Growth rates and survival of western rock lobster (*Panulirus cygnus*) at two temperatures (ambient and 23^{o}C) and two feeding frequencies

Danielle Johnston[1]*, Roy Melville-Smith[1], Blair Hendriks[1], Bruce Phillips[2]

[1] Western Australia Department of Fisheries, Western Australia Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia 6920.

[2]Department of Environmental Biology, Muresk Institute, Curtin University of Technology, Western Australia, 6845.

*Corresponding Author: Tel: 61 8 9203 0248, Fax: 61 8 9203 0199; Email Address: djohnston@fish.wa.gov.au

ABSTRACT

Wild caught post-pueruli, year one and year two post settlement juvenile western rock lobster, *Panulirus cygnus*, were held at ambient temperatures (15.6^{o}C to 23.1^{o}C; mean 19.0 ± 0.07^{o}C) or at 23^{o}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^{o}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^{o}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^{o}C than at ambient temperatures ($p<0.05$), with the growth of post-pueruli almost double at 23^{o}C (weight gain at 23^{o}C vs ambient: 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^{o}C weight and carapace length were significantly greater when fed once per day ($p<0.05$). Feed intake (g pellet dry matter lobster^{-1} day^{-}

^{1}) of pellet was higher at 23^{o}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^{o}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^{o}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^{o}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 co-incide with nocturnal feeding behaviour is recommended.

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

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 to achieve market size in the shortest period possible. However, mortalities under elevated temperatures 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^{o}C and 26^{o}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^{o}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, 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 (5-22g) *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 to co-incide with nocturnal feeding behaviour.

P. cygnus requires daily feeding for optimum growth and survival (Chittelborough, 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 temperature (23^{o}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*.

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} (10 post-pueruli, 20 year 1 juveniles and 20 year 2 juveniles per tank). 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^{o}C ± 1^{o}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, 2400h 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. Pellet diet was 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^{o}C. The proximate composition on a % dry matter basis was protein 55%, lipid 10%, carbohydrate 24%, ash 11% (see Johnston et al., 2007 for details on ingredients and formulation of pelleted feed). 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 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., 2007) 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 were fed mussels for the first two months of the trial before being weaned onto the pellet diet.

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 (Brunsen et al., 1997) and drying overnight at 70^{o}C. Apparent feed intake (g DM day^{-1}) calculations accounted for leach rates (stability) of the diet. Estimates of feed intake refer only to that of pellet feed and for 2 out of the 7 days of the week (28% of the time) the lobsters were fed on fresh mussels, the intake of which was not quantified. Apparent feed intake calculations for post-pueruli do not include the initial 2 month period when these animals were fed entirely on mussels and represents feed intake of pellets only.

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^{o}C and 23.1^{o}C (mean 19.0 ± 0.07^{o}C). Heated water was held at 23.0 ± 0.35 ^{o}C, apart from occasional instances when there were fluctuations to as low as 21.8 ^{o}C and as high as 24.4 ^{o}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 bi-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 (mean pellet loss 20%). The feed remaining was collected into a sieve, washed with fresh water to remove salt (Brunsen et al., 1997) and dried in an oven overnight at 70^{o}C.

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

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 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.

RESULTS

Mean survival across all temperature and feed frequency treatments after 12 months was highest for year 2 lobsters (81 ± 3.3 %), with lower but similar survival between year 1 lobsters (63 ± 4.2 %) and post-pueruli (63 ± 7.3 %). 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 $p<0.05$; 14% higher for year 2, $p<0.05$) (Table 1; Fig 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).

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 (Fig 2).

All lobsters grew faster at 23^{o}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, Fig 3). In particular, growth (% weight gain and g/day) of post-puerulus at 23^{o}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^{o}C tanks, however growth in ambient tanks slowed during winter and was negligible for year 1 and 2 lobsters (Fig 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 growth rates of all lobsters in 23^{o}C tanks steadily increased throughout the 12 month trial (Fig 3).

Feeding frequency did not influence the growth of year 1 and year 2 lobsters over 12 months (Table 2; Fig 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^{o}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) (Fig 5).

Mean apparent feed intake (AFI, g DM lobster^{-1} day^{-1}) of pellets by post-pueruli, year 1 and year 2 lobsters was significantly higher at 23^{o}C than at ambient temperatures (post-pueruli $F_{(1,6)}$=8.57, p=0.03; year 1 $F_{(1,6)}$=5.90, p<0.05; year 2 $F_{(1,6)}$=23.48, p<0.01; Table 2; Fig. 6). 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 (Fig 6). Feed intake of year 1 and 2 lobsters held at 23^{o}C also declined in winter but not to the same extent, with a plateau in intake for post-pueruli (Fig. 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; Fig 7).

