Reproduction in the Tropical Rock Lobster *Panulirus ornatus* in Captivity

Thesis submitted by

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Nikolas Graham Sachlikidis

Statement on the Contribution of Others

I would like to acknowledge the significant contribution of others to this work. This includes Darella Chapman, Dr. Clive Jones and Dr. Jamie Seymour for significant input into intellectual properties of this work as stated at the beginning of each chapter. Also, funding from the Fisheries Research and Development Corporation (FRDC) and M.G. Kailis Pty. Ltd. through the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) and the Department of Primary Industries and Fisheries (QDPIF) for provision of the facilities where these studies took place.

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For Anna

Who gave completely and without question, endured tenaciously without complaint, and whose eyes shine blue like the sea of her home shore eternally….

Abstract

The Australian spiny lobster fishery is Australia's most economically valuable fishery, presently worth around $450 million per annum (Anon., 2008a). However, while many are currently well managed, most Australian spiny lobster fisheries are at their maximum sustainable capacity whilst demand for lobster continues to increase (Jones, 2009a).

Globally, spiny lobster catch rates have stabilised or even declined since 2002 (FAO, 2007; 2009). Furthermore, due to anthropogenic perturbations, wild fishery production may actually drop significantly as highlighted by recent projections for western rock lobster catch and marked decreases in catch per unit effort (CPUE) for may lobster species (Anon., 2008b; FAO, 2009). The only real potential for significant production growth appears to be through aquaculture.

Internationally, lobster aquaculture to date has consisted of capturing juvenile wild seed stock for on-growing to market size (Jones, 2009b). However, the long-term viability and sustainability of spiny lobster aquaculture depends on closing the life cycle and developing an economically viable method for raising lobsters from eggs through to market size (Grove-Jones *et al.*, 2002).

The ornate spiny lobster (*Panulirus ornatus*) has been identified as having clear commercial potential, largely due to its abbreviated life history compared to other spiny lobster species (Grove-Jones *et al.*, 2002; George, 2005). More specifically, its relatively short larval phase (4 months) and fast juvenile growth rate make it a suitable candidate for commercial aquaculture if techniques and

technologies, to consistently produce large numbers of juveniles from eggs, are developed.

A Fisheries Research and Development Corporation (FRDC) commissioned review (Grove-Jones *et al.*, 2002) stated that development of viable puerulus production technology for lobsters would be a 5+ year prospect, which should be addressed through three discrete phases to most effectively achieve the commercialisation of spiny lobsters in Australia (see Chapter II).

The experiments carried out as a part of this study address phase I as specified by the Grove-Jones *et al.* (2002). This work fills important knowledge gaps previously unknown for this species, in brief, the major findings include:

- The control of breeding, gonad development, moulting and egg extrusion though the photoperiodic and water temperature manipulation. In particular, that the lengthening of photoperiod is the cue to gonad development and breeding for this species.

- The optimal egg incubation temperature defined for this species and a model to predict the hatch timing and developmental period of embryos when incubated at a range of temperatures.

- Techniques to quantitatively assess phyllosoma quality at hatch, including the development of a ten minute salinity stress test.

- Larval husbandry techniques to achieve high growth and survival through to phyllosoma stage V.

These findings are important towards the development of hatchery technology and commercialisation for *P. ornatus*. Ultimately, these findings are fundamental to successful puerulus culture.

Results from these studies have made a significant contribution to the culture for *P. ornatus* within Australia. Furthermore, they have formed the foundation of continuing successful work in the area of rock lobster larval rearing which has resulted in the completion of the larval cycle of *Panulirus ornatus* in captive culture.

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Chapter I: Study rationale

The Australian spiny lobster fishery is an important marine resource making up 25% of Australia's total fishery landings (Anon., 2008a). While many are currently well managed, most Australian spiny lobster fisheries are at their maximum sustainable capacity whilst demand for lobster continues to increase (Jones, 2009a).

Globally, spiny lobster catch rates have stabilised or even declined since 2002 (FAO, 2007; 2009). Indeed, due to the impacts of anthropogenic perturbations, some wild fishery production may drop significantly as evidenced by recent projections for western rock lobster catch and marked decreases in catch per unit effort (CPUE) for many lobster species (Anon., 2008b; FAO, 2009).

Spiny lobsters are Australia's most economically valuable fishery, presently worth around $450 million per annum (Anon., 2008a). Future increases in the value of the current production are possible by increasing sales of higher value live lobsters and/or targeting periods of high demand, but total production gains are likely to be relatively small. The only real potential for significant production growth appears to be through aquaculture.

Globally, spiny lobster aquaculture to date consists of capturing post larval juveniles, or 'puerulus', from the wild for on-growing to market size (Jones, 2009b). However, the long-term viability and sustainability (Bell, 2004) of spiny lobster aquaculture depends on developing an economically viable method for raising lobsters full life cycle, from egg through to market size (Jones, 2009a).

Due to the considerable interest in spiny lobster aquaculture in Australia, the Fisheries Research and Development Corporation

(FRDC) established the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) in order to develop a cohesive and nationally coordinated approach to spiny lobster research and development.

In 2002, FRDC RLEAS commissioned a review to examine the most likely research steps required to successfully produce puerulus through culture (Grove-Jones *et al.*, 2002). The Grove-Jones *et al.* (2002) review stated that development of viable puerulus production technology for lobsters would be a 5+ year prospect, which should be addressed through 3 discrete phases. In chronological order they are:

Phase I

- Primary focus on achieving high growth and survival through to phyllosoma stage V.

Goal 1:

- Establish a reliable supply of stage one larvae at any time of year using the following suggested methods:
  - Manipulate photoperiod to control gonad maturation and timing of extrusion.
  - Manipulate incubation temperature to control the developmental period of embryos.
  - Produce larvae monthly
  - Assess the effect of broodstock diet on phyllosoma quality

Phase II

- Primary focus on the physical and chemical assessment of formulated diets ready for assessment with stage V+ phyllosoma

Phase III

- Diet optimisation studies and commercialisation of culture techniques.

The need for this research was clearly stated by the RLEAS Steering Committee in May 2002, which indicated that greater emphasis on one of the six tropical lobster species in Australia was appropriate. Over 50% of the global lobster market is in sub-tropical or tropical species and as the vast majority of lobster product is destined for export there is a need to supply this market (Hart, 2009).

The ornate spiny lobster (*Panulirus ornatus*) has been identified by market research as having strong market demand, especially in Chinese markets (Hart, 2009). Biologically, *P. ornatus* has also been identified as a favourable aquaculture candidate due to its relatively short larval phase (4 months) (George, 2005) and rapid post-juvenile growth rate, reaching 1 kg within 20 months compared to 0.25 kg in 24 months for the temperate species *Jasus edwardsii* (Crear *et al.*, 2000; Jones *et al.*, 2001). It is estimated that growth from egg to market size (600g) achieved in less than 2 years (Phillips *et al.*, 1992; Jones *et al.*, 2001).

The favourable attributes of *P. ornatus* were also clearly stated by the RLEAS propagation review (Grove-Jones *et al.*, 2002). Both the RLEAS steering committee and Grove-Jones, *et al.*(2002) recommended a change in focus of lobster larval rearing research, to address the key blockages to commercial culture of this species, in logical sequence, to rapidly achieve commercially relevant outcomes.

Consequently, a logical series of experiments was devised towards the ultimate goal of consistent and commercial culture of *P. ornatus* for aquaculture. The experiments reported in this thesis address the following previously identified knowledge gaps:

- The control of gonad maturation, breeding and timing of egg extrusion.
- The control of the embryonic developmental period by incubation temperature manipulation.
- Development of the tools to assess phyllosoma quality
- Improvement of larval husbandry to maximise growth and survivorship through to phyllosoma stage V.

Results from these experiments significantly add to the knowledge of the biology of this species and have laid the platform for subsequent work adapting larval culture techniques for the successful culture of this species from egg to puerulus.

Chapter II: Introduction

Lobsters are a diverse and widely distributed group of ecologically, recreationally and economically important crustaceans. Their value as a food and an economic resource comes from their reputation as a highly desirable food source and are undoubtedly the benchmark for fine dining fare the world over. Aesthetically pleasing, they are instantly recognisable in restaurants globally, and subsequently attract commercial interest and high market prices. It is this demand which drives the 230 000 tonne (annual) global wild harvest, estimated to be worth over US $2.163 billion (FAO, 2009).

Since the 1990s, demand for this valuable commodity has increased, whilst supply from wild capture fisheries has remained static or decreased (FAO, 2009). This differential has prompted a surge of interest to commercially culture lobsters, and is reflected in a significant increase in lobster aquaculture research over the past decade.

Although the market driven stimulus is strong, the biological constraints inherent with captive lobster culture have so far prevented the commercial culture of hatchery-reared lobsters. Specifically, this has been because of both aggression and cannibalism in juvenile life stages, as has been the case for clawed lobster species, or through difficulties in larval cultivation, especially in spiny lobsters which generally have protracted larval phases (George, 2005).

To this end there have been promising advancements over the past decade. Research has resolved some knowledge gaps about the biology of many lobster species and, more practically, how this is applied and adapted for the purpose of culture. This is especially

true for spiny lobster species, for which there has been great advancement in understanding and culture techniques over recent times (Kittaka, 2000; Jones, 2009a).

Successful larval culture has now been achieved for a handful of spiny lobster species at research scale (see reviews: Kittaka, 1997; 2000; Kittaka and Booth, 2000; Phillips and Melville-Smith, 2006; Jones, 2009a), although achieving success at commercial scale has so far proven elusive. Understanding, optimising and adapting aspects of lobster biology to the culture scenario is likely to be the key to unlocking the true aquaculture potential of lobsters.

The basic biology of tropical spiny lobster species is only now beginning to be understood. For these species, interest is largely commercially driven as their great potential for commercialisation has been recognised due to their comparatively fast growth rates (Lozano-Alvarez, 1996; Jones *et al.*, 2001; Kulmiye and Mavuti, 2005; Murugan *et al.*, 2005; Vijayakumaran *et al.*, 2009) and abbreviated larval cycles relative to other spiny lobster species (George, 2005).

Increased commercial interest has subsequently resulted in increased aquaculture research in these species over recent years, though many biological knowledge gaps still exist. Specifically, research needs to focus on how biological traits can be used, managed and adapted towards the practical management strategies essential for reliable commercial aquaculture. Biological information on reproductive, egg and basic larval biology underpins any advancement in culture technology and is critical to achieve the consistency of production required for commercialisation of lobster culture.

Biology

The generic term "Lobster" refers to four distinct family groups: The clawed lobsters (Nephopidae), the spiny lobsters (Palinuridae), the slipper lobsters (Scyllaridae) and the coral lobsters (Synaxidae). One of the most widespread and commercially important groups is the spiny lobsters, so called because of the abundance of spines on the carapace and on the anntenular peduncle. This group has been classified into eight genera inhabiting marine environments from 0-50 degrees of latitude, depths to 1000 metres and temperatures between 5 - 30ºC (Figure 1)(George and Main, 1967). Species of the genera *Jasus*, *Palinurus* and *Panulirus* are the three most commercially sought after spiny lobsters largely due to their large size, distribution and abundance (Figure 2).

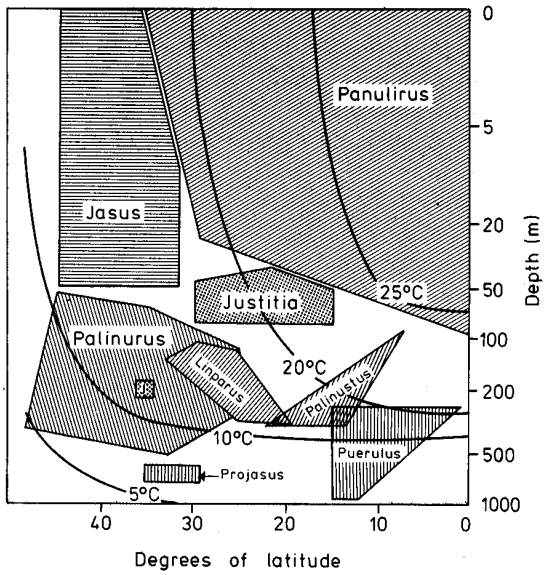

Figure 1: The distribution of lobsters of the family Palinuridae by latitude, temperature and depth according to George & Main (1967).

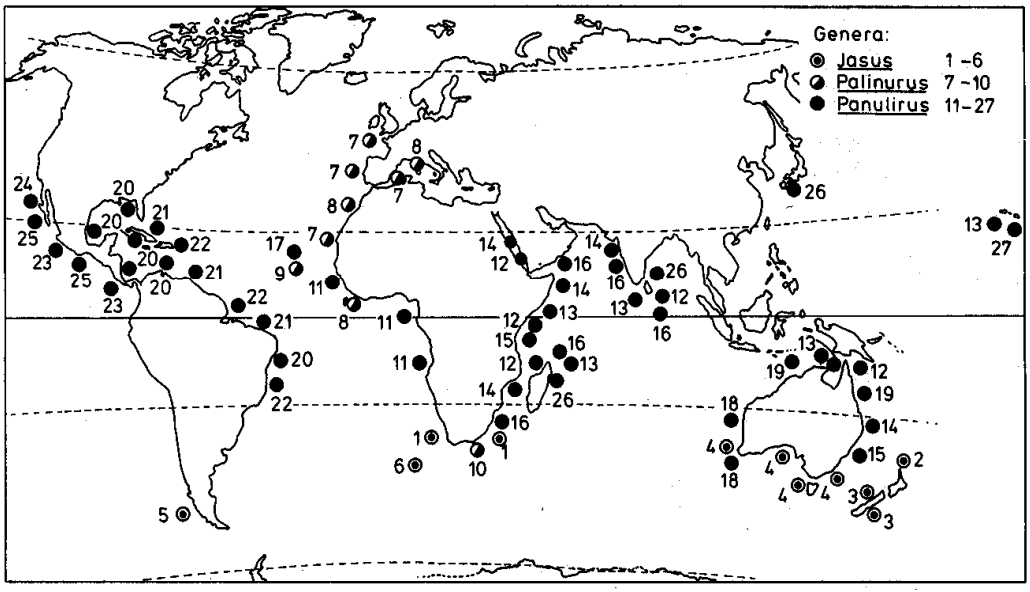

Figure 2: Distribution of the main commercial species of the genera Jasus, Palinurus and Panulirus in the world's oceans according to Phillips, Cobb & George (1980). **Genus *Jasus*:** (1) *J. lalandii* (2) *J. verreauxi* (3) *J. edwardsii* (4) *J. novchloandiae* (5) *J. frontalis* (6) *J. tristani*. **Genus *Palinurus*:** (7) *P. elephus* (8) *P. mauritanicus* (9) *P. charestoni* (10) *P. gilchristi*. **Genus *Panulirus***: (11) *P. regius* (12) *P. ornatus* (13) *P. penicillatus* (14*) P. versicolor* (15*) P. longipes* (16) *P. homarus homarus* (17) *P. echinatus* (18) *P. cygnus* (19) *P. polyphagus* (20) *P. argus* (21) *P. laevicanda* (22) *P. guttatus* (23) *P. gracilis* (24*) P. interruptus* (25) *P. inflatus* (26) *P. japonicus* (27) *P. marginatus*.

