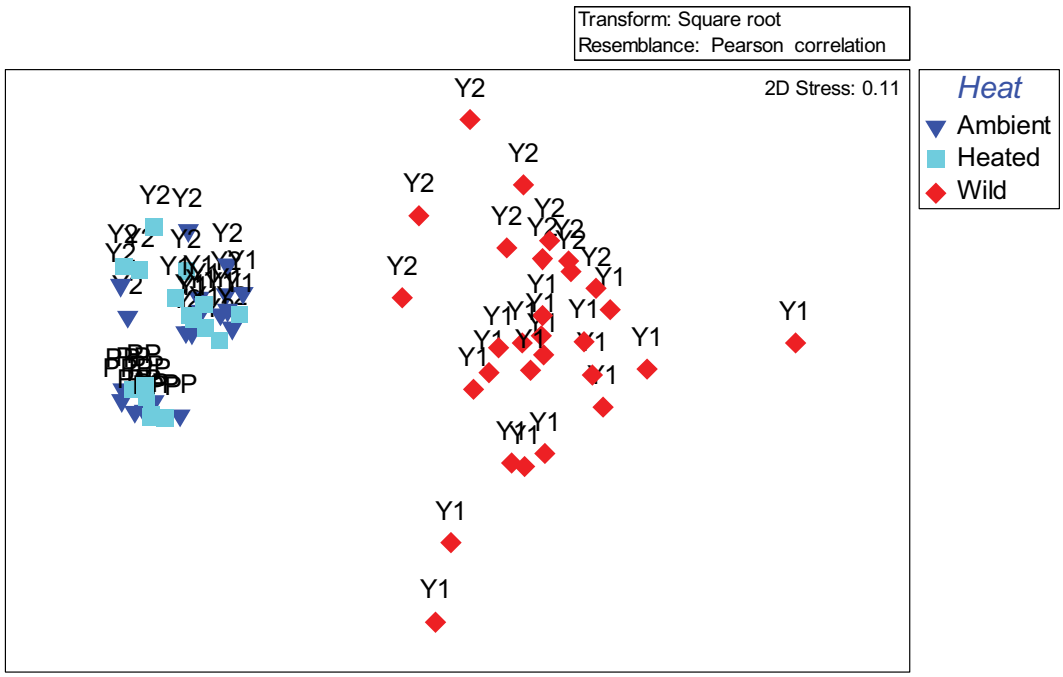

Figure 6. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* hepatopancreas for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured and wild animals which were held at ambient temperature and at 23°C. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