DISCUSSION

Temperature

For *Panulirus cygnus* culture to be economically viable, it is essential to reach market size in the shortest period possible. 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^{o}C - 26^{o}C (Chittleborough, 1975). In this study, *P. cygnus* post-pueruli, year 1 and 2 juveniles held at 23^{o}C grew significantly faster than those held at ambient temperatures, with no significant reduction in the survival of post-pueruli, and only small reductions in survival of year 1 (11%) and year 2 juveniles (14%). 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^{o}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^{o}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 (1974; 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^{o}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^{o}C would not contribute significantly to their increased growth at elevated temperatures in this study.

Marked increases in growth of all lobsters held at 23^{o}C were consistent with their significantly higher feed consumption compared to lobsters held at ambient temperatures. Similar feed intake responses by *J. edwardsii* held at elevated temperatures have also been reported by Crear et al. (2000) with these trends most likely linked to increased metabolic rate.

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, 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 little or no difference in 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. Smith et al., (2005) 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 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 greater 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 utilization 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).

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 and only minimal 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, 1974; 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, particularly with respect to the costs of heating water. There are no obvious benefits to feeding *P. cygnus* juveniles multiple times per night in terms of growth or survival. Feeding the *P. ornatus* pellet diet once daily to excess just prior to dusk to co-incide with the noturnal feeding behaviour of *P. cygnus* is therefore recommended.

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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**
PostPuerulus	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 < 0.05		**P < 0.05**	
Year 2	74.2 ± 4.4	88.3 ± 2.8	82.5 ± 3.1	80.0 ± 6.1
	P < 0.01		P = 0.97	

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 temperature or feeding frequency over time of trial (SGR, %WG, growth coefficient, AFI), and two way ANOVA to determine significant differences between final data (initial weight, final weight)

Size Class	Parameter	Temperature		Feeding Frequency	
		23^{o}C	Ambient	1	3
PostPuerulus	**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
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
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

Figures

Figure 1. Differences in survival of *Panulirus cygnus* at two temperature regimes for each size class over 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.

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

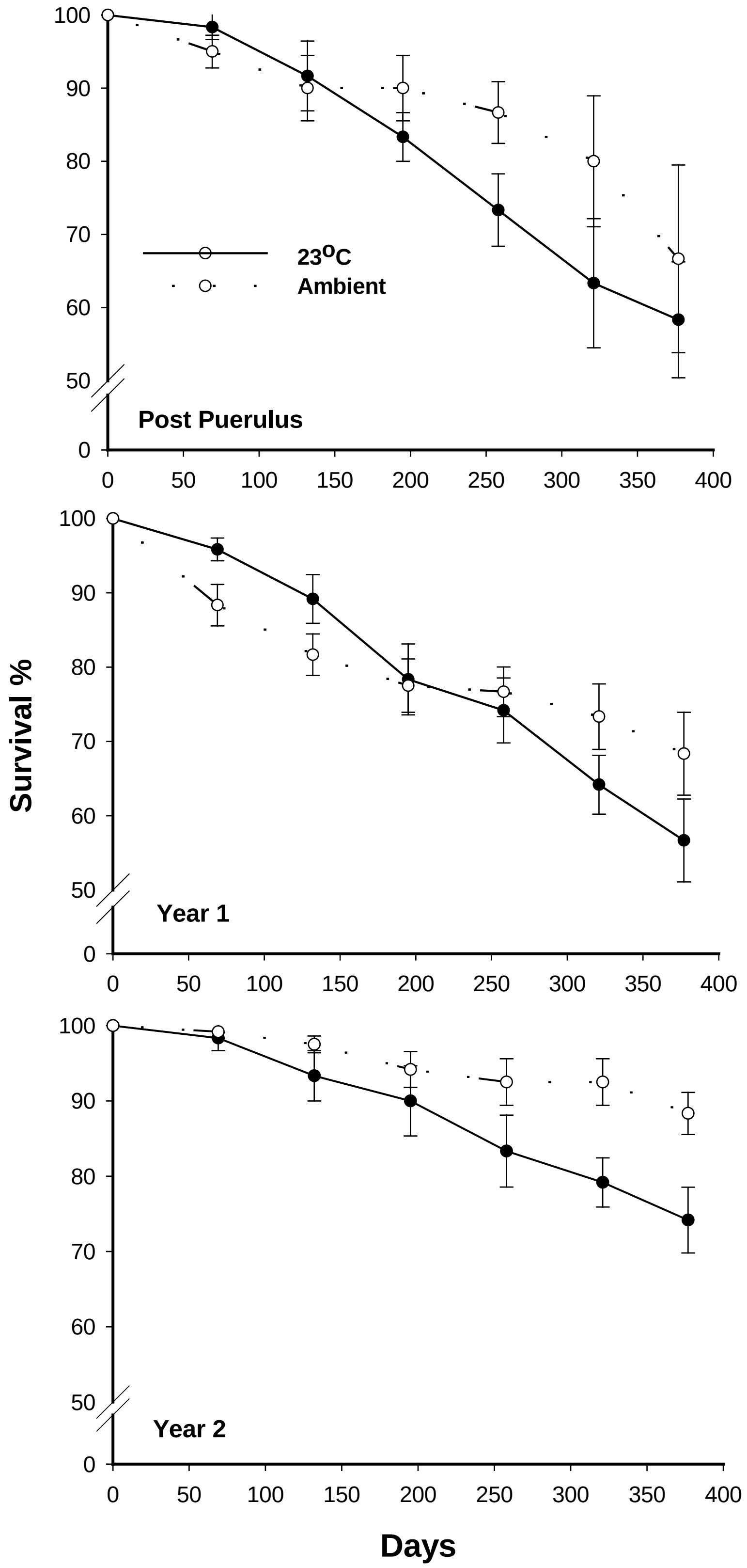
Figure 3. Growth rates of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two temperature regimes after 12 months (December 04 – December 05). Data are mean and standard error. Weight gain in grams per day between each time period is indicated. Ambient temperatures ranged between 15.6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