Breeding

Breeding in lobsters varies widely but is predominantly seasonal and is controlled by environmental factors such as water temperature and day length, usually occurring in summer months within a species known range (Aiken and Waddy, 1980; Aiken and Waddy, 1989; MacDiarmid and Kittaka, 2000).

Tropical species such as *P. homarus, P. versicolour, P. inflatus, P. gracilis* and *P. penicillatus*, have been reported by several authors to be reproductively active throughout the year through parts of their natural range (See MacDonald, 1982; Juinio, 1987; Briones-

Fourzan and Lozano-Alvarez, 1992; Vijayakumaran *et al.*, 2005). The seasonality of temperate lobsters is typically more temporally defined, though the timing of breeding is still impacted upon by special factors. A good example of this is *P. japonicus*, which has been observed to be reproductively active seasonally earlier at the more equatorial parts of its range, with breeding delayed towards higher latitudes and has been attributed to water temperature (Ino, 1950; MacDiarmid and Kittaka, 2000).

Seasonal breeding migrations have been recorded as a breeding strategy for many lobster species. Adult lobsters of both temperate and tropical species have been observed walking in large groups up to 500 km to their preferred breeding sites (Herrnkind and McLean, 1971; Herrnkind, 1980; Moore and MacFarlane, 1984; Bell *et al.*, 1987; Evans and Lockwood, 1994; Groeneveld and Branch, 2002). However, this is not the case with all species, and some species such as are reported as having an annual range of less than 2 km (Gardner *et al.*, 2003; Follesa *et al.*, 2009).

This difference in breeding strategy could define the importance of different environmental breeding cues (including photoperiod and temperature) between different species, and be important when considering potential controlling factors for breeding in *P.ornatus*.

Reproductive physiology

Male reproductive physiology

Spermatophore properties

This is a topic of significant previous study (see review: Subramoniam, 1993) and as such, only the basic physiology and behaviour are covered in this section.

As in other crustacean species, lobster sperm ducts are simple paired tubes that open at the base pair of trunk appendages (Berry, 1971; Ruppert and Barnes, 1994). Lobster spermatozoa are spherical with several pointed spines and are non-motile (Berry, 1970a; Berry and Heydorn, 1970; Ruppert and Barnes, 1994). Spermatozoa adhere directly to the fresh ova with their spines and this likely occurs when the ova roll over the spermatophore down to the brood chamber therefore coming into contact with the spermatozoa (Berry, 1970a). Berry (1970a), also suggests that the spines on the spermatozoa are to increase surface area so as to be washed by currents (from the pleopods) into the brood chamber where they can also attach themselves to the ova. Sperm is transferred from the male to the female in the form of a spermatophore (Berry, 1970a; Ruppert and Barnes, 1994).

Spermatophore properties vary between lobster species but are essentially similar. In all lobster species, with the exception of *Jasus*, there is a basal adhesive layer followed by a gelatinous matrix containing the spermatophores (a pair of highly convoluted tubules containing the spermatozoa) all covered by a harder, protective outer layer (Berry and Heydorn, 1970; Radha and Subramoniam, 1985; Subramoniam, 1993). The outer layer can be very tough and has been described as "putty like" (See Berry, 1970a; Berry and Heydorn, 1970; Chittleborough, 1976; Lipcius and Herrnkind, 1985; MacFarlane and Moore, 1986; Juinio, 1987; Lipcius and Herrnkind, 1987; Juinio-Menez and Estrella, 1995).

In *Jasus sp.*, the outer protective layer of the spermatophore is lacking, causing it to begin to deteriorate within a few hours of being deposited (Paterson, 1969; Berry and Heydorn, 1970; MacDiarmid, 1989).

For all lobsters, the spermatophore is arranged in the *vas deferens* prior to ejaculation (Berry and Heydorn, 1970; Radha and Subramoniam, 1985). The characteristics of the spermatophoric mass may correspond with, or may dictate, when mating occurs in relation to oviposition (MacDiarmid, 1989). This relates to female mate timing as discussed later in this review (Chapter II).

Multiple mating

Multiple mating between an individual pair of spiny lobsters is common, with as many as four per night having been observed *P. argus* (Lipcius *et al.*, 1983) and *P. penicillatus* (Juinio, 1987). Although the spermatophore is arranged in the *vas deferens* prior to ejaculation (Berry and Heydorn, 1970), MacDiarmid and Kittaka (2000) postulated that multiple mating might be necessary for male ejaculation and the secure attachment of the spermatophore to the sternum of the female.

Another aspect of multiple mating is the ability of a single male to inseminate multiple females(Kittaka and MacDiarmid, 1994). In *P. argus,* males have been shown to vary the amount of sperm deposited on each female, increasing with increased female size (MacDiarmid and Butler, 1999). Sperm economy has been shown to have a greater influence on clutch size (the number of eggs in a brood) than female size (MacDiarmid and Butler, 1999). In captivity, males of *P. ornatus* are capable of breeding with at least five different females over multiple broods within a breeding season (Sachlikidis *et al.*, 2005).

Female reproductive physiology

Oviposition

Just prior to oviposition, female lobsters from the genus *Palinurus* and *Panulirus* have been observed picking at the outer, protective layer of the sperm matrix (Berry, 1970a; Lipcius *et al.*, 1983). This exposes the spermatozoa to the freshly laid eggs for fertilisation. During oviposition, the female assumes a vertical position on the substrate with her tail fanned out and pressed against the thoracic sternum or covering the genital aperture at the base of the third walking legs (Berry, 1970a; McKoy, 1979).

During oviposition the eggs move to the exopodites from the gonopores via the pre scratched spermatophoric mass where they are fertilised by the spermatozoa (Berry, 1970a). This is achieved by gravity and currents created by the endopodites of the pleopods (Berry, 1970a; McKoy, 1979). Upon arrival at the pleopods, eggs are attached to the ovigerous setae with glue from the cement glands at the base of the pleopods (Berry, 1970a). Here they are fanned by the pleopods to keep up a constant supply of oxygen to the eggs.

Fecundity

Female spiny lobsters produce large numbers of small eggs. The highest recorded fecundity is for *J. verreauxi,* which is able to produce up to 2 000 000 eggs in one brood (Kensler, 1967). Generally, fecundity is positively related to female size (MacFarlane and Moore, 1986; Annala and Bycroft, 1987; Fonseca-Larios and Briones-Fourzan, 1998; MacDiarmid and Butler, 1999; Freitas *et al.*, 2007; Smith and Ritar, 2007). In wild populations of *P. ornatus*, egg production increases from approximately 270 000 from an animal of 80 mm carapace length (CL) to nearly 700 000 for a female of 120

mm CL and can be predicted approximately through the equation: Fecundity = 10 416.68 x CL – 561 793.71 (Macfarlane & Moore, 1986).

Size plays a major role in determining the fecundity of the female lobster, although the male may also play a role(MacDiarmid and Kittaka, 2000). Macdairmid and Butler (1999) showed that in *P. argus* and *J. edwardsii* sperm outputs limit female fecundity. This is so pronounced for *P. argus* that spermatophore area has a bigger effect on clutch weight than does female size (MacDiarmid and Butler, 1999). The results from that study suggest that to avoid reducing clutch size due to sperm limitation, females should mate either with larger males or mate multiple times prior to oviposition.

A number of species of spiny lobster (particularly tropical) reproduce multiple times between moults (MacFarlane and Moore, 1986; Juinio, 1987; Murugan *et al.*, 2005; Vijayakumaran *et al.*, 2005; Villalejo-Fuerte and Velazquez-Abunader, 2007). Successive broods within the same breeding season have however, been shown to be smaller for *P. japonicus, P. ornatus* and *P. penicillatus* (Ino, 1950; MacFarlane and Moore, 1986; Juinio, 1987).

Egg and ovary development

Ovarian development

Ovaries in the spiny lobster are similar and are "H" shaped running dorsally, parallel to the midgut, starting anteriorly at the cephalic area and running to the fourth abdominal segment when mature (Nakamura, 1994). A lobe slightly to the anterior of the middle connects the left and right sides of the ovary. The oviducts

are slender semi-transparent tubes that connect the ovaries to the coxopodites on the third walking legs where the ripe ova are extruded during spawning (Berry, 1971; Nakamura, 1994). These tubes derive from the mid-lateral region of each ovary (Nakamura, 1994).

When in season, the ovaries of the spiny lobster become enlarged and swollen with colouration turning from white to orange/red. Berry (1971) developed a system of macroscopic classification for *P. homarus* that has been used as the basis of ovary assessment (with slight modification) in other species both in the field and under culture conditions (MacFarlane and Moore, 1986; Bell *et al.*, 1987; Minagawa and Sano, 1997). Classification according to Berry (1971) is as follows:

Stage I - Immature

Ovaries flattened and strap-like; ova when seen with a hand lens tend to be uniform in diameter but range from 0.07 to 0.15 mm; overall colour is white with a slightly granular appearance.

Stage II - Inactive

Ovaries flattened dorso-ventrally; ova not uniform in diameter when seen with a hand lens, the smaller ones being white and the larger ones being pale pink or light orange, giving an overall pinkish-orange colouration; ova range in diameter from 0.07 to 0.21 mm.

Stage IIa - Active

Ovaries slightly swollen, tending to become rounded in cross-section; ova just visible to the naked eye and range in diameter from 0.19 to 0.29 mm; overall colour deep orange.

Stage III - Active/ripe

Ovaries swollen to fill most of the available space in the cephalothoracic cavity; ova easily visible to the naked eye and range in diameter from 0.24 to 0.38 mm; overall colour bright coral red.

Stage IV - Ripe

Ovaries grossly swollen, filling all available space in the cephalothoracic cavity; ova easily visible, tending to appear uniform in diameter and some are usually present in the oviducts; small ova are still present, however, and size of ova is 0.35 to 0.46 mm diameter; overall colour bright coral red.

Stage V - Spent

Ovaries similar in appearance to stage II, but may be distinguished macroscopically by the presence of a few large residual ova from stage IV, usually retained at the extremities of the ovary lobes and in the oviducts. These ova are soon resorbed, in which case this stage is indistinguishable macroscopically from stage II.

Stage I ovaries only occur in sexually immature animals. Mature animals cycle though stages II to V throughout the breeding season (Berry, 1971). Minagawa and Sano (1997) and Minagawa (1997) developed a similar scale for *P. japonicus* but included detail about the ova themselves and a gonadosomic index (GSI) as a measure of ovary maturity. Minagawa and Sano (1997), calculated GSI using the formula:

$$I = W \times 100{,}000/L^3$$

Where:

I = GSI W = Gonad weight (g) L = Carapace Length (mm)

Their study divides the stages of ovary development into seven steps. These are Inactive, Developing, Ripe, Re-developing, Re-ripe, Spawned and Recovery (Minagawa and Sano, 1997). Details of the stage of egg development in each of the stages (with reference to the yolk platelet of the ova within each stage of ovary development) are incorporated in the final assessment of gonad maturation.

Eyestalk ablation

Eyestalk ablation has been shown to accelerate gonad development and increase growth rate in palinurid lobsters (Quackenbush and Herrnkind, 1981; Quackenbush and Herrnkind, 1983; Radhakrishnan and Vijayakumaran, 1984; Juinio-Menez and Ruinata, 1996). However, stress indicators increase significantly post ablation affecting survivorship (Verghese *et al.*, 2008). Juinio-Menez and Ruinata (1996), researching *P. ornatus*, found that survivorship was greatly decreased in bilaterally ablated lobsters while unilateral ablation had much better survival rates that were comparable to those of un-ablated lobsters.

Reproductive behaviour

Courtship

After finding a mate, lobsters engage in a pre-copulatory courtship period. Courtship has been observed and has been described for *P. argus*, *J. edwardsii* and *P. homarus* (MacDiarmid,

1989). In these species, the male positions himself in front of the female, who is often hiding in shelter, and touches antennules with the female. The touching and scraping of the females antennules by the male increases in frequency and intensity until the female emerges from cover when the male pursues her (Berry, 1970a; McKoy, 1979; Lipcius *et al.*, 1983). Often, this is into another shelter where the process is then repeated until copulation begins (Berry, 1970a; McKoy, 1979; Lipcius *et al.*, 1983).

Studies of *P.argus* showed that males initiated 91.5 % of all frontal approaches, however females did initiate mating if they had not copulated and were near the time of spawning (Lipcius *et al.*, 1983; Lipcius, 1985; Lipcius and Herrnkind, 1987). Initiation was mostly by the largest male (99.5% of the time) in each tank (Berry, 1970a; McKoy, 1979; Lipcius *et al.*, 1983; Lipcius and Herrnkind, 1987). Similar trends were also observed for *P. homarus* (Lipcius and Herrnkind, 1985; 1987) and *J. edwardsii* (Berry, 1970a).

Female choice

Female choice may occur when either the female initiates mating behaviour or chooses to accept or reject (or flee) male pre-copulatory advances (McKoy, 1979). Rejection or fleeing may not be possible in tank conditions and may lead to forced copulation (Lipcius and Herrnkind, 1985). Females may also become less particular over mate choice when they approach the time of egg extrusion and no mature male has been supplied (Lipcius *et al.*, 1983).

This may not be as profound in species where the spermatophore is long lived, enabling mating and oviposition to occur independently allowing time for mate choice (Lipcius and

Herrnkind, 1985; 1987). One such example is *P. ornatus* where successive broods have been hatched from a single spermatophore (Juinio-Menez and Estrella, 1995).

Copulation

Copulation has been observed and documented for many lobster species (Berry, 1970a; McKoy, 1979; Lipcius *et al.*, 1983; Lipcius, 1985; Waddy and Aiken, 1991; Van der Meeren *et al.*, 2008). In *P. homarus*, Berry (1970a) identified three major activity patterns in copulation. After a lengthy courtship (5-13 hours), the male attempts to move in front of the female and face her head on. The female's response to this is to stop immediately with her anterior cephalothorax and antennules touching the ground (Berry, 1970a). The male then takes up a position in front of the female and extended his legs laterally. If the female remains stationary for more than 30 seconds he withdraws slightly after which time the male rushes at the female gripping and pulling her legs towards him in an attempt to lift her up and expose her sternum (Berry, 1970a).