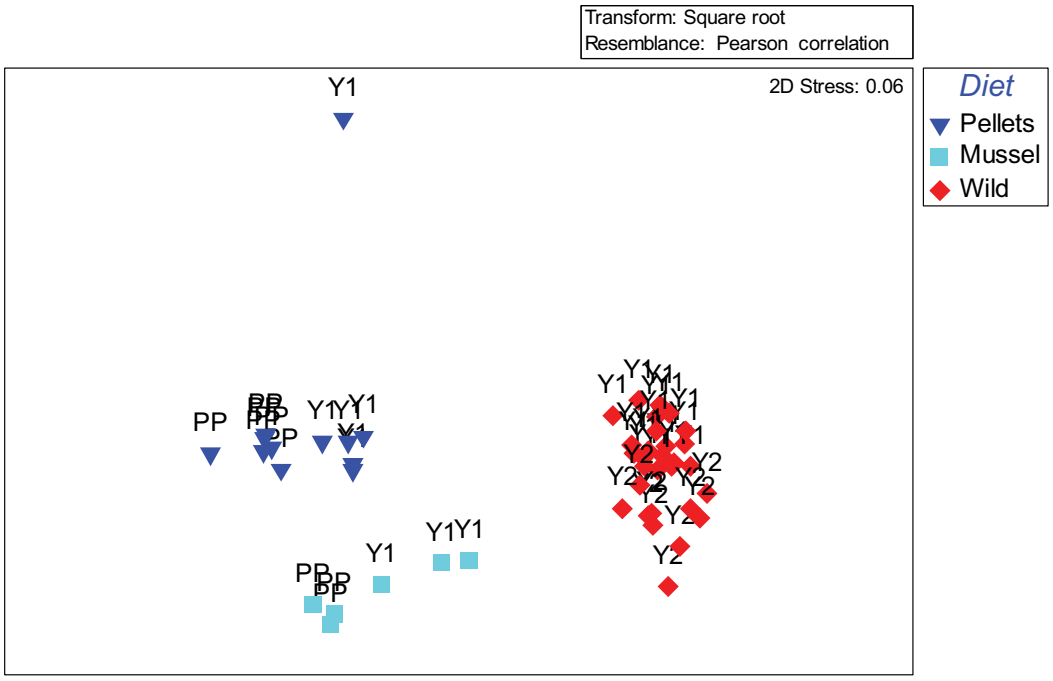

Figure 7. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* muscle tissue for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured animals fed mussel or pellets vs wild lobsters that had been consuming a natural diet. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

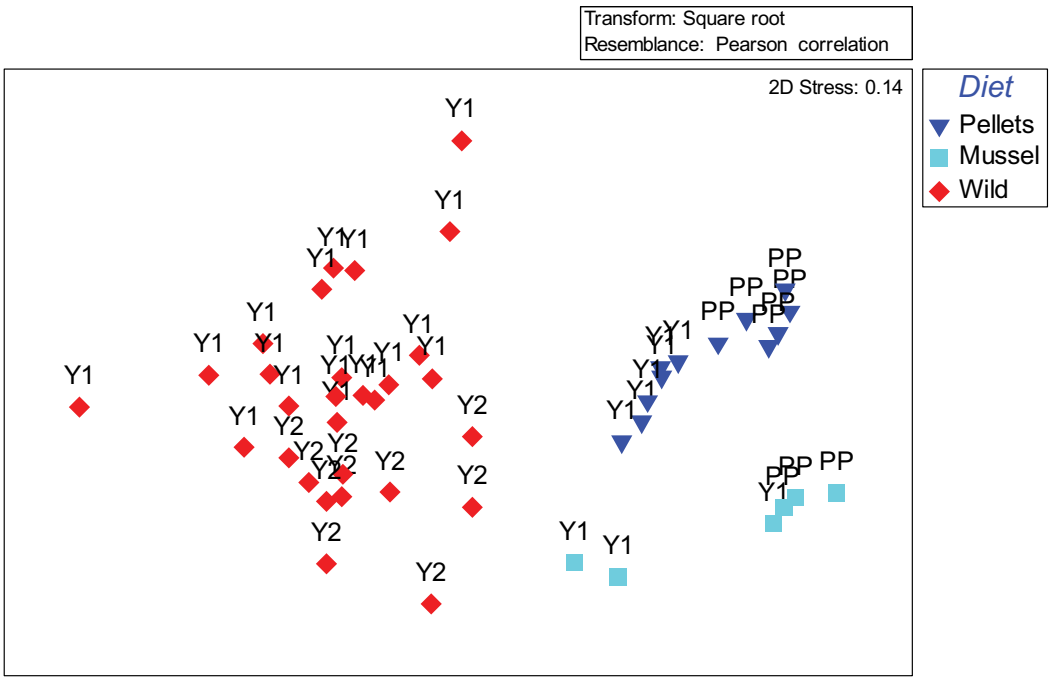

Figure 8. MDS scatterplot comparing the fatty acid composition of *Panulirus cygnus* hepatopancreas for three year classes (post pueruli, year 1 and year 2 post settlement) of aquacultured animals fed mussel or pellets vs wild lobsters that had been consuming a natural diet. PP indicates post pueruli; Y1 and Y2 are juvenile lobsters from Year 1 and Year 2 cohort respectively.

5.0 Discussion

The aim of the research was to determine the influence of lipid content, lipid class composition and fatty acid composition of western rock lobster muscle tissue and hepatopancreas on aquaculture-held lobsters of three different cohorts, fed a diet of mussel compared to pellet; (ii) fed once per day compared to three times a day; (iii) held at ambient temperatures compared to 23 °C; and (iv) compared to wild caught animals that had lived on a natural diet. In crustaceans, the digestive gland stores lipids and the composition of the lipid classes as well as the fatty acid distribution can be used as an indicator of health and nutritional status of the animal (D'Abramo 1997). The biochemical composition of abdominal muscle has also been used as an indicator of energy reserve (Cockcroft 1997).