Figure 4. Growth rates of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two feeding frequencies over 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.

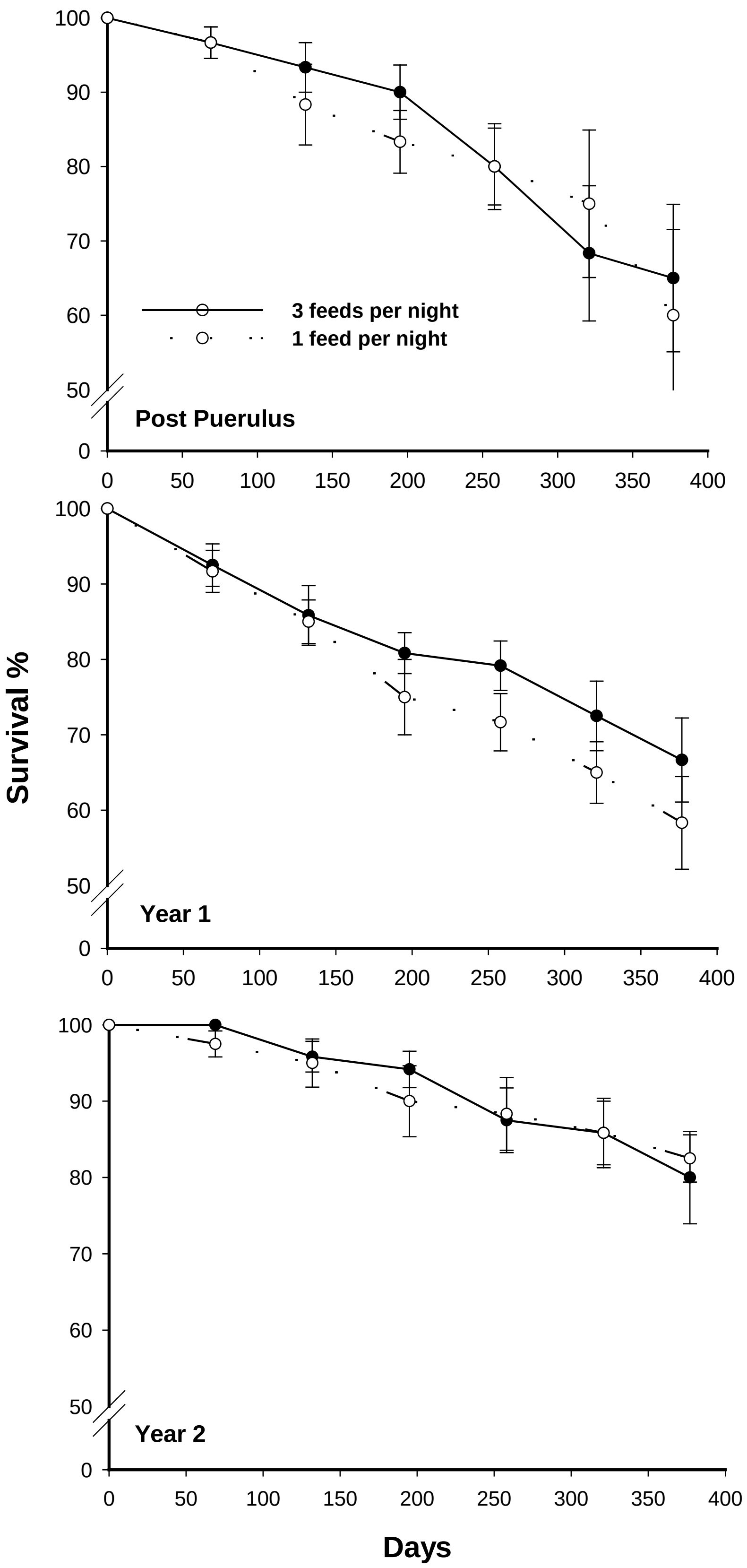
Figure 6. Apparent feed intake of *Panulirus cygnus* post-pueruli, year 1 and year 2 juveniles at two temperature regimes over 12 months (December 04 – December 05). Data are mean and standard error. Time period: D1-2, 8 week period between consecutive tank drains and measurements. Ambient temperatures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