Female timing

Macdiarmid & Kittaka (2000) suggest that spiny lobster females must find a mate when her eggs are ready to be extruded and are fully ripe, to avoid running the risk of a significant reduction of egg fertility. Moreover, the need to find a mate at exactly this time varies with species in accordance with the longevity of the male spermatophore (MacDiarmid and Kittaka, 2000). For example, Macdiarmid (1989) suggests that females of the species *J. edwardsii* only remain at peak fertility for 1-2 days and for maximum fertilisation mating must also occur at this time due to short spermatophore life. *P. cygnus* however, has a longer lasting

spermatophore. This enables mating up to 69 days before egg extrusion without missing the peak fertility period (Chittleborough, 1976). The ability to fertilise multiple egg clutches from a single mating also extends the period of successful mating(Vijayakumaran *et al.*, 2005).

Moult timing vs mating timing

Male lobsters are reported not to mate in the pre-moult stage, only at inter-moult (MacDiarmid and Kittaka, 2000). This has repercussions for captive breeding, especially for species that moult all year round compared to species that moult before breeding season (Chittleborough, 1976; Juinio, 1987; Briones-Fourzan and Lozano-Alvarez, 1992).

Numerous studies have developed systems to assess male moult stage and therefore reproductive suitability for successful captive breeding (Lyle and MacDonald, 1983; Turnbull, 1989; Isaacs *et al.*, 2000; Groeneveld and Branch, 2001). Additionally, an alternative male within each breeding tank can be provided to increase the probability of a reproductively active male being available when females are active.

For adult females, breeding occurs post-moult (MacDiarmid and Kittaka, 2000). Individuals do not always need to be newly moulted to mate, provided that ovigerous setae on the endopods and pleopods (to which eggs attached) are fresh (Turnbull, 1989).

There is significant variation in the time between ecdysis and first mating after ecdysis. In aquarium held *P. cygnus* the time between ecdysis and first mating varied between 2 and 97 days (Chittleborough, 1976). Similar variation has been seen in *Jasus*

sp. where the time varied from 2 hours to 63 days after ecdysis. (Chittleborough, 1976).

Egg development

In lobsters, incubation period between egg fertilisation and egg hatch is temperature dependant (Perkins, 1972; Phillips and Sastry, 1980; Aiken and Waddy, 1985a; Tong *et al.*, 2000; Smith *et al.*, 2002; Moss *et al.*, 2004). Incubation period can vary widely both between species and individuals within a species depending on the incubation temperature (Table 1). For example, the incubation period of *Sagmariasus verreauxi* ranges between 55 (20 °C) and 130 (13°C) days (Moss *et al.*, 2004). Tropical species generally have shorter incubation periods than their temperate relatives (Table 1).

Predictive hatch models have been developed for a number of species using sequentially developing embryonic features to estimate time until hatch at a specified temperature.

Specifically, many hatch models involve the measurement of the eyespot of developing embryos within the egg. An eye index is calculated based on the measurements of eye spot length and width and, because developmental rate is a function of incubation temperature, hatch date can then be predicted for a given temperature. This method has been successfully used for both clawed and spiny lobster species (Perkins, 1972; Beltz *et al.*, 1992; Tong *et al.*, 2000; Smith *et al.*, 2002; Moss *et al.*, 2004)

In studies of captive lobsters, larval quality can also affected by incubation temperature (Aiken and Waddy, 1985a; Smith *et al.*, 2002). Egg incubation at a relatively high temperature in *J.*

edwardsii, although expediting development, produced smaller larvae (Tong *et al.*, 2000; Smith *et al.*, 2002) with reduced levels of eicosapentanoic acid (20:5n-3) and sterols compared with those incubated at lower temperatures. In this instance, expediting incubation may actually hamper the production of consistent, high quality larvae rather than enhance it.

Number of broods

The number of broods produced by each female annually is variable both between and within species. Temperate species generally produce only one brood per year, moulting between broods to refresh the pleopods follicles to which the fertilised eggs adhere (Kittaka and MacDiarmid, 1994; MacDiarmid and Sainte-Marie, 2006)(Table 1). Tropical species are generally more reproductively active with some species capable of reproducing almost continuously, bearing multiple broods between moults (Juinio, 1987; Gomez *et al.*, 1994)(Table 1).

For species with wide geographical distributions, the number of broods produced annually can vary between locations. For *P. argus, P. cygnus*, *P. interruptus* and *P. japonicus* latitudinal breeding trends have been observed in wild populations (Ino, 1950; Kanciruk and Herrnkind, 1976; Velazquez, 2003). The number of annual broods per female is generally increased in individuals which live in the more equatorial areas of each species geographic range, suggesting that environmental conditions may influence brood number (Chittleborough, 1976; Phillips *et al.*, 1983; Melville-Smith *et al.*, 2009).

Food availability is a possible factor affecting the number of inter-moult broods. Captive reared *P. cygnus* produced multiple

broods between moults, a vast increase when compared to wild populations, which generally produce a maximum of two broods per season (Chittleborough, 1976; Phillips *et al.*, 1983). Chittleborough (1976), suggests that this phenomena is a result of the increased food supply, and in turn nutritional condition in captive reared broodstock.

Seasonality factors

Photoperiod and temperature

Photoperiod and temperature have been identified as major factors controlling breeding of many crustaceans (Meusy and Payen, 1988). Lobsters use environmental factors as cues for endocrine regulation and therefore the regulation of reproduction and moulting, which can be used to synchronise breeding in captive lobster populations (Aiken and Waddy, 1980; Lipcius and Herrnkind, 1987; Olive, 1995; Matsuda *et al.*, 2002; Sachlikidis *et al.*, 2005).

Strong evidence of latitudinal breeding trends exists, with breeding occurring seasonally earlier in more equatorial areas within the range of a single species (Relini *et al.*, 1998; Velazquez, 2003; de Lestang and Melville-Smith, 2006; Gardner *et al.*, 2006). Some tropical species show limited seasonality and are able to breed almost year-round (Gomez *et al.*, 1994; Hearn and Toral-Granda, 2007).

Observations of lobsters held under experimental conditions suggest that photoperiod controls the development of the gonads and spawning frequency in *P. argus and P. japonicus* (Lipcius and Herrnkind, 1987; Matsuda *et al.*, 2002). Longer photoperiods may also increase the frequency of mating behaviours in *P. argus*

(Lipcius and Herrnkind, 1985). Temperature has been shown to influence the rate of both of the above conditions in *P. argus* and *P. japonicus*, and has been implicated as a major breeding cue in *P. cygnus* and *P. interruptus* (Chittleborough, 1976; Lipcius and Herrnkind, 1987; Matsuda *et al.*, 2002; Velazquez, 2003). Similar trials on the American lobster (*Homarus americanus)*, with some debate (Nelson *et al.*, 1983), have also shown temperature to be responsible for the seasonal regulation of ovarian maturation and spawning (Aiken and Waddy, 1985b; 1989). Photoperiod and temperature was shown not to affect aggression and male gonad development in *P. argus* (Lipcius and Herrnkind, 1987).

In *P.* argus, photoperiod was shown not to affect moulting rates significantly, however temperature did, with sub-adults and young adult lobsters having the highest rates followed by large adult males then reproductively active females (Lipcius and Herrnkind, 1987).

Table 1: The number of broods/year possible and egg incubation periods for a range of temperate and tropical spiny lobster species.

Species	***Broods between moults***	***Incubation duration (days)***	***Incubation temperature range observed (ºC)***	Source
Panulirus homarus	≤4	15-30	26.1-29.8 ºC	Radhakrishnan (1977) Berry (1970a; 1971)
Panulirus penicillatus	≤4	35.5±1	24-27 ºC	Plaut (1993) Chang *et al* (2007)
Panulirus ornatus	≤4	22-36	24-30 ºC	Sachlikidis (Chapter 4)
Panulirus cygnus	1-3	19-68	19-25 ºC	Chittleborough (1976) Phillips *et al*(1983)
Palinurus elephas	1	150-280*	-	Ansell and Robb (1977) Hunter (1999)
Sagmariasus verreauxi	1	55-130	13-20 ºC	Moss *et al* (2004)
Jasus edwardsii	1	65-163	12-18 ºC	Tong *et al.*(2000)
Panulirus inflatus	≤4	-	-	Grano-Maldonado and Alvarez-Cadena (2010)
Panulirus polyphagus	≤4	60-90*	-	Kagwade (1988)
Panulirus argus	1-3	20-30*	-	Kanciruk and Herrnkind (1976) Fonseca-Larios and Briones-Fourzan(1998)
Panulirus longipes longipes	>2	40-80*	-	Gomez *et al.*(1994)
Panulirus japonicus	1-2	-	16-18 ºC *	Minagawa (1997)
Panulirus interruptus	1	-	12.5-24 ºC	Velazquez (2003)
Panulirus gutattus	≤4	14-21	-	Negrete-Soto *et al* (2002)

** Estimated*

The tropical rock lobster *Panulirus ornatus*

Biology

The tropical rock lobster *Panulirus ornatus*, is a large (<5 kg), high value spiny lobster. Well-established markets and limited capacity for increased fishery production have prompted interest in development of aquaculture for this species. Over the past decade, research effort in Australia has examined the various life history phases of this species, in particular the complex and protracted larval phase, with the ultimate goal of commercial hatchery production (refer to chapter 1: Study rationale).

Widely distributed, its natural range includes the Indo-west Pacific region from the Red Sea to east Africa (south to Natal) to southern Japan, the Solomon Islands, Papua New Guinea, S.W., N.W., N.E. and E Australia, New Caledonia and Fiji and has been recorded from catches from between 1 and 50 metres depth from coastal and coral reef areas throughout this range (Holthuis, 1991)(Figure 3).

P. ornatus becomes sexually mature at around 90mm carapace length or 800 grams, though broodstock used for culture are generally over 1 kg (MacFarlane and Moore, 1986; Sachlikidis *et al.*, 2005). In wild populations from N.E. Australia, the natural breeding season is from November through to March, coinciding with longer day lengths and warmer water temperatures (Bell *et al.*, 1987).

Captive animals can now be bred throughout the year through the manipulation of captive environmental conditions (Sachlikidis *et al.*, 2005). Broods can produce between 200 - 800 thousand larvae with each female lobster capable of producing up to four broods per

season (MacFarlane and Moore, 1986). Spermatophore structure is similar to that of other tropical spiny lobster species consisting of a long lasting, white putty-like mass (MacFarlane and Moore, 1986; Murugan *et al.*, 2005).

Figure 3: The geographical distribution of *P. ornatus* according to the FAO Species catalogue vol. 13: Marine lobsters of the world (reproduced with alteration from Holthuis (1991)).

Adults of this species have previously been held in captivity for up to 5 years (Sachlikidis, unpublished data) on a mixed diet of live and frozen pipis *(Plebidonax deltoids)*, frozen green mussels (*Perna canaliculus)* and squid *(Loligo opalescens)* provided once per day at the rate of 3% live body weight.

The growth rate for this species is fast, compared to other spiny lobster species, with experiments concluding that small (3g) juveniles are capable of growing to 1kg in under 18 months (Phillips *et al.*, 1992; Jones *et al.*, 2001). Through dietary manipulation it is

thought that significant increases in growth rate are possible (Smith *et al.*, 2003a; Smith *et al.*, 2005).

Larval development

The larval life history of *P. ornatus* is short relative to other species of spiny lobster (George, 2005). To date, the fastest developmental time from hatch to puerulus is 115 days under culture conditions (Sachlikidis, pers. obs). Ecological studies suggest that larval development may take between four and seven months in the wild (Dennis *et al.*, 2001). However, Smith *et al.*(2009a), on comparing cultured to wild caught late stage *P. ornatus* phyllosoma, suggested that this period may be as short as three months under suitable nutritional conditions.

The larval development of *P.* ornatus has been divided into 11 recognisable stages consisting of approximately 24 instars or moults (Jones *et al.*, 2003; Smith *et al.*, 2009a). The stages are somewhat arbitrary, based on gross morphological changes, but the transition between some stages involves significant physiological changes that are closely tied to condition (Table 2) (Figure 4). These descriptions are used in this dissemination as a guide to growth of early stage phyllosoma under culture conditions.

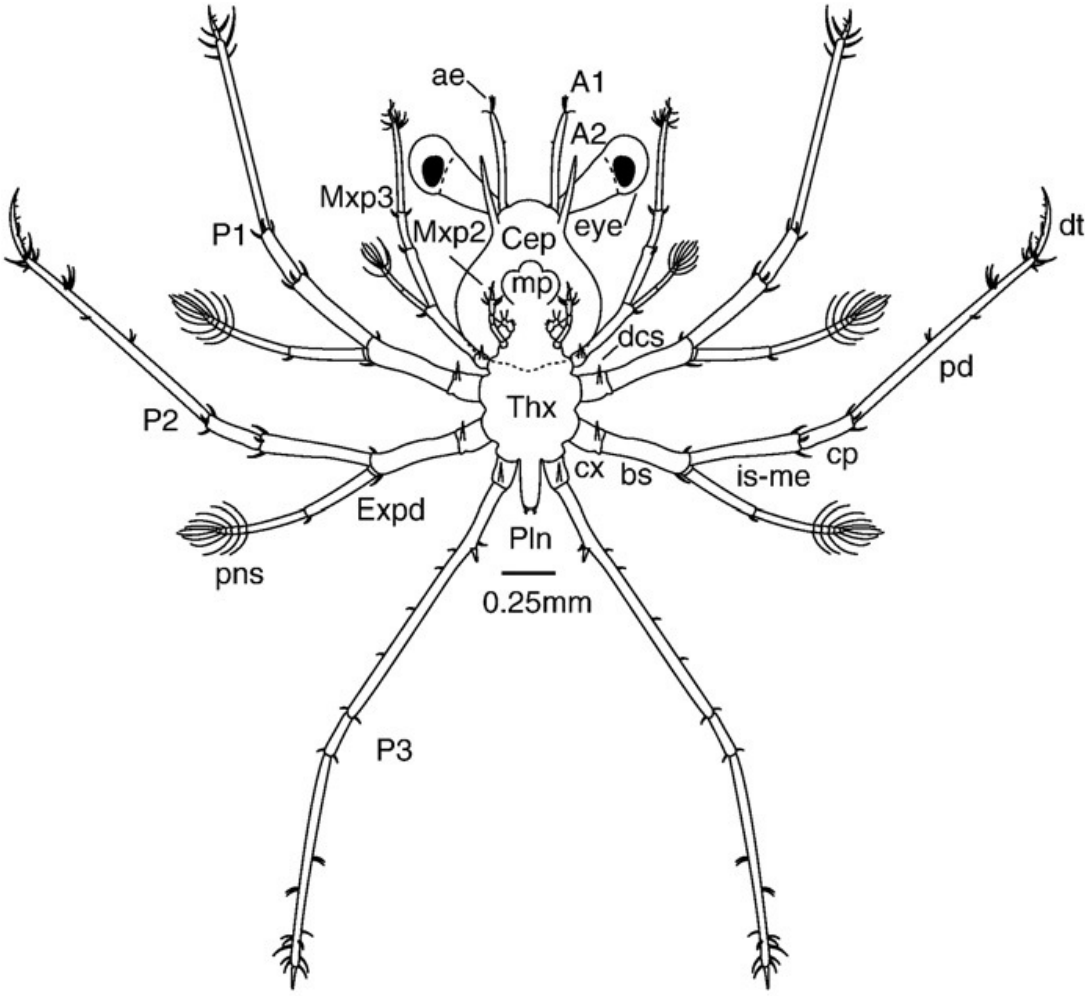

Figure 4: Stage I Panulirus ornatus phyllosoma ventral surface with external morphological features illustrated; Cephalic shield (Cep); Thorax (Thx); Pleon (Pln); Maxilliped (Mxp); Pereiopod (P); Antennule (A1); Antenna (A2); exopod (Expd); dcs (dorsal coxal spine); plumose natatory setae (pns); aesthetasc (ae); eye; mouth parts (mp); coax (cx); basis (bs); ischio-merus (is-me); carpus (cp); propodus (pd); dactylus (dt). Reproduced from Smith *et al.* (2009a).