The MDS scatterplots showed much greater scatter for hepatopancreas compared to muscle tissue samples. Other authors (Trendall and Prescott 1989; Cockcroft 1997) have found the hepatopancreas to be a more sensitive indicator of physiological stress than muscle tissue, however the abdominal muscle is more important as an energy reserve (Dall 1974; Trendall and Prescott 1989). The biochemical composition of the crustacean hepatopancreas fluctuates at different stages of the moult cycle (Cockcroft 1997; Barclay et al. 1983), and it is possible that the scatter for hepatopancreas samples was due to variations in moult stage between individuals within a cohort. Given the similar trends in lipid and fatty acid response to the different treatments compared in this study, we would consider muscle tissue to be a more reliable overall indicator of fatty acid response to holding conditions that were compared in this study.

In all cases the differences in scatter plots in fatty acid composition between year 1 and year 2 tank held animals shown by the MDS scatterplots was less marked than when these two-year classes were compared to the post pueruli year class. We believe that this may be due to the post pueruli being fed a mussel-only diet for the first two months after stocking before being weaned onto the pellet weekdays, mussel weekend feeding regime to which the other year-classes were subjected for the full duration of the trial.

There was a degree of contradictory information in the effect that temperatures had on the fatty acid profiles of the cultured animals. It has been shown in other species that the higher the temperature, the greater the reduction in the percentage of long chain fatty acids, in particular EPA and DHA (Miller et al. 2006). Increased amounts of unsaturated oil in animals at lower temperatures are due to a function of the melting temperature and digestibility of these lipids at reduced temperatures (Olsen and Ringo 1998). The fatty acid profiles of tail muscle tissue from animals fed once per day conformed with what has been recorded in other species, namely higher percentages of EPA and DHA in animals held at 23˚C compared to ambient temperatures. However, the converse situation occurred in the western rock lobsters fed three times a day. We believe this difference may be due to increased dietary bioavailability in the diet of the animals that were fed more frequently.

The major difference in fatty acid composition across the treatments compared in this study, were the differences related to diet. This ability to separate tissue samples taken from wild-caught and cultured rock lobsters using multivariate analysis based on fatty acid profiles, has also been reported for *Jasus edwardsii* (Nelson et al. 2008). As with many oil replacement trials the old adage "you are what you eat" holds true (Glencross et al., 2002; Glencross et al., 2003; Miller et al. 2007). The bioconversion of dietary fatty acids to longer chain PUFA in higher order animals is inefficient, due to the rate of the reactions of enzymes involved in this process, in particular the omega 6 desaturase (Tocher et al. 1998). Furthermore, their natural diet tends

to be mostly abundant in LC-PUFA, which largely cancels the need to convert fatty acids to this form. The difference in fatty acid composition in aquaculture reared, compared to wild caught animals, possibly indicates that long chain fatty acids were more limited in the wild compared to the formulated feed supplied to the aquaculture-held lobsters.

With the granting of an experimental permit to harvest pueruli for aquaculture in Western Australia, there have been concerns about the Regional Services Branch (the Branch responsible for fisheries enforcement) being able to distinguish between aquaculture and wild caught product in the event of illegal activity. This study has shown that the fatty acids of the two products are likely to be distinctive enough for any perceived illegal activity to be resolved by biochemical analysis of muscle or hepatopancreas tissue samples.

6.0 Literature Cited

Ackman, R.G. 1981. Lipids Part D. In: Methods in Enzymology. Academic Press, New York.

Anon. 2008. Lobsters: feeding the pinch. Seafood International, June 2008. 31-33.

Barclay, M.C., Dall, W. and Smith, D.M. 1983. Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn. J. Exp. Mar. Biol. Ecol. **68**, 229-244.

Bligh, E.G. and Dyer, W.G. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**, 911-917.

Cockcroft, A.C. 1997. Biochemical composition as a growth predictor in male west-coast rock lobster (*Jasus lalandii*). Mar. Freshwater Res. **48**, 845-856.

D'Abramo, L.R. 1997. Triacylglycerols and fatty acids. In: D'Abramo, L. R., Conklin, D.E., Akiyama, D.M. (Eds). Advances in World Aquaculture - Crustacean Nutrition, Vol 6. World Aquaculture Society, Baton Rouge, LA, USA. pp 587.

Dall, W. 1974. Indices of nutritional state in the western rock lobster *Panulirus longipes* (Milen-Edwards). II. Gastric fluid constituents. J. Exp. Mar. Biol. Ecol. **18,** 227-238.