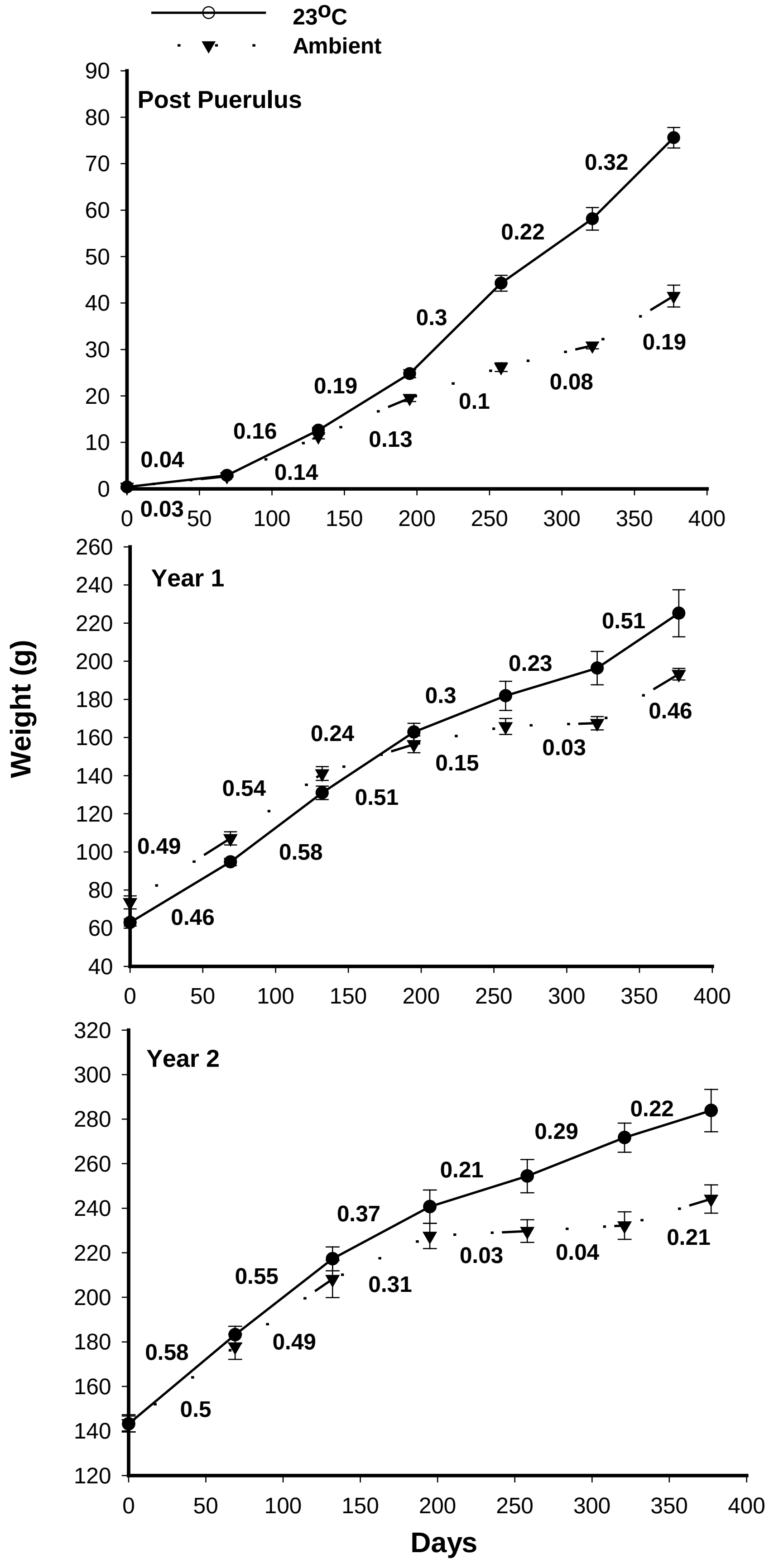
Survival %
Days
23°C
Ambient
Post Puerulus
Year 1
Year 2
0
50
60
70
80
90
100
0 50 100 150 200 250 300 350 400