Table 2: The defining morphological features of Panulirus ornatus phyllosoma during development to puerulus. Reproduced from Smith *et al*. (2009a).

Stage	Instar	Defining morphological features
I	1	Eyestalk unsegmented.
II	1	Eyestalk segmented, pereiopod 3 biramous but not setose.
III	1–3	Pereiopod 3 biramous and setose (1), pereiopod 4 bud (1)→cleft (2)→biramous (3).
IV	1–2	Pereiopod 4 biramous and setose, 4 segments (1) , antennule 3 weak segments (2), maxilla 1 basal endite intermediate between bidentate and tridentate (2),
V	1–2	Antennule biramous and setose (1), antennae 2 segments (1), Pereiopod 4 has 5 segments (1), pereiopod 5 is a bud (1), maxilla 1 basal endite tridentate (1), maxilliped 1 is a bud (1), exopod bulge on maxilliped 2 (2).
VI	1–2	Telson differentiated (1), uropod buds (1)→cleft (2), maxilliped 2 exopod bud (1), 5 antennae segments (2).
VII	1–2	Maxilliped 2 exopod bud setose (1), uropods bifid (2), pleopods weakly defined (2).
VIII	1–5	Uropods segmented (1), pleopod buds (1)→segmented (4), abdomen weak (1)→strong segmentation (2), spines on margin of last pleon segment and telson (1), maxilliped 1 apex sides undergo flattening (3)→ indentation (4), pereiopod 5 has 2 (1)→3 (5) segments, antennae pigmentation on tip (5)→.
IX	1–2	Pereiopod 5 has 4 segments (1), pleopods cleft (1)→with basal joint (2), outgrowth of maxilliped 1 apex (1–2), antennae pigmentation on tip.
X	1–2	Segmentation of pleopods (1), rudimentary appendix interna on pleopods (2), maxilliped 1 trilobe bud, single (1)→segmented (2) gill buds midway along the coxa of pereiopods 1–4 and maxilliped 3. Single gill buds at the base of maxilliped 2 and 3 and pereiopods 1–4 (2), paired gill buds on the thoracic region adjacent to pereiopods 2–4 (2) and single gill buds adjacent to pereiopods 1 and 5 and maxilliped 3 (2).
XI	1–2	Pleopod appendix interna elongated (1), inner and outer rami bearing setae (2), gill buds previously single midway along the coxa of pereiopods 1–4 and maxilliped 3 are bifid (1)→papilla like (2), pleopods setose with appendix interna well defined (2), spatula shape on terminal end of antenna (2), antennae pigmented at the tip (1) and midway along (2).

*Stages are represented by Roman numerals, while instars are represented by Arabic numbers, additionally their location within parenthesis is indicative of when specific development occurs.

Culture

Biological suitability, combined with high market value, has made *P. ornatus* popular as an aquaculture species throughout Vietnam, where sea cage farms grow wild seed through to market size by feeding fishery by-catch or "trash fish" (Jones, 2009b). Other South-East Asian countries with access to significant wild seed stock, such as Indonesia, are beginning to invest in similar farming operations (Jones, 2009b).

Concerns have been raised about the long-term sustainability of this practice. Significant lobster stock mortality, caused by disease outbreaks, have already been seen throughout Vietnam and have been linked to current lobster farming practises (Jones, 2009b; a). Efforts are now being made to discourage farms from using "trash fish" and to instead use a formulated pellet diet (Smith *et al.*, 2003a) to increase food conversion and decrease disease-promoting organic load around sea-cage facilities (Jones, 2009b).

The sustainability of this industry also relies on wild caught puerulus, the seed stock of all currently farmed lobsters. The collection of wild seed stock relies on the successful maintenance of significant wild broodstock populations. Should source broodstock populations become threatened, through continued anthropogenic perturbations such as overfishing and marine degradation, and fail to produce significant numbers of puerulus, this form of farming will be unviable.

These threats, combined with high market price and demand for this species, have led to significant interest in the development

of suitable techniques and technology for the hatchery rearing of this species (Grove-Jones *et al.*, 2002; Jones, 2009a).

The tank based larval culture of this species has now been completed (Anon., 2006; Sachlikidis *et al.*, 2009; Smith *et al.*, 2009a), though hatchery production is not yet at commercial levels. Progress so far has been achieved by research in all areas of the captive rearing of this species, specifically broodstock management and breeding, basic larval biology and system design.

For rearing technology to achieve commercialisation, methodology will have to be developed which delivers consistency in all areas of rearing. This includes consistent and timely generation of high quality larvae from captive broodstock and a more complete understanding of the basic biology and husbandry of this species including the adaptation of this knowledge to culture situations.

The research outlined in the following chapters has helped to advance understanding of some aspects of the biology of this species. Additionally, the following chapters describe practical techniques to increase the consistent production of larvae and early larval stage husbandry of *P. ornatus*.

Chapter III: Reproductive cues in *Panulirus ornatus*

The work presented in this chapter has been published as:

Sachlikidis, N. G., Jones, C. M. & Seymour, J. E. (2005). Reproductive cues in *Panulirus ornatus*. *New Zealand Journal of Marine and Freshwater Research* **39**, 305-310.

Abstract

Two experiments were performed to assess the effect of photoperiod and temperature on spawning of *Panulirus ornatus*. To elucidate the effects of light and temperature on breeding, two photoperiod treatments (summer and winter) were applied to sexually mature lobsters taken from the wild in summer and maintained at either summer or winter temperatures (24°C and 29°C) (Experiment 1).

Spawning was significantly greater when animals were exposed to summer photoperiod than to winter photoperiod irrespective of temperature. Although a higher percentage of lobsters spawned when placed under a higher temperature, this trend was not statistically significant.

To determine if natural season also influenced captive breeding, sexually inactive lobsters taken from the wild in winter and maintained under the above photoperiod treatments at a summer temperature of 29°C (Experiment 2).

Under these conditions, breeding started earlier and was more successful when animals were held at the summer photoperiod. Under this photoperiodic regime, the time to first breeding was 17 weeks after exposure to summer photoperiod, compared with less than 1 week in experiment 1, and did not occur until individuals had moulted. Moulting occurred in 81% of lobsters, primarily after an increase in temperature to 29°C.

The time between moulting and mating varied and there was no significant difference in moult frequency between the two experimental photoperiods. After the lobsters had moulted,

breeding success was reached earlier if photoperiod was lengthened.

Results suggest photoperiod is the primary cue for the onset of gonad maturity and mating activity. Animals held under summer photoperiods showed either increased instances of breeding and faster time to first breeding irrespective of temperature. Physiological rest and possibly a moult may be required between breeding seasons before spawning can occur. The importance of temperature and it's potential role in pre-reproduction moulting is also discussed.

Keywords ***Panulirus ornatus*; photoperiod; temperature; breeding; moulting**

Introduction

In marine lobsters, the relative importance and magnitude of temperature and photoperiod that induce gonad development, differs between species according to natural environment and breeding season (Muesy and Payen, 1988).

Chittleborough (1976) identified temperature alone as the primary stimulus for gonad development in *Panulirus cygnus* which has a distinct spring/summer breeding season. This is in contrast to *Panulirus japonicus* which depends on both photoperiod and temperature to cue the onset of gonad maturation (Matsuda *et al.*, 2002).

Although temperature was important, gonad development in *P. japonicus* was retarded at photoperiods of less than 12.5 h of light per day. Findings were similar for *Panulirus argus* for which gonad development and mating behaviours were significantly enhanced in animals held at longer day-length, irrespective of temperature (Lipcius and Herrnkind, 1985).

Breeding in many lobster species has been controlled in captivity through the manipulation of environmental factors in particular photoperiod and temperature (Lipcius and Herrnkind, 1985; 1987; Matsuda *et al.*, 2002). To be able to successfully breed *P. ornatus* in captivity it is important that the breeding cues are first properly identified for this species.

P. ornatus is known to take part in a mass annual summer breeding migration from the Torres Strait, across the Gulf of Papua

to Yule Island (Moore and MacFarlane, 1984). However, breeding populations of *P. ornatus* on the north-east Queensland coast are non-migratory (Bell *et al.*, 1987). In both instances, breeding is nonetheless seasonal with berried females reported in summer months when photoperiod is at its annual longest and water temperature at its highest level.

This paper aims to asses the use of photoperiod and/or temperature by *P. ornatus* as cues to the onset of breeding.

Methods

To determine the effects of photoperiod and temperature on spawning in *P. ornatus*, wild-caught mature lobsters were exposed to different combinations of these factors. As the reproductive condition of lobsters brought into captivity for experimentation would likely influence the response to controlled environmental stimuli, lobsters were exposed to winter conditions of water temperature and photoperiod (as measured from their typical natural environment), before the application of experimental conditions for a period of 4 weeks.

To mimic photoperiods that *P. ornatus* would be exposed to in nature, mean summer (14.5 Light (L): 9.5 Dark (D)) and a mean winter (13 Light (L):11 Dark (D)) photoperiods were used. These were chosen as they represent nautical day lengths as recorded at Cockburn Reef (11°49'E 143°21'S) on the east coast of Queensland, from where experimental animals were collected and where breeding individuals had been found (Anon., 2002b). Similarly, water temperature equivalent to summer (29 ± 0.3°C) and winter (24 ± 0.3°C) of this location were also used (Anon., 2002a).

To elucidate the effects of light and temperature on breeding, two photoperiod treatments (summer and winter) were applied to sexually mature and active lobsters maintained at summer or winter (24°C and 29°C) (Experiment 1). To determine if time of year (i.e., season) also influenced breeding, the above photoperiod treatments were also applied to seasonally sexually inactive lobsters maintained, in winter, at a summer temperature of 29°C (Experiment 2).

Both experiments were conducted in six 2000 litre round polyethylene tanks supplied with semi-recirculated sea water, within an environmentally controlled room. Three tanks were applied to each of the two treatments within a separate recirculation system. Each system was connected to a 2000 litre sump. Water in each system was recirculated continually at the rate of 330 litres per hour providing a total replacement of water in each tank four times per day.

Water from each tank drained to the sump through a screen to collect larger solids, and was then pumped through bead and fluidised bed filters, a protein skimmer and a heat pump before delivery back to the tanks. Water temperature for each of the two systems was controlled using Aqua hort™ heat exchange units and logged in each system by Gemini Tinyview™ temperature loggers.

Light was applied by single 20-watt halogen waterproof lights mounted on the wall of each covered tank. Maximum light levels of an intensity of 110 lux were used to simulate typical light levels found in the natural environment of *P. ornatus*. Light was remotely controlled by a Clipsal Pty. Ltd. C-Bus™ home automation system which ramped tank light levels up and down at specific programmed times. Sunrise and sunset were programmed to occur over 17 min

each daily within each tank. Light was monitored and logged by Stowaway SLA-08™ light loggers.

Each tank was equipped with a freestanding PVC table shelter measuring 600 mm x 800 mm and standing 250 mm high. A 1 m² strip of plastic mesh (10 mm mesh size) was suspended from the edge of each tank and weighted so as to partially cover an area of the tank wall and floor to enable spawning females to hang vertically, as an aid for oviposition (Berry, 1970b).

Previous studies have show that in wild breeding populations females with carapace lengths (CL) of >100 mm comprise the majority of reproductive animals (MacFarlane and Moore, 1986). Consequently, only females >100 mm and <130 mm CL were used in these experiments. Similarly, as mating success has been demonstrated to improve with increased male relative to females size (Berry, 1970b), only males greater that 130 mm CL were used.

In the wild, at recognised breeding areas for *P. ornatus*, males tend to be larger and females more abundant (ratio of males to females is 1:1.5 or greater)(MacFarlane and Moore, 1986). Lobsters for the experiments were chosen accordingly. Seven adult lobsters, two male and five female were assigned to each tank (male to female ratio of 1:2.5). Animals were selected from commercial landings from Cockburn Reef (11° 49'E. 143° 21'S), north-eastern coast of Australia in October 2002 and 2003 and April 2003.

Individual weight, carapace length (CL), and sex were recorded at the time of stocking to the experimental tanks. Lobsters were identified using individually numbered tags, consisting of small round pieces of waterproof paper glued to the base of the rostrum using Locktite 454™ instant adhesive. Moulted individuals were

recognised during daily tank checks as those without a tag, and based on the tagged exuvium were identified (by patterns between the frontal horns) and re-tagged.

Lobsters were stocked to the experimental tanks and then conditioned to average winter temperature and photoperiod by incrementally adjusting water temperature to 24C ± 0.3C and photoperiod to 13 L : 11 D over a period of one week to stimulate them to a non-breeding condition. For experiment 1, this involved a reduction from ambient summer conditions, and for experiment 2, from ambient winter conditions. Lobsters were held in these conditions for 4 weeks.

For initiating the treatment effect in both experiments, photoperiod was increased immediately from the winter (13 L : 11 D) condition to summer (14.5 L : 9.5 D) photoperiod in half of the experimental tanks. For Experiment 1, water temperature was maintained at either 24°C ± 0.3 or increased to 29°C ± 0.3 overnight. For Experiment 2, the water temperature in all tanks was increased overnight to 29°C ± 0.3 at this point.

Experimental conditions (post conditioning) were maintained until a majority had spawned (three weeks for Experiment 1). In Experiment 2, at 16 weeks post conditioning, the photoperiod for the winter, short day treatment was increased to 18 L : 6 D to impose a large photoperiodic increase to attempt to shock the animals into breeding. Females were examined on a weekly basis to check for spawning activity.

At the end of the experiments, weights and CL were re-recorded. Animals from Experiment 1 that had not spawned were

dissected and the ovaries macroscopically staged from 1-4 as follows:

Stage 1: Immature. Ovaries white, flattened dorso-ventrally.
Stage 2: Developing. Ovaries pink to pale orange, noticeably enlarged.
Stage 3: Ripe. Ovaries bright orange to red, greatly enlarged.
Stage 4: Spent. Ovaries white, yellow or pale pink, often with a few enlarged ova retained from stage 3 at overall lobe extremities (often indistinguishable from stage 1).