Glencross, B.D., Smith, D.M., Thomas, M.R. and Williams, K.C., 2002. Optimising the essential fatty acid and total neutral lipid requirements for weight gain of the prawn, *Penaeus monodon*. Aquaculture **204**, 85-99.

Glencross, B.D., Hawkins, W.E. and Curnow, J.G., 2003. Restoration of the fatty acid composition of red seabream (*Pagrus auratus*) after grow-out on plant oil based diets. Aquaculture Nutrition **9**, 409-418.

Glencross, B.D., Booth, M. and Allan, G.L. 2007. A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquacult. Nutr. **13**, 17 – 34.

Johnston, D., Melville-Smith, R., Hendriks, B. and Phillips, B. 2008. Growth rates and survival of western rock lobster (*Panulirus cygnus*) at two temperatures (ambient and 23°C) and two feeding frequencies. Aquaculture **279**, 77-84.

Miller, M.R., Nichols, P.D., Barnes, J., Davies, N.W., Peacock, E.J. and Carter, C.G. 2006. Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar* L.) grown at elevated temperature, Lipids **41**, 865-876.

Miller, M.R., Nichols, P.D., Carter, C.G. 2007. Replacement of fish oil with thraustochytrid *Schizochytrium sp.* L. oil in Atlantic salmon parr (*Salmo salar* L) diets. Comp. Biochem. Physiol. A. **148**, 382-92.

Nelson, M.M., Olley, J., Crear, B.J., Lewis, T. and Nichols, P.D. 2005. Comparison of wild and cultured adult southern rock lobster *Jasus edwardsii*: Growth, sensory analysis and oil composition. Food Australia **57**, 499-508.

Olsen, R.E., Ringo, E. 1998. The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. Aqua Res. **29**, 695-701.

Sargent, J.R., Bell, M.V., Bell, J.G., Henderson, R.J., and Tocher, D.R. 1995. Origins and functions of n-3 polyunsaturated fatty acids in marine organisms. In: Cevc, G., Pactauf, F. (Eds). Phospholipids: Characterisation, metabolism and novel biological applications. Amer. Oil. Chem. Press., Champaign, USA, pp 248-259.

Tocher, D.R., Leaver, M.J., Hodgson, P.A. 1998. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Prog.

Lipid Res. **37(2-3),** 73-117 .

Trendall, J.T. and Prescott, J. 1989. Sever physiological stress associated with the annual breeding emigration of *Panulirus ornatus* in the Torres Strait. Mar. Ecol. Prog. Ser. **58**, 29-39.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture **177**, 191-199.

Volkman, J.K. and Nichols, P.D. 1991. Application of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine environmental samples. J. Planar Chromatogr. **4**, 19-26.

Appendix IV

Abbreviations and the major fatty acid nomenclature

Monounsaturated
18:1n-9, OA, oleic acid

Omega 3 (n-3) series
18:3n-3, αLNA, α-linolenic acid
18:4n-3, SDA, stearidonic acid
20:4n-3, ETA, eicosatetraenoic acid
20:5n-3 EPA, eicosapentaenoic acid
22:5n-3 DPA(3), docosapentaenoic acid
22:6n-3 DHA, docosahexaenoic acid

Omega 6 (n-6) series
18:2n-6 LOA, linoleic acid
20:4n-6 ARA, arachidonic acid
22:5n-6 DPA(6), docosapentaenoic acid

The following abbreviations are used in this report.
ARA, arachidonic acid
αLNA, α-linolenic acid
DHA, docosahexaenoic acid
DM, dry matter
DPA, docosapentaenoic acid
EPA, eicosapentaenoic acid
ETA, eicosatetraenoic acid
FA, fatty acid(s)
FALD fatty aldehyde
FAME, fatty acid(s) methyl ester
FFA, free fatty acids
GC, gas chromatography
GC-MS, gas chromatography mass spectroscopy
LNA, linolenic acid
LC, long chain (≥ C_{20})
MUFA, monounsaturated fatty acid(s)
NMI, nonmethylene-interruped
OlA, oleic acid
PL, polar lipid
SDA, stearidonic acid
SFA, saturated fatty acid(s)
ST, sterol(s)
TAG, triacylglycerol
TLC-FID, thin layer chromatography-flame ionisation detection
TLE, total lipid extract
n-3, omega 3
n-6, omega 6
WW, wet weight