Figure 1.



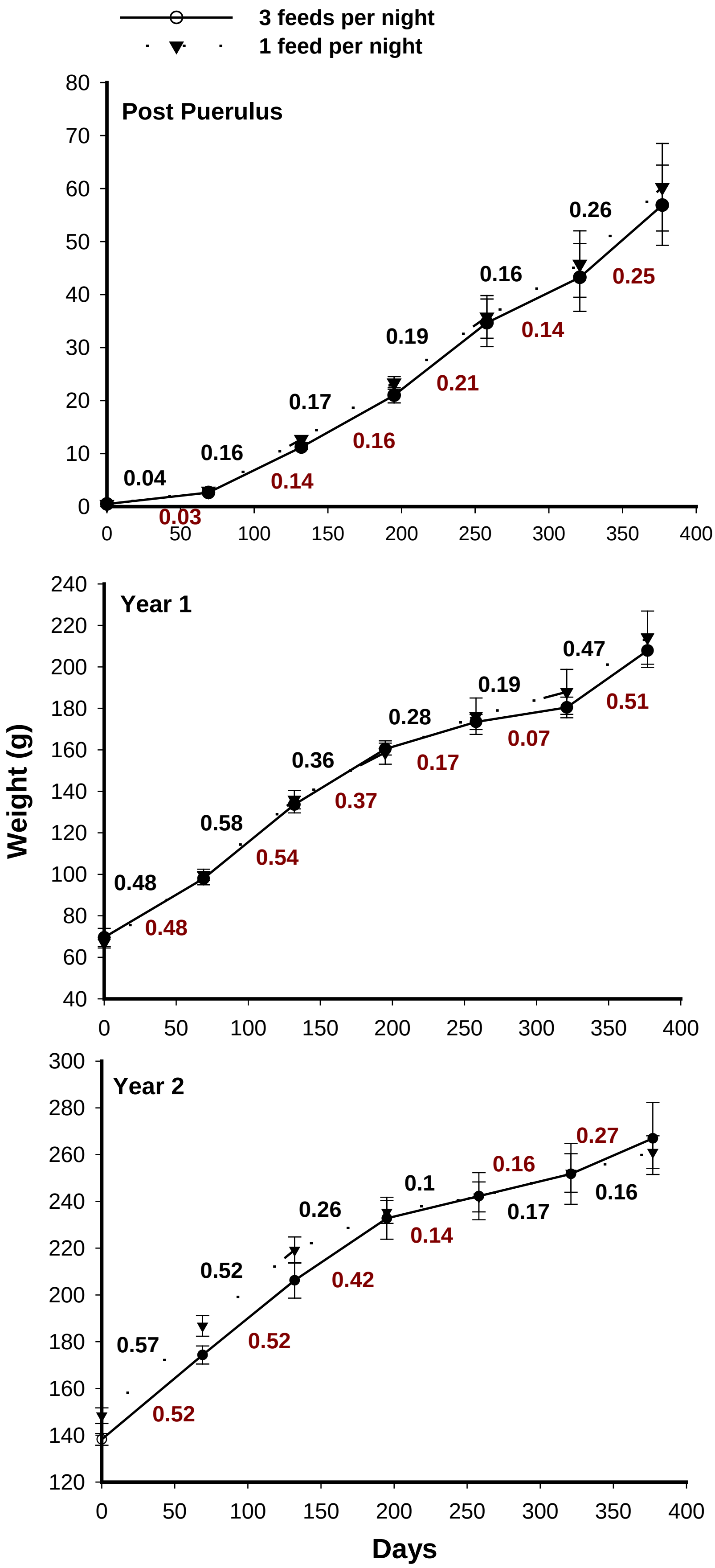
3 feeds per night
1 feed per night
Post Puerulus
Year 1
Year 2
Survival %
Days
0
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400