Water quality parameters of pH, salinity, dissolved oxygen, ammonia, and nitrite were measured and recorded weekly and more frequently if outside the desirable range. Food provided to the experimental lobsters consisted of live and frozen pipis (*Plebidonax deltoides*), frozen green mussels (*Perna canaliculus*) and squid (*Loligo opalescens*) provided once per day after 1500 h. Animals were fed at the rate of 3% body weight per day consistently for all tanks (approximate maximum food intake from pilot study).

The proportion of females within each treatment that had spawned was analysed by application of a two-way ANOVA using Genstat 5th ed. (Lawes Agricultural Trust). Data was first ArcSin transformed to normalise the distribution. Additionally, ANOVA was used to assess differences in weight and CL between both sexes and tanks.

Results

Experiment 1

Of the original female stock held at 24°C, 30% unexpectedly produced unfertilised eggs during the conditioning period and were excluded from the trial. As a consequence, only 4 of the 6 proposed replicates were run with unspawned animals repositioned into 4 tanks to maintain the initial stocking density. A similar phenomenon was observed for the 29°C trial however more animals were conditioned which therefore enabled the stocking of the full six available tanks.

Three weeks after the application of the two photoperiod treatments, significantly more females had spawned under the summer photoperiod (14.5 L : 9.5 D) than at the winter photoperiod (13L : 11 D) (p = 0.030) irrespective of temperature (Figure 5). Temperature slightly increased breeding success however not significantly (p = 0.380) (Figure 5). Dissections of the remaining animals found significantly greater ovary development in animals held under summer photoperiod (p = 0.004) (Figure 6).

There was no significant difference in weight within or between tanks for each sex. Males were significantly heavier and had significantly longer CL than females within each tank (p = 0.001). Neither CL nor weight was found to have affected breeding (p = 0.324 and 0.176 respectively).

Experiment 2

Moulting rate increased dramatically 4 weeks after the conditioning period was completed, i.e. after the temperature had

been increased to 29 ºC (Figure 7). No significant difference in frequency of moulting was evident between winter and summer photoperiod (p = 0.505, n = 42). When no spawning occurred after 16 weeks, but the bulk of lobsters moulted, it was postulated that a pre-reproduction moult was necessary before reproduction could be triggered.

In an attempt to cue the onset of reproduction the winter (13 L : 11 D) treatment was increased to 18 L : 6 D (Figure 7). Breeding occurred first in the animals held in the summer photoperiod treatment, 17 weeks after the end of conditioning (Figure 7). Breeding began 2 to 3 weeks later for populations exposed initially to winter photoperiod.

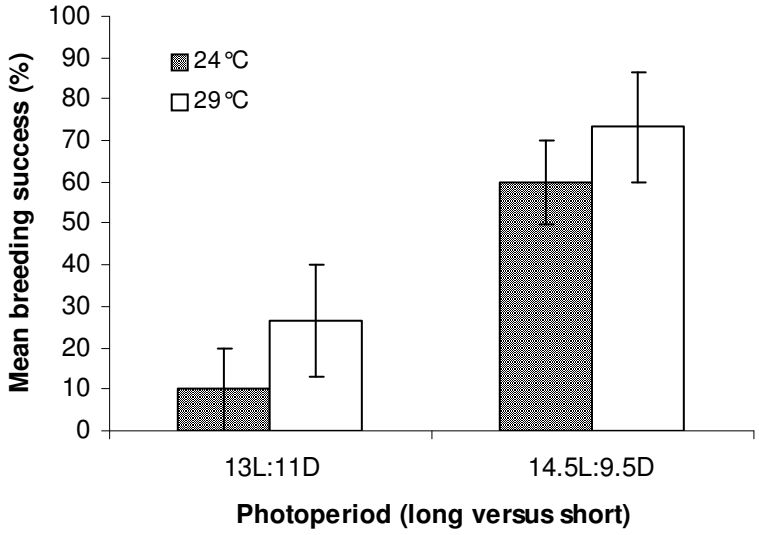

Figure 5: Percentage of lobsters (*Panulirus ornatus*) (female) spawning in each tank under two photoperiod treatments; short day (13:11 L:D) and long day (14.5:9.5 L:D) at either summer or winter average temperature.

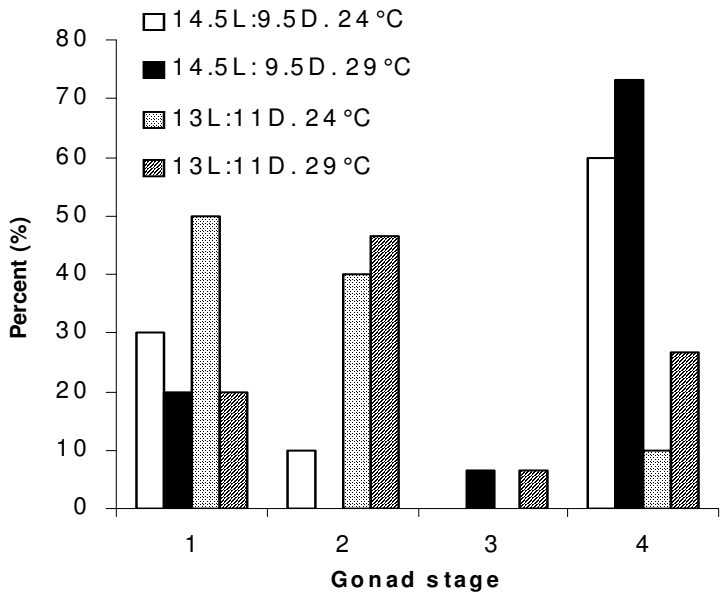

Figure 6: Stages of ovary development for *P. ornatus* from animals held under long or short day lengths at both temperatures (%). Spawned animals were assumed to be at a spent (stage 4) condition and recorded as such. Stages are described under methods section.

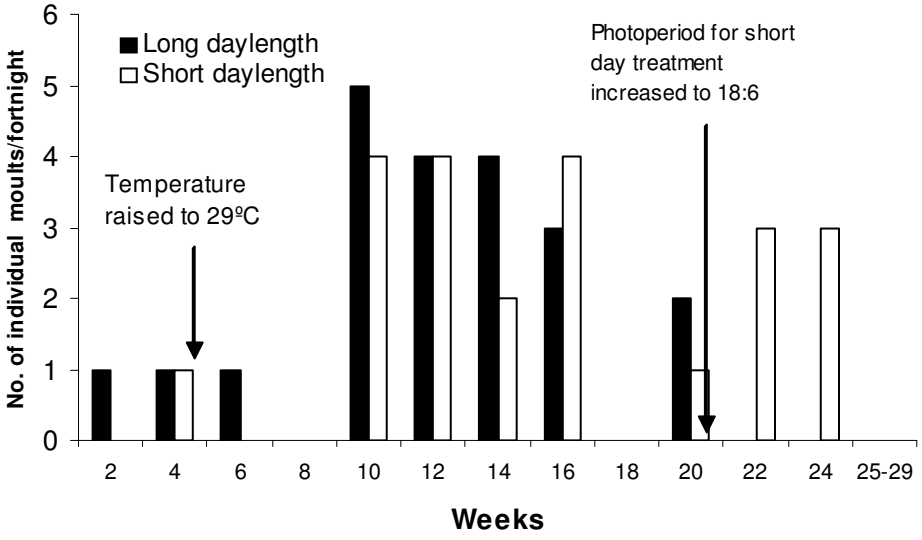

Figure 7: Overall number of moults *P. ornatus* per fortnight through the experiment for each treatment (long and short photoperiod). Conditioning period ended after 4 weeks, at which time water temperature was raised to 29°C.

Discussion

P. ornatus is known to take part in migration to breeding aggregation sites during summer (Moore and MacFarlane, 1984). Breeding is seasonal with berried females reported within summer months annually. Seasonally changing environmental parameters may be used by this species as cues to aid synchrony of migratory behaviour/breeding however possibilities are numerous and may include, for example, seasonal changes in water temperature, photoperiod, or other abiotic stimuli (Herrnkind, 1980).

Temperature and photoperiod were tested in this experiment. As breeding occurs in *P. ornatus* populations when photoperiod is at its annual longest and water temperature at its highest, different combinations of these factors were assessed as cues for breeding in *P. ornatus*.

Results from Experiment 1 implicate photoperiod as a significant cue for breeding in *P. ornatus*. Photoperiod is used to cue processes such as diapause, hibernation, breeding, and migration in many species of animals including marine invertebrates resulting most probably from annual reliability (Herrnkind, 1980; Gwinner, 1981; Olive, 1995).

Findings from this study compare with other similar studies which have also determined photoperiod to be an important environmental cue to breeding in other palinurid species (Lipcius and Herrnkind, 1985; 1987; Matsuda *et al.*, 2002).

The reasons why this photoperiod is used as a primary cue and not temperature may be related to the occurrence of mass migrations for spawning *P. ornatus*. General trends in water

temperature, although most likely important to moulting and gonad maturation, are subject to significant variation within season or local geographic area. Although mass spawning populations of *P. ornatus* are restricted within the species distribution, local variations in water temperature across this range, especially at small isolated reefs, may not allow for synchronised timing of the migratory and spawning event. However, photoperiod is a feature of latitude and would be expected to vary little over the range of this species, and as such may be a more reliable cue, allowing for breeding synchrony across the population (Olive, 1995).

Temperature appears to be of less significance. However, it is possible that this was shown to be non-significant because of low sample size within this trial. Spawning rates were increased (non-significantly) and gonad was further developed in animals held under high temperatures. It is likely that temperature is in some way related to the onset of breeding. However, based on the results of this study it is probably a less important breeding cue for this species.

The absence of breeding activity in Experiment 2 (17 weeks exposed to the experimental treatments) was unexpected. In all respects, Experiment 2 was equivalent to Experiment 1 with only one major difference: the experimental lobsters were obtained from the wild during winter (in a non-breeding condition) relative to those of Experiment 1, which were obtained in summer.

Although the summer photoperiod cue may be appropriate to stimulate spawning, it may only work for lobsters physiologically prepared. An important part of this may be a necessity to moult before breeding. The dramatic increase in moulting of lobsters in Experiment 2 after the temperature was raised suggests that the

increase in temperature may be a cue for pre-reproductive moulting.

Twelve weeks after the sudden increase in moulting, spawning activity was still not evident and the photoperiod of the winter photoperiod treatment (13 L : 11 D) was increased to 18 L : 6 D to provide a further cue to trigger onset of reproduction. This appeared to have an effect with breeding occurring in subsequent weeks.

It may be possible to somewhat hasten breeding through the induction of a moult and subsequent increase in photoperiod post moult.

The speed with which these treatments had their desired effect has important implications to the establishment of management protocols for year-round breeding. Although it may be possible to shift the reproductive season, using controlled conditions, to enable breeding out of phase with wild populations, it may not be possible to significantly condense the reproductive cycle into a period less than 12 months.

Additionally, condensation of the breeding season may have consequences in terms of larval quality. Although several other tropical Palinurid species have been shown to breed year round (Juinio, 1987; Briones-Fourzan and Lozano-Alvarez, 1992), continuous breeding of the same captive lobsters, many successive times, may not generate high quality eggs and larvae. For example, it is possible that lobsters forced to breed for two or more seasons in one year may hatch larvae of lesser quality than those that undergo the usual one breeding season annually.

The establishment of multiple breeding populations that are out of phase with wild season is likely to be the most effective way to generate high quality larvae year round.

The necessity for a pre-reproduction moult is well documented for many crustaceans. In lobsters, moulting provides the female with fresh ovigerous setae on the endopods of the pleopods which are critical for attachment of the freshly laid eggs (MacDiarmid and Kittaka, 2000). In establishing protocols for year-round breeding, it may be necessary to stimulate a moult before the initiation of mating and spawning.

Although this preliminary work has isolated some of the cues important to breeding, further research into the endocrinology of moulting and gonad development as well as flow-on research into effects of different breeding regimes on larval quality may provide a more complete understanding of *P. ornatus* reproduction.

Acknowledgments

Thanks to the ever-reliable W. Bowman, D. Horton and L. Linton for their technical advice and help during laboratory experiments and also to S. Shanks and D. Toon for manuscript revision. Thanks also to M.G. KIALIS for their support. This research was supported by the Fisheries Research and Development Corporation (FRDC): project number: 2003/211, through the Rock Lobster Enhancement and Aquaculture Subprogram.

Chapter IV: The effect of temperature on the incubation of eggs of the tropical rock lobster *Panulirus ornatus*.

The work presented in this chapter is published as:

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Abstract

The period over which spiny lobster eggs develop varies widely and is related to incubation temperature. For the tropical rock lobster *(Panulirus ornatus)*, this relationship is found to be: *Incubation period (days) = 95.444-2.482 x Temperature (ºC)* for incubation temperatures between 24 and 30 ºC. Incubation temperatures ≥32ºC are unsuitable for this species and result in the termination of egg clutches. Additionally, egg clutches incubated at lower temperatures hatched over more nights than those at higher temperatures. These findings mean that lead times to larval hatch can now be predicted for this species, allowing for preparation time prior to larval culture.

Keywords: **Lobster; egg incubation; temperature; *Panulirus ornatus*; hatch prediction.**

Introduction

The tropical rock lobster, *Panulirus ornatus*, is a large (>4kg), reef-dwelling lobster fished commercially in north-eastern Australia (Holthuis, 1991). This fishery is extensively managed (Ye *et al.*, 2008), but has limited capacity for increased production (Ye and Dennis, 2009), whilst demand for the species into export markets exceeds supply prompting interest in the development of aquaculture technology for this species.

Over the past decade, a concerted research and development effort in Australia has examined the various life history phases, in particular the complex and protracted larval phase (George, 2005; Jones *et al.*, 2006; Jones, 2009a). A focal point of this research has been the development of larval culture techniques that increase larval survivorship towards the commercial culture of this species (Grove-Jones *et al.*, 2002; Jones *et al.*, 2006; Jones, 2009a).

In a culture situation, uncertainty of larval hatch date and variability in larval quality is undesirable and may cause expensive disruptions to production schedules and human resourcing. As a management tool, accurate, long-range prediction of hatch date and production of high quality larvae are advantageous as they allow for the determination of lead times and output consistency.

Culture preparation time is important for spiny lobsters whose larval culture requires the preparation of expensive and time consuming live feeds (Kittaka, 2000). Time and effort is wasted if larval production is either stopped or delayed due to inconsistencies in larval quality or the inaccurate prediction of hatch date. Under these circumstances, the ability to predict the date of hatch would add to culture success and reduce associated costs.