600 **Figure 2.**



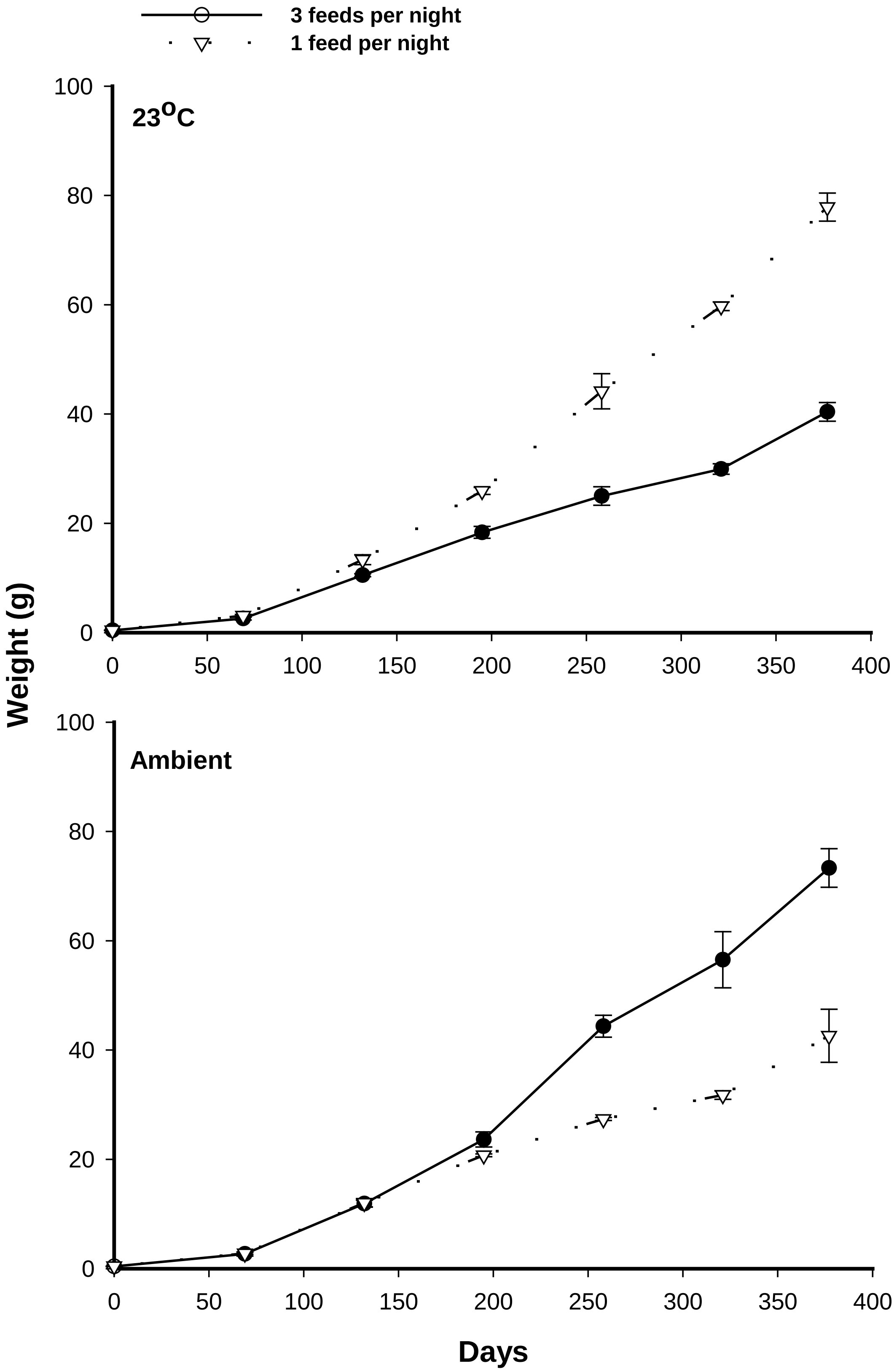
23°C
Ambient
Post Puerulus
Year 1
Year 2
Weight (g)
Days
0.04
0.03
0.16
0.14
0.19
0.13
0.3
0.1
0.22
0.08
0.32
0.19
0.49
0.46
0.54
0.58
0.24
0.51
0.3
0.15
0.23
0.03
0.51
0.46
0.58
0.5
0.55
0.49
0.37
0.31
0.21
0.03
0.29
0.04
0.22
0.21

Figure 3.



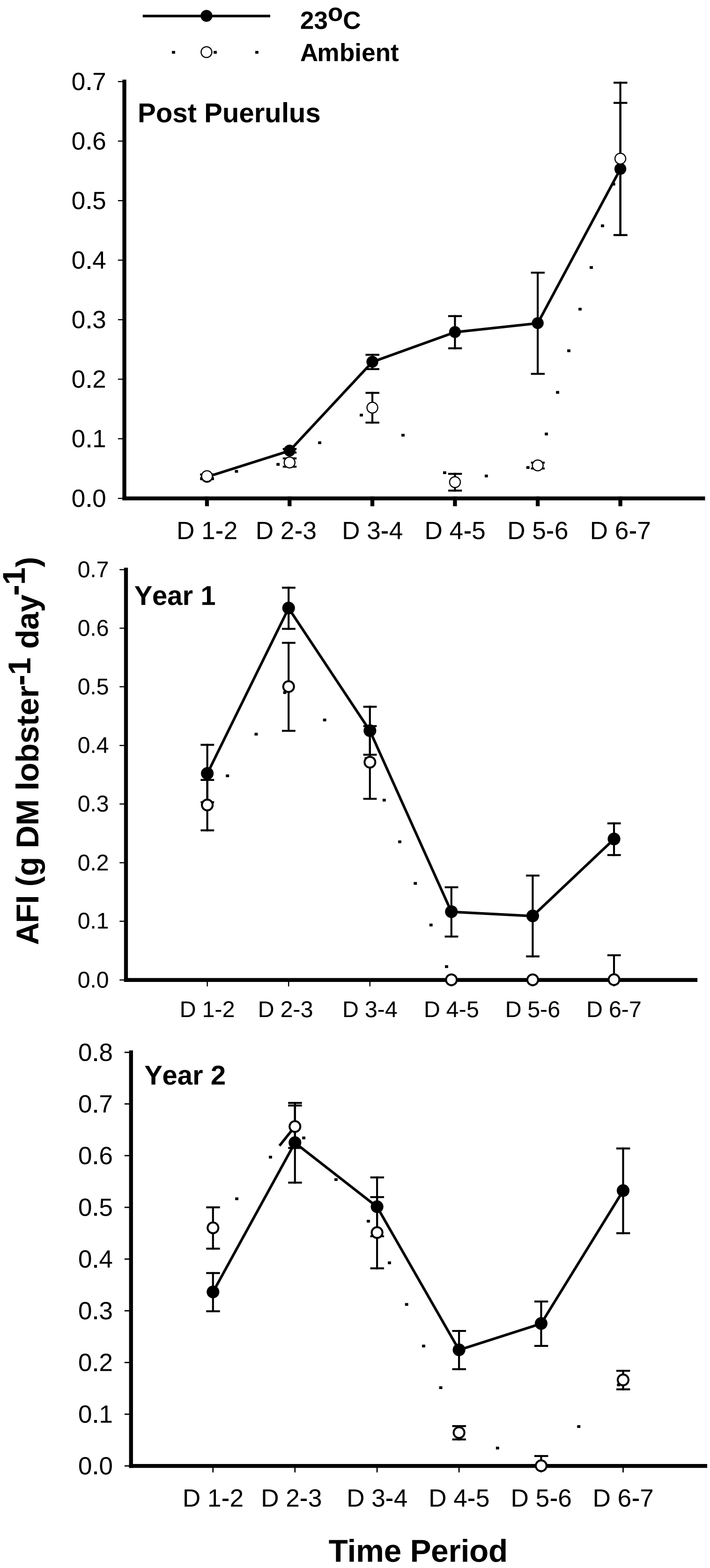
3 feeds per night
1 feed per night
Post Puerulus
0.04
0.03
0.16
0.14
0.17
0.16
0.19
0.21
0.16
0.14
0.26
0.25
Year 1
0.48
0.48
0.58
0.54
0.36
0.37
0.28
0.17
0.19
0.07
0.47
0.51
Year 2
0.57
0.52
0.52
0.52
0.26
0.42
0.1
0.14
0.16
0.17
0.27
0.16
Weight (g)
Days

Figure 4.