In marine lobsters, the incubation period between egg fertilisation and egg hatch is temperature dependant (Perkins, 1972; Phillips and Sastry, 1980; Aiken and Waddy, 1985a; Tong *et al.*, 2000; Smith *et al.*, 2002). Incubation period is negatively correlated with temperature and can vary widely, for example, the incubation period of *Sagmariasus verreauxi* can range between 55 (20 °C) and 130 (13°C) days depending on the incubation temperature (Moss *et al.*, 2004).

Incubating eggs at a temperature that produces larvae in the shortest time period may be advantageous under some circumstances, however, larval quality can also be affected by incubation temperature (Aiken and Waddy, 1985a; Smith *et al.*, 2002).

The effect incubation temperature has on larval quality is unknown for *P. ornatus* and the rate at which eggs develop may not be the most important factor in determining the temperature at which an egg clutch should be reared for optimal results.

Egg incubation at a relatively high temperature in *Jasus edwardsii*, although expediting development, produced smaller larvae (Tong *et al.*, 2000; Smith *et al.*, 2002) with reduced levels of eicosapentanoic acid (20:5n-3) and sterols compared with those incubated at lower temperatures (Smith *et al.*, 2002). In this instance, expediting incubation may hinder rather than enhance the production of consistent, high quality larvae (Smith *et al.*, 2002).

It is possible that for *P. ornatus,* incubating eggs at lower temperatures may take longer, however the larvae produced may

be of superior quality and result in increased overall culture success.

Successful predictive hatch models have been developed for some lobster species. These models use sequentially developing embryonic features, such as the eyespots, to gauge time until hatch at a specified temperature (Perkins, 1972). By relating indexed embryonic eyespot measurements to the developmental rate at a given temperature, the date of hatch can be predicted for that temperature (Perkins, 1972).

This method of hatch prediction has been successfully developed and used for a number of clawed and spiny lobster species (Perkins, 1972; Phillips and Sastry, 1980; Aiken and Waddy, 1985a; Helluy and Beltz, 1991; Tong *et al.*, 2000; Smith *et al.*, 2002; Moss *et al.*, 2004).

For this hatch model to work however, the developing embryos must first develop eyespots before the eye index can be measured and subsequent hatch date predicted. Presently, this model is used for temperate species, where the time between the initial development of eye spots and hatch can be protracted. For example, the eye spots of *S. verreauxi* can be measured up to 60 days prior to hatch when eggs are incubated at 13 °C which gives significant notice of the impending hatch for management purposes (Moss *et al.*, 2004).

Tropical species have comparatively more rapid egg development. Adult female *P. ornatus* have an incubation period of approximately three weeks before the developed larvae hatch when held at ambient summer temperatures (Jones *et al.*, 2003). Eye spots do not develop until around 12 days prior to hatch (Jones *et*

al., 2003), giving only short notice of the impending hatch date and insufficient time for hatchery preparation.

An alternative technique that may provide a longer forecast for hatch date prediction is a model based on developmental rate. By using the date of egg extrusion and the developmental rate at a known temperature the hatch date can be theoretically predicted (Tong *et al.*, 2000). In addition to providing more preparation time, this technique has the added advantage of precluding repeated sampling of the egg clutch through the late stages of development, and its associated handling stress.

The aims of this experiment are to measure the effect of incubation temperature on the egg incubation period of *P. ornatus* and to generate a practical predictive hatch model for a range of culture temperatures by assessing the thermal characteristics of fertilised eggs of this species.

Materials and methods

Captive breeding

To provide the necessary egg bearing (berried) lobsters, captive breeding was achieved using methods described in Sachlikidis *et al.* (2005). In brief, adult lobsters were bred under a 14 l:10 d photoperiod in 28ºC sea water. Broodstock lobster size was consistent between treatments and female size (100-130mm carapace length) was smaller than male (>130mm carapace length). All lobsters were selected from commercial landings from the northeastern coast of Australia.

Each lobster was individually identified with a tag consisting of a numbered waterproof paper dot glued between the frontal horns. As berried females were identified during daily checks, they were transferred to the incubation system within 24 hours of egg extrusion.

Incubation

The experimental system consisted of five independent flow through systems operated at a fixed temperature of either 24, 26, 28, 30 or 32°C. This temperature range was chosen to represent the range of average water temperatures *P. ornatus* is exposed to seasonally at known breeding grounds in North-eastern Australia and the Gulf of Papua New Guinea (MacFarlane and Moore, 1986; Bell *et al.*, 1987) (http://www.metoc.gov.au/ims/website/woa01/viewer.htm). Fifteen lobsters were sequentially allocated between each temperature treatment according to date of egg extrusion, equating to three per treatment. Each berried lobster represented an experimental unit and was housed individually for the duration of egg incubation.

Individual flow-through systems consisted of a 200 L pre-heated chamber, a 200L broodstock container (in which each experimental lobster was housed) and finally a larval collector (Figure 8). Supply water was flow through and was first cooled to 24°C in a 500L sump using a Kirby™ water heat/chill unit before being heated to the prescribed treatment temperature with titanium immersion heaters and introduced to the broodstock container.

Water was delivered to each tank at the rate of 16.6 L/minute^{-1} providing a 200% water exchange per tank per day^{-1}. Used water

was put to waste. Hatched larvae were collected in the larval collection chamber post hatch (Figure 8).

Tanks were cleaned on a daily basis. Water quality parameters were measured daily and maintained within acceptable limits for the duration of the experiment, these were: pH 7.8-8.4, salinity 30-34 ‰, dissolved oxygen 6-7 mg/l, ammonia 0.0-0.1 mg/L and nitrite 0.0-0.01 mg/L. Half-hourly logged temperatures within the broodstock chambers were within ±0.2°C of the prescribed treatment temperatures.

Diet for the broodstock in the breeding tanks consisted of pipis (*Plebidonax deltoids*), green mussels (*Perna canaliculus*) and squid (*Loligo sp.*) provided once per day after 3pm at around 3% bodyweight adjusted according to observation. Feed rate remained consistent between tanks, and was monitored daily.

Once berried females were moved into the egg incubation system, feeding consisted of one green mussel every second day to minimise fouling and to mitigate any negative effects such as the dropping of the egg mass which can be caused by bacterial and fungal infection of the egg mass under high organic load (MacDiarmid and Sainte-Marie, 2006).

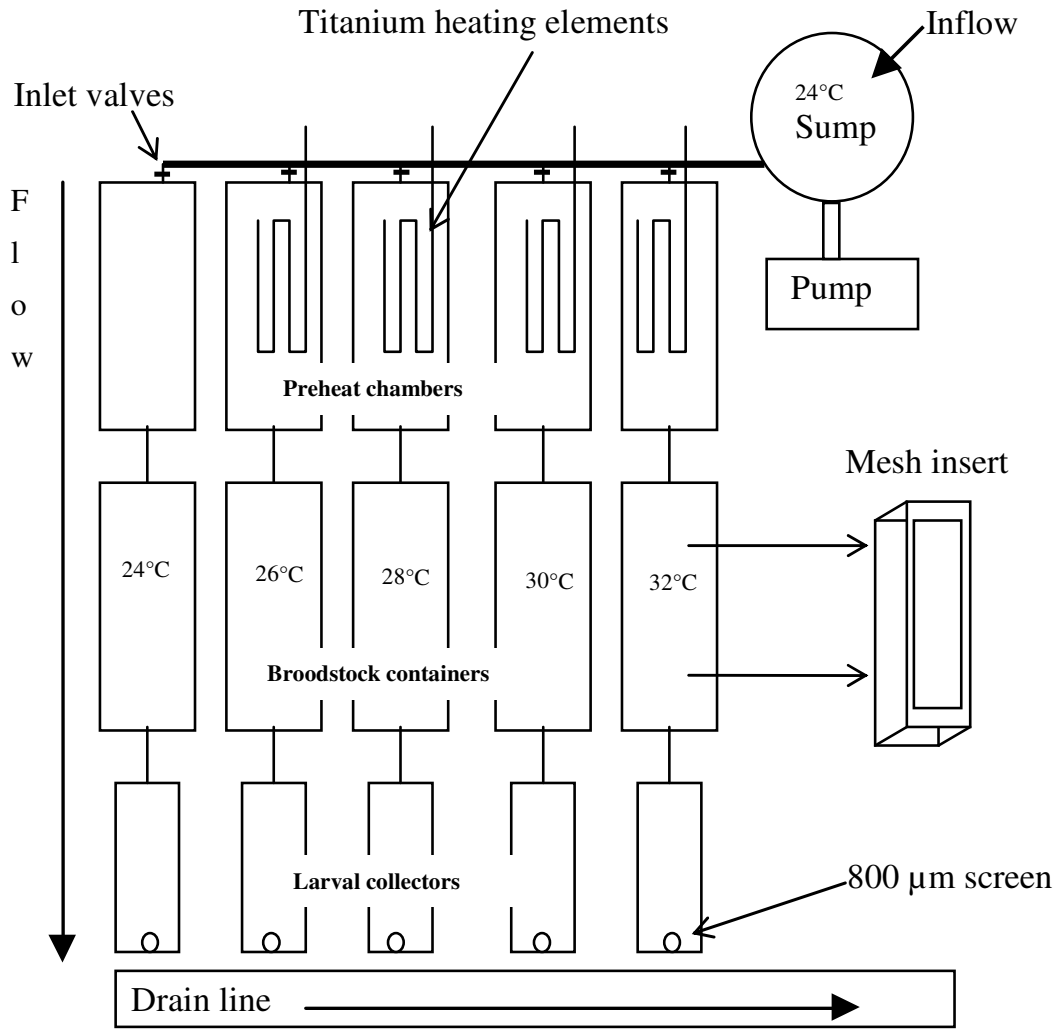

Figure 8. An illustration of the incubation system consisting of pre-heat chambers, where incoming water is heated to the desired treatment temperature, broodstock containers, for the holding of berried broodstock throughout the incubation period and larval collectors where hatched larvae collect prior to testing. Water flow is denoted by arrows.

Data recorded for each lobster included date of egg extrusion (berry date) and hatch date. Incubation period was recorded as the period in days from egg extrusion to the first night of larval hatch.

Larval size and fatty acid sampling

After hatching, larval size was measured for 20 individual larvae by microscopy (Leica MZ6 microscope, Leica DFC 320 Camera, Leica CLS50X camera and Leica Applications Suite Software Version 3.3.1, copyright Leica Microsystems (Switzerland) limited), including the total length, carapace length and carapace width (to 0.01mm) for each hatch.

The total length (TL) was defined as the distance from the anterior cephalic shield; between the first antennules, to the central posterior pleon. Carapace length (CL) was defined as the distance from the anterior to the posterior margins on the cephalic shield and carapace width (CW) was defined as the distance at the widest point of the cephalic shield.

100 larvae from each clutch were also sampled and frozen at -80°C for subsequent analysis of fatty acid content. Lipids were extracted from samples with chloroform/methanol by the method of Folch et al (1957). Total lipid was determined gravimetrically on an aliquot of the extract by drying for 4 hours at 80^{o}C in a pre-weighed glass vial.

A further aliquot of the extract was taken for fatty acid analysis. The lipid fatty acids in the extract were derivitised to their fatty acid methyl esters (FAME) using 14% boron trifluoride-methanol (Van Wijngaarden, 1967). FAME were analysed on an Agilent Technologies 6890 gas chromatograph using split injection

with helium carrier gas and a flame ionization detector. The column used was a DB23 fused silica capillary column, 30m x 0.25mm, with a 0.25µm coating (Agilent Technologies, USA). Column oven temperature was held at 140°C for 5 minutes and then elevated at 3°C/minute to 210°C where it was held until all FAME had been eluted. FAME were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich Co, USA), and were quantified by comparison with the response of an internal standard, heneicosanoic acid.

Statistical analysis was conducted using one-way analysis of variance (ANOVA) and regression analysis statistical tests. ANOVA's were validated by examining residual plots and means comparisons were performed for all significant ANOVA's ($P<0.05$) by LSD post-hoc testing. Probabilities of <0.05 were considered significant and analysis were carried out using Genstat 11th edition. Morphometrics are presented as mean ± S.E.M.

Results

Incubation period

The incubation period of *P. ornatus* was significantly related to temperature ($R^2=0.938$, d.f. 1, 11, $p< 0.001$) (Figure 9). Incubation duration was shorter when broods were exposed to higher temperatures ranging from an average of 22 days (30°C) to 36 days (24°C), an average range of up to 14 days. Of the 15 broods tested, 12 hatched successfully and all broods of eggs incubated at 32°C were aborted before larval hatch. The predictive equation for *P. ornatus* egg incubation period between 24 and 30 °C is:

Incubation period (days) = 95.444 (95%CL 83.919 to 106.97) - *2.482* (95%CL: - 2.907 to -2.058) *x Temperature (ºC)*

Hatching of eggs incubated at 24°C usually extended over two nights while hatching of eggs incubated at higher temperatures was more succinct, generally occurring over one night (Figure 10). Larval hatches occurred only at night and were therefore over a discreet number of days.

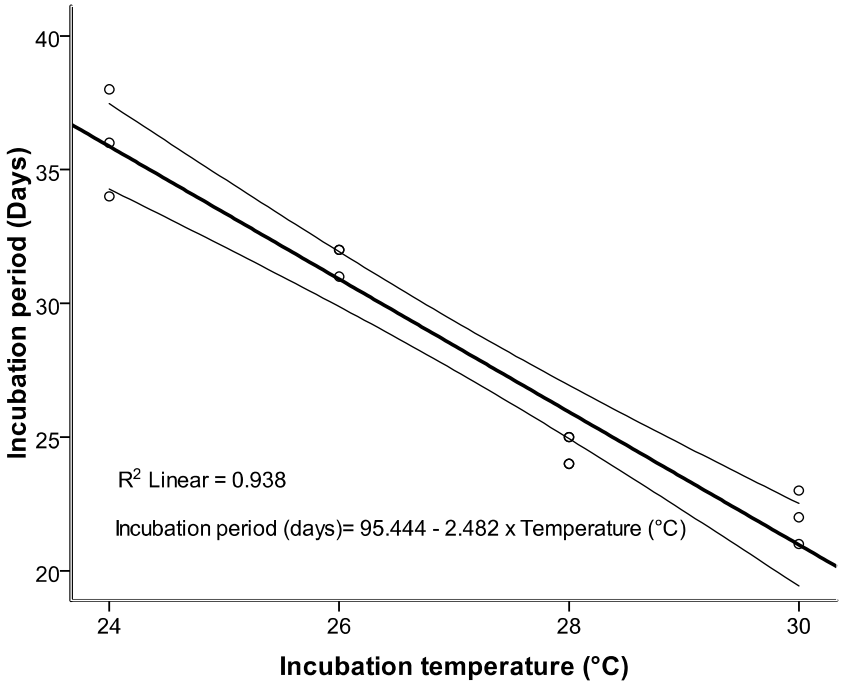

Figure 9. Relationship between incubation period and incubation temperature for P. ornatus (R^2=0.938, d.f.1,11, p=<0.001, ±95% CL).