3 feeds per night
1 feed per night
23°C
Ambient
Weight (g)
Days
0
20
40
60
80
100
0
50
100
150
200
250
300
350
400

605

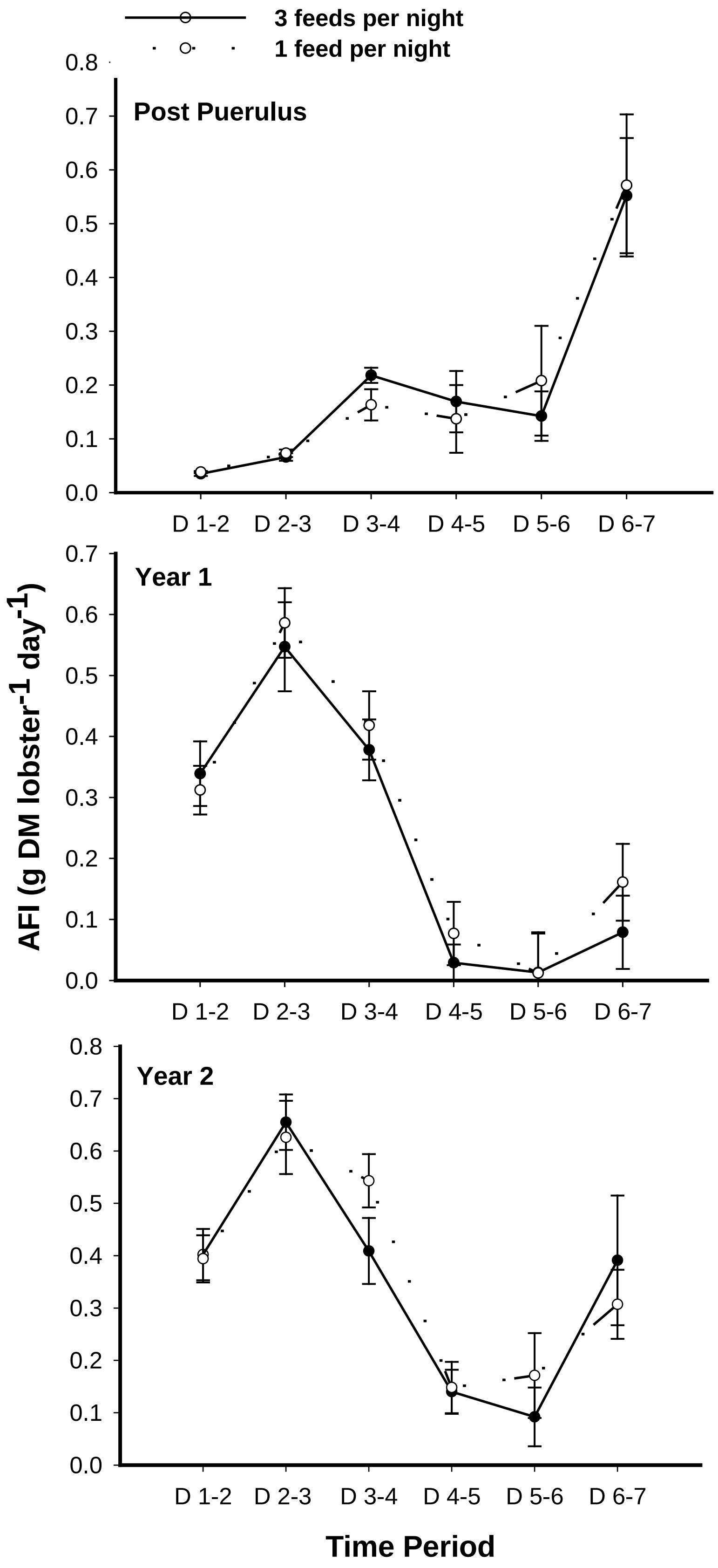
Figure 5.



23°C
Ambient
Post Puerulus
Year 1
Year 2
AFI (g DM lobster-1 day-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
D 1-2
D 2-3
D 3-4
D 4-5
D 5-6
D 6-7
Time Period

Figure 6.

610



3 feeds per night
1 feed per night
Post Puerulus
Year 1
Year 2
AFI (g DM lobster-1 day-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
D 1-2
D 2-3
D 3-4
D 4-5
D 5-6
D 6-7
Time Period

Figure 7.