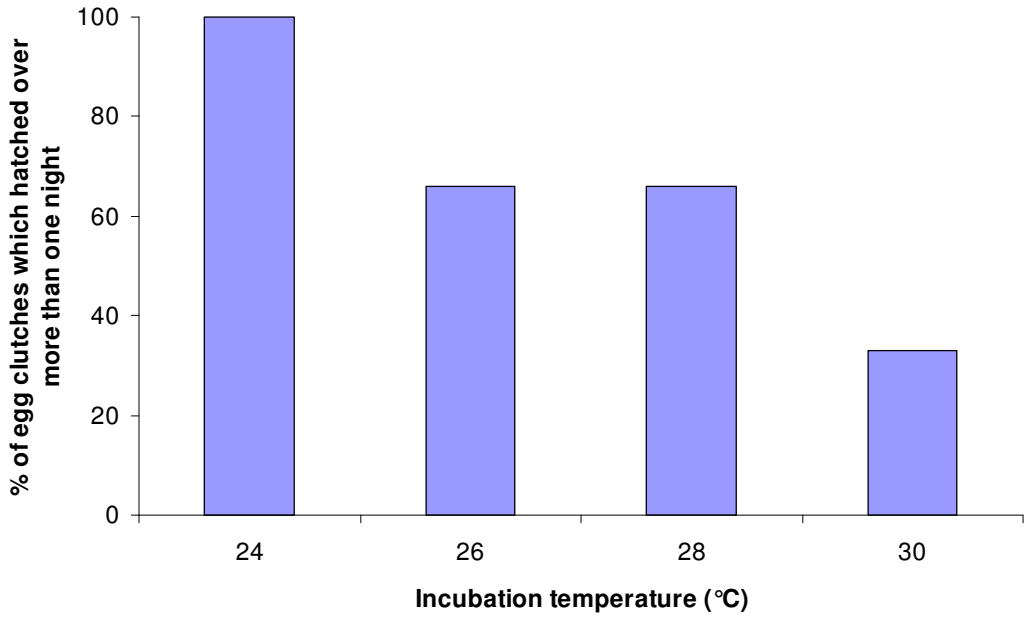

Figure 10. The percentage of broods which hatched over more than one night in relation to incubation temperature. Hatchings were more succinct when eggs were incubated at higher temperatures and spread out to a maximum of two nights at lower incubation temperatures.

Table 3. Fatty acid composition (mg/g) of newly hatch phyllosoma of Panulirus ornatus incubated at different temperatures throughout embryonic development.

Fatty acid	Incubation temperature			
	24℃ (*n=3*)	26℃ (*n=3*)	28℃ (*n=3*)	30℃ (*n=3*)
14:0	0.17±0.040	0.18±0.004	0.23±0.038	0.19±0.005
14:1n-5	-	-	-	-
15:0	0.16±0.041	0.14±0.008	0.19±0.029	0.16±0.005
16:0	5.71±1.079	5.45±0.088	7.11±0.767	6.15±0.326
16:1n-7	0.74±0.296	0.76±0.019	1.02±0.120	0.84±0.061
17:0	0.41±0.097	0.38±0.020	0.48±0.065	0.42±0.033
17:1n-8	-	-	-	-
18:0	3.99±0.573	3.91±0.053	4.98±0.655	4.38±0.050
18:1n-9	4.30±0.801	3.58±0.090	4.13±0.315	3.73±0.348
18:1n-7	1.27±0.277	1.01±0.022	1.14±0.114	1.00±0.015
18:2n-6	0.34±0.103	0.33±0.012	0.43±0.015	0.36±0.042
18:3n-6	0.13±0.053	0.09±0.007	0.11±0.017	0.11±0.013
18:3n-3	0.08±0.026	0.09±0.020	0.11±0.015	0.11±0.028
20:0	0.15±0.019	0.14±0.003	0.19±0.026	0.18±0.003
20:1n-11	0.11±0.051	0.10±0.013	0.13±0.004	0.12±0.025
20:1n-9	0.84±0.010	0.92±0.029	1.09±0.094	0.87±0.053
20:1n-7	0.12±0.000[a]	0.12±0.009[ab]	0.13±0.008[ab]	0.15±0.004[b]
20:2n-6	0.41±0.049	0.38±0.016	0.44±0.048	0.43±0.042
20:3n-6	-	-	-	-
20:4n-6 (AA)	2.13±0.701	1.33±0.161	1.67±0.261	1.50±0.091
20:3n-3	0.15±0.016	0.18±0.012	0.22±0.027	0.20±0.038
20:4n-3	-	-	-	-
20:5n-3 (EPA)	5.87±0.692	5.49±0.041	6.26±0.631	4.87±0.154
22:0	0.06±0.007	0.07±0.012	0.08±0.015	0.08±0.007
22:1 isomer	0.27±0.030	0.19±0.011	0.31±0.072	0.20±0.010
22:5n-6	0.11±0.043	0.09±0.019	0.10±0.021	0.08±0.006
24:0	-	-	-	-
22:5n-3	0.13±0.050	0.13±0.015	0.14±0.021	0.16±0.027
22:6n-3 (DHA)	4.52±0.488	4.73±0.191	5.62±0.611	4.41±0.086
24:1n-9	-	-	-	-
∑Saturated	10.65±1.856	10.24±0.138	13.26±1.592	11.57±0.272
∑Monounsaturated	7.70±1.524	6.68±0.084	7.95±0.699	6.95±0.335
∑Polyunsaturated	13.94±2.256	12.87±0.344	15.11±1.623	12.25±0.392
∑n-3	10.77±1.267	10.64±0.187	12.35±1.279	9.76±0.203
∑n-6	3.17±0.989	2.23±0.190	2.76±0.352	2.50±0.189
∑n-3/n-6	3.63±0.735	4.84±0.389	4.50±0.153	3.94±0.203
Total fatty acid	32.30±5.636	29.80±0.410	36.32±3.876	30.77±0.577

Data presented as mean ± S.E. fatty acids as mg/g dry weight; "–" represents trace fatty acids which are present at levels below <0.05 mg/g; Different superscript denotes fatty acids which are significantly different from one another ($P<0.05$).

Larval size and fatty acid sampling

There was no significant difference in larval size between incubation temperatures (P>0.05). Average morphometrics measurements were; total length 1.434 ± 0.007 mm, carapace length 0.777 ± 0.005 mm and carapace width 0.744 ± 0.004 mm.

No significant differences were measured in the fatty acid content of larvae between incubation treatments (P>0.05) (Table 3) with the exception of 20:1n-7, which was found in significantly greater amounts in larvae incubated at 30 °C than those incubated at 24 °C (p<0.05)(Table 3).

Discussion

Larval incubation period

The developmental rate of *P. ornatus* embryos could be sped up or slowed down by manipulating the incubation temperature of the developing embryos. The egg incubation period for *P. ornatus* varied up to an average 15 days when incubated between 24 and 30ºC and could be predicted when the date of egg extrusion and fertilisation was known.

These results are in line with previously reported findings for other clawed and spiny lobster species where incubation temperature has been shown to govern the embryonic developmental rate (Perkins, 1972; Phillips and Sastry, 1980; Aiken and Waddy, 1985a; Tong *et al.*, 2000; Smith *et al.*, 2002; Moss *et al.*, 2004). An understanding of the effect of temperature on egg incubation period makes the management of the incubation process possible for this species.

From a larval culture perspective, this information allows for the deliberate timing of larval cultures and planning for provision of live prey items, system setup and preparation prior to larval hatch when the eggs are incubated at temperatures of between 24ºC and 30ºC.

Hatch synchronicity

The number of days over which a single brood hatches highlights subtle differences between temperate and tropical spiny rock lobster species. Temperate species such as *J. edwardsii* have been shown to hatch larvae from the same brood of eggs over a possible eight consecutive nights (Smith *et al.*, 2002) as compared to a maximum of two nights for *P. ornatus* in this study. For temperate species, this gives greater flexibility in choosing either suitable larvae or a suitable stocking date when compared to tropical species. This also places greater importance on the accuracy of hatch modelling for tropical species as there are relatively fewer opportunities for larval collection when compared to temperate species.

Larval fitness

Studies by Tong *et al.* (2000) and Smith *et al.* (2002) whose work on the temperate lobster *J. edwardsii* used larval size and, in the case of Smith *et al* (2002), fatty acid profiles as measures of larval fitness, showed that expediting larval development can reduce larval fitness. Temperature was not shown to affect larval size or fatty acid content in this study. A neutral effect on larval fitness due to incubation temperature could be due to the short duration of the incubation period for tropical rock lobsters or

alternately broodstock lobsters being conditioned identically up to the point of berry. Firm conclusions on the effect of incubation temperature on the larval quality of *P. ornatus* are beyond the scope of this study. However, the consistent production of high quality larvae is an important part of the future of culture for this species and should be a focus of future research.

The limited sample size of this study is acknowledged and the lack of statistical difference in measures of larval fitness may be due to sampling variability caused by low replication and a more complete assessment of larval quality may require further study. Additionally, as hatch times were not recorded at night, the hatch dates are discrete. In reality, broods may hatch continually at any time of day, potentially adding some measurement error.

The predictive hatch model presented in this paper is a practical tool for the management of the incubation period of *P. ornatus*. The consistent production of larvae at predictable times will result in an increased ability to forward plan and prepare for larval culture. It is hoped that this will ultimately result in significant time saving, materials and labour cost when culturing the larvae of this species.

Acknowledgements

The authors would like to acknowledge funding from the Fisheries Research and Development Corporation (FRDC) and M.G. Kailis Pty. Ltd. through the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) that made this study possible. Also, the technical expertise of Darella Chapman, Shaun Mayes and Larnie Linton and Ian Brock for biochemical analysis and support. We also wish to thank anonymous reviewers whose useful comments have helped to improve this manuscript.

Chapter V: Assessing the competency of newly hatched phyllosoma of the tropical rock lobster *Panulirus ornatus*.

The work presented in this chapter is currently *under review* for publication as a research paper for *Aquaculture* journal.

Abstract

Due largely to their protracted larval phase, spiny lobsters are difficult to culture in captivity. To maximise the opportunity for successful larval culture, a test which quickly identifies good quality lobster larvae prior to or at an early stage of culture is a desirable management tool for the culture of rock lobster species.

Acute stress tests, using either ammonia or salinity stressors, have previously been used for other crustacean species to distinguish between strong and weak larvae prior to continued larval culture.

This study assesses the suitability of both ammonia and salinity as environmental stressors in short-term, repeatable stress tests. Findings suggest that both stressors are technically suitable for the determination of the physiological condition of early stage *Panulirus ornatus* larvae, and that larvae of lesser condition are less able to withstand higher concentrations of ammonia or lower salinities. However, salinity is more practical as it is less variable with fluctuations in water pH, resulting in a more consistent and repeatable determination of larval quality.

This paper describes an acute (10 minute) test suitable for testing the competency of early stage *P. ornatus* phyllosoma. The results of this study can be used as a guide to maximising the quality of larvae used in the culture of *P. ornatus*.

Keywords: **Stress testing; larval competency; *Panulirus ornatus*; lobster larvae.**

Introduction

The tropical rock lobster, *Panulirus ornatus*, is a large (>4kg), reef-dwelling lobster fished commercially in north-eastern Australia (Holthuis, 1991). This fishery is extensively managed (Ye *et al.*, 2008) but has limited capacity for increased production (Ye and Dennis, 2009). Demand for the species into export markets exceeds supply (Hart, 2009) prompting interest in the development of aquaculture technology for this species. Over the past decade, a concerted research and development effort in Australia has examined the various life history phases, in particular the complex and protracted larval phase (George, 2005; Jones *et al.*, 2006; Jones, 2009a).

For the tropical rock lobster *P. ornatus*, the larval culture period from egg hatch to the puerulus stage is around 4 months (Anon., 2006; Sachlikidis *et al.*, 2009; Smith *et al.*, 2009a). This is considerably longer than most commercially reared crustaceans such as penaeid shrimp species which complete their larval phase in less than 3 weeks (Jory and Cabrera, 2003).

From a hatchery perspective, considerable resources are required to rear lobster larvae through their protracted larval phase, and consequently, there is great importance placed on maximising the quality of the larvae used for culture. Highly competent larvae increase the chances of successful larval culture and maximise the efficiency of hatchery operations. A test which quickly measures the competency of newly hatched lobster larvae prior to, or at an early stage of culture is therefore a desirable management tool for the culture of rock lobster species (Smith *et al.*, 2003b).

Many different criteria have been proposed for the evaluation of crustacean larval condition (Samocha *et al.*, 1998). Other than measures of spawn size, egg size, and hatching rate, time to hatching and larval survival, very few standardised methods have been developed for early larval stages (Cavalli *et al.*, 2000; Cavalli *et al.*, 2001).

Stress testing is one method of testing larval competency that may have a more practical application (Dhert *et al.*, 1992). The principle of a stress test is to expose larvae to a short but extreme environmental condition in which the physiological condition of the larvae determines their survival (Dhert *et al.*, 1992; Cavalli *et al.*, 2000). Environmental stressors can be anything that affects the physiological condition of the proposed subject. For aquatic crustacean larvae, previously used environmental stressors have included ammonia, salinity and temperature.

Acute stress tests, using ammonia over 24 hours, have previously been used to distinguish between strong and weak post larvae of *Macrobrachium rosenbergii* (Cavalli *et al.*, 2000; Cavalli *et al.*, 2001) and *Litopenaeus vannamei* (Racotta *et al.*, 2004). Smith *et al.* (2003b; 2004) used salinity and temperature based stress testing to determine strong from weak early stage larvae for the rock lobster species *Jasus edwardsii*. In that study, there was a strong relationship between activity levels and survival of larvae cultured up to 42 days, proving the usefulness of stress testing for lobster larvae (Smith *et al.*, 2003b). Cavalli *et al.* (2000) also successfully used the principle of a salinity stress test using cumulative totals of mortality to determine post larval quality of *M. rosenbergii*.

The aim of this study was to assess both ammonia and salinity for suitability as environmental stressors in short-term, repeatable stress tests to determine the physiological condition of early stage *P. ornatus* larvae. It is proposed that through the use of a stress test, more fit larvae can be identified and used in culture, subsequently saving time and resources as well as increasing the success rate of *P. ornatus* larval cultures.

Materials and methods

Larval quality variability

For a practical competency test, larvae of different competency should show different test results. Larvae from various broods and different females are inherently likely to be of various qualities and should therefore generate different test scores. When developing a competency test, this very difference may make it difficult to distinguish between variations in larval quality and variation in the test itself over different larval batches and females. To develop a stress test to distinguish between more or less competent larvae, larvae of known competency are required. As it is logical to assume that unfed larvae decrease in competency over time, samples of larvae from each hatch were starved and tested over multiple days to represent larvae of decreasing condition.

Samples were taken from each brood on days one (hatch), two, and three days post-hatch for competency testing. If a test is representative of larval competency, test results should show the competency of the larvae decreasing over the starvation period for each separate hatch. Because variations in larval quality due to maternal influences are likely to be seen in the test results, data from each individual female was analysed independently to make an

assessment of the accuracy of the competency test exclusive of paternal and/or maternal factors.

Larval production

The larvae used in this experiment were produced from broodstock lobsters commercially caught at Cockburn Reef (11°49'E 143°21'S), Northeast Queensland, Australia. These lobsters were held at the Queensland Primary Industries and Fisheries, Northern Fisheries Centre (QPI&F, NFC), Cairns.

Captive breeding was achieved using methodology described in Sachlikidis *et al.*(2005). In brief, adult lobsters were bred under a 14 light:10 dark photoperiod in 28ºC water. Broodstock lobster size was consistent between treatments with females (100-130mm carapace length) being slightly smaller than males (>130mm carapace length). Broodstock diet consisted of frozen pipis (*Plebidonax deltoids*), green mussels (*Perna canaliculus*) and squid (*Loligo sp.*) provided once per day after 3pm at around 3% bodyweight adjusted according to observation.

Berried females were removed from their tanks two days before egg hatch and transferred to a larval hatching system, which was a flow-through system comprising one 100L and two 50L polyethylene rectangular bins interlinked with hosing. The inflowing water passed through a Titan-Series™ polyester cartridge filter and ultra-violet sterilisation before flowing into the first bin (50L); the water then flowed through to the second bin (100L) where the berried female was placed; and flow continued through to the third bin (50L) where a 500µm banjo screen covered the outlet to prevent loss of the newly hatched larvae. Water was delivered to each tank

at the rate of 16.6 L/minute^{-1}. Used water was not recycled through the system and was put to waste.

Air stones were used to provide aeration in all chambers. Larvae produced by this method were then subjected to ammonia and salinity testing.

Experiment 1: Ammonia

Ammonia in the aquatic environment exists in equilibrium as un-ionised ammonia (NH_3), ammonium (NH_4^+), and the hydroxide ion. NH_3 is the most toxic form of ammonia and its concentration is dependent on a number of factors in addition to the amount of total ammonia present (Emerson *et al.*, 1975). Temperature and pH are two of the most important factors, as the concentration of NH_3 will increase with an increase in pH and with increasing temperature. The term total ammonia nitrogen (TAN) refers to the sum of NH_3 and NH_4^+.

Using reagent grade NH_4Cl (ammonium chloride) a stock solution was made to 118.6g/L with distilled water. The concentrations used for the experiment were determined from a pilot trial, and the chosen range consisted of 8 amounts (1.0; 1.2; 1.4; 1.6; 1.8; 2.0; 2.2; and 2.4ml) of NH_4Cl taken from the stock solution.

Each morning of the two 3 day experiments, a set of circular 2.5L bowls (dark in colour for easy observation of larvae) were each filled to a marked 2L line with filtered seawater previously sterilised with ultra-violet radiation. The bowls were set randomly within a block of floating PVC piping framework and the frame was tied to the edge of a 2000L water bath held at a temperature of 26°C

(±0.5°C). For each 2L vessel, one concentration amount was added, with four replicates of each. The ammonia solution was transferred to the larval bowls by measuring with an accurate automatic pipette. Once the ammonia was added to the bowl, the water was stirred with a glass rod to ensure even distribution. A Gemini Tinyview™ temperature logger was used to record the water temperature throughout the experiment and was placed in a bowl (without larvae) within the block. The pH, salinity and temperature were recorded for each bowl before the selected ammonia concentration for each was added.

Larvae were transferred from the hatch system using a light source (as a photopositive response is associated with phyllosoma at hatch) and scooping the larvae from the surface into a 1L jug. The larvae (n=100) were gently poured into 2L experimental bowls. Each bowl was returned to the water bath and maintained at a constant temperature of 26°C (±0.5°C) for one hour. The pH was recorded at the beginning and end of the trial. Larvae were observed at one hour and mortalities were recorded. Mortality was determined if the phyllosoma showed no movement through the water when stimulated by either a light or when mechanically stimulated with a plastic pipette.

From the total ammonia added to each bowl the concentration of NH_3 was estimated by using the final pH, salinity, and temperature values of each vessel, and derived from equations used by Bower and Bidwell (1978) and Hampson (1977). Genstat (Genstat, 2008) was the statistical program used to graph and analyse the data. Based on the inactivity (estimated mortality) rates the median lethal concentrations (LC_{50}) were estimated using a linear regression probit analysis. Significance was ascertained

between replicates of each day using logistic regression analysis of the mortality vs NH_3 concentration.

Experiment 2: Salinity

It was determined from Experiment 1 that smaller clear vessels would allow for easier larval observation. Subsequently, a set of 19 x 250ml clear vessels were each filled to 100ml with tap water made up to a range of salinities using aquarium grade salt on the morning of each hatch. The concentrations used for the experiment were determined from a pilot trial, and the chosen range consisted of 5 salinities (16; 17; 18; 19; and 20 ppt), conducted in triplicate.

Larvae were transferred from the hatch system using a light source to attract and condense larvae (as a photopositive response is associated with phyllosoma at hatch) then scooping the larvae from the surface into a 1L jug. Twenty larvae were then gently transferred into the experimental units from the jug using a plastic pipette under a point light source. The test larvae were observed, using a light box, at six minute intervals over a one hour period and inactivity was recorded at each interval giving a cumulative total at completion. The maximum total that could be obtained over one hour was 200 and the minimum was 0 (thus the highest cumulative total would show the most inactivity). Inactivity was determined if the phyllosoma showed no movement of appendages when stimulated by light.

The results were analysed for significance between days for each trial and graphed using a Gompertz model of regression analysis. Controls for replicates in each trial all showed zero mortality and are therefore not displayed in the results. The cumulative totals achieved from the trials have been converted to

percentage of inactivity to allow for clear representation. Genstat was the statistical program used for data analysis (Genstat, 2008).

Experiment 3: Acute salinity stress test - Developing a 10 minute test

Using the results of the first two experiments, a quick, practical competency test was conceived and trialled. Given ease of use of salinity compared with ammonia, salinity was chosen as the stressor and concentrations identified that would likely provide discernment of larval competency over a ten minute period.

Larvae were removed at hatch and 50 were counted into each of five different salinities (11, 12, 13, 14 and 15ppt) in triplicate. Larvae were then subjected to a test period of ten minutes and then counted for mortality in a Petri dish against a black background using a point light source. Additional larvae from the same hatch were kept unfed, over successive nights and tested as previously stated each day after hatch for two further days. These larvae were assumed to be of decreased quality due to the effects of starvation.

Results

Experiment 1: Ammonia

Two trials were conducted. These are referred to as trial 1 and trial 2. Statistically both trial 1 and 2 showed significant differences ($P<0.001$) between larval mortality over days 1, 2, and 3 against un-ionised ammonia (NH_3) concentrations (Figure 11 and Figure 12) The standard error (S.E.) for mortality in trial 1 across all days was 9.08; and trial 2 had an overall standard error of 4.04. All control mortalities were subtracted from each data point during statistical

analyses and graphing to obtain the most accurate curve starting with the first concentration above zero.

The median lethal concentration (LC_{50}) of NH_3 for each day was determined by using probit analysis, including upper and lower confidence intervals (Table 4). The results of the probit analysis can be seen below in Figure 11 and Figure 12 for both trial 1 and trial 2. The median lethal concentrations between days 1 and 3 for both trials show a clear competency assessment of unfed larvae on these days.

The ammonium chloride concentrations used were converted to TAN (mg/L) and these values were constant across the trials. The NH_3 (mg/L) concentrations were estimated and clearly varied across days and between replicates, due to the differences in observed pH values.

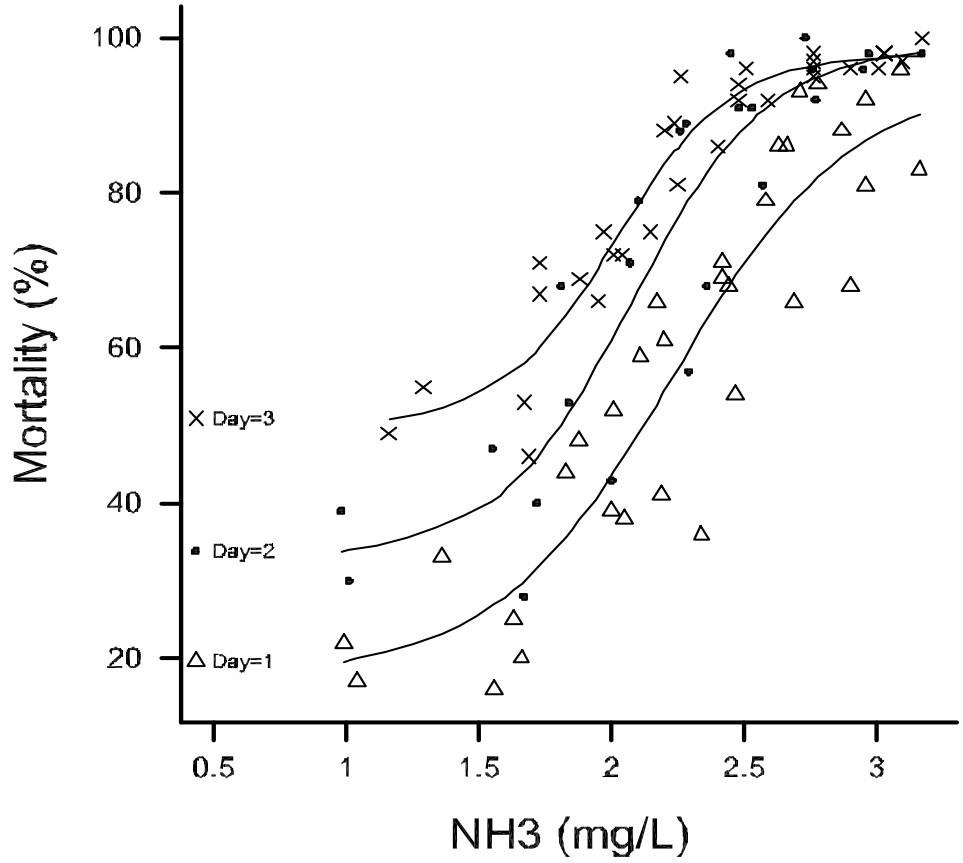

Figure 11: NH_3 (mg/L) vs mortality for Trial 1, days 1, 2, 3 (including all replicates) including logistic regression with a line of best fit for each successive day ($P<0.001$). Percentage of variance accounted for = 85.9 with an estimated SE of 9.08.

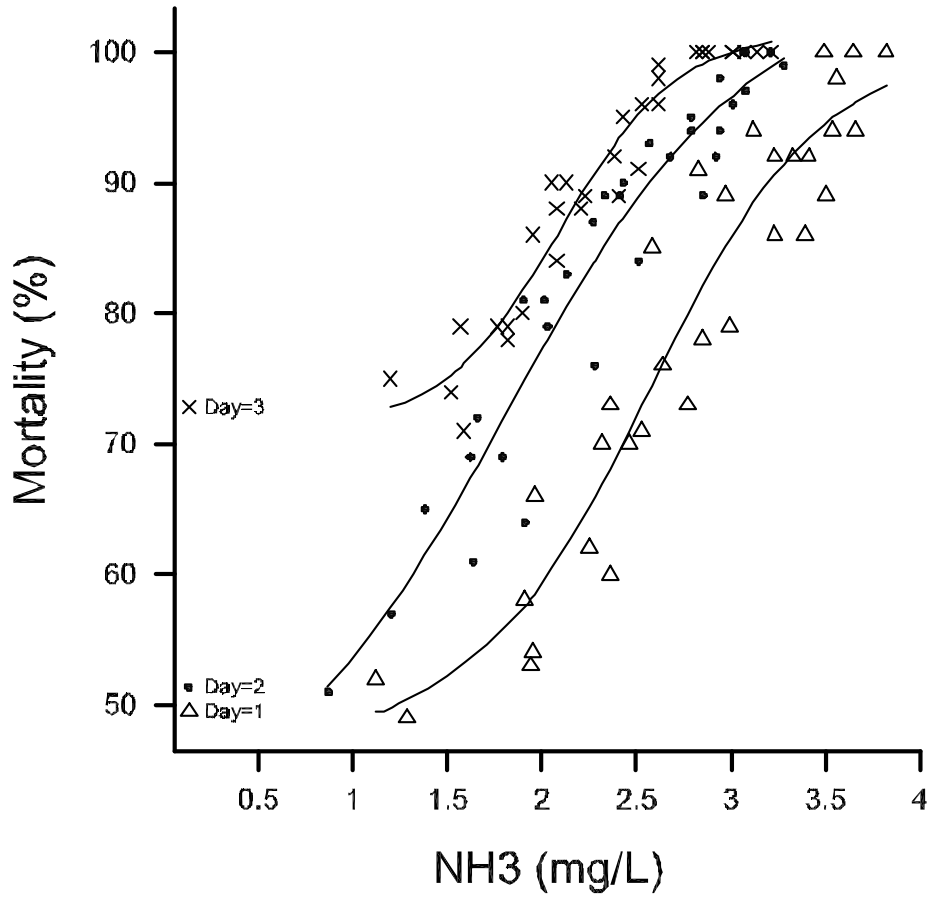

Figure 12: NH_3 (mg/L) vs mortality for Trial 2, days 1, 2, 3 (including all replicates) including logistic regression with a line of best fit for successive each day ($P<0.001$). Percentage of variance accounted for = 91.6 with an estimated SE of 4.04.

Table 4: Median lethal concentrations (LC_{50}) of NH_3 (mg/L) for Trial 1 and 2 across all replicates for each day and their 95% confidence intervals. The LC_{50} for each brood reduces for each day after hatch corresponding with a reduction in competency.

Trial	*Day*	*LC_{50}*	*Lower 95% C.I.*	*Upper 95% C.I.*
1	1	1.957	1.808	2.086
1	2	1.543	1.293	1.721
1	3	1.430	1.310	1.528
2	1	1.555	1.311	1.741
2	2	1.138	0.9752	1.270
2	3	1.144	0.9745	1.275

Experiment 2: Salinity

Starved larvae from days one (hatch), two, and three were used to represent larvae of decreasing condition. Four trials were conducted each using a brood from separate females, these are referred to as trial 1, 2, 3, and 4. All trials showed significant difference ($P<0.01$) between days and a clear assessment of quality was determined from the results. A regression analysis using the Gompertz model can be seen for all trials in Figure 13.

Experiment 3: Acute salinity stress test

Larvae starved over successive days from days one (hatch), two, and three were used to represent larvae of decreasing condition. Four trials were conducted each using a brood from separate females. All trials showed significant difference ($P<0.01$) between days and a clear assessment of larval competency was determined from the results. A regression analysis using the Gompertz model can be seen for all trials in Figure 14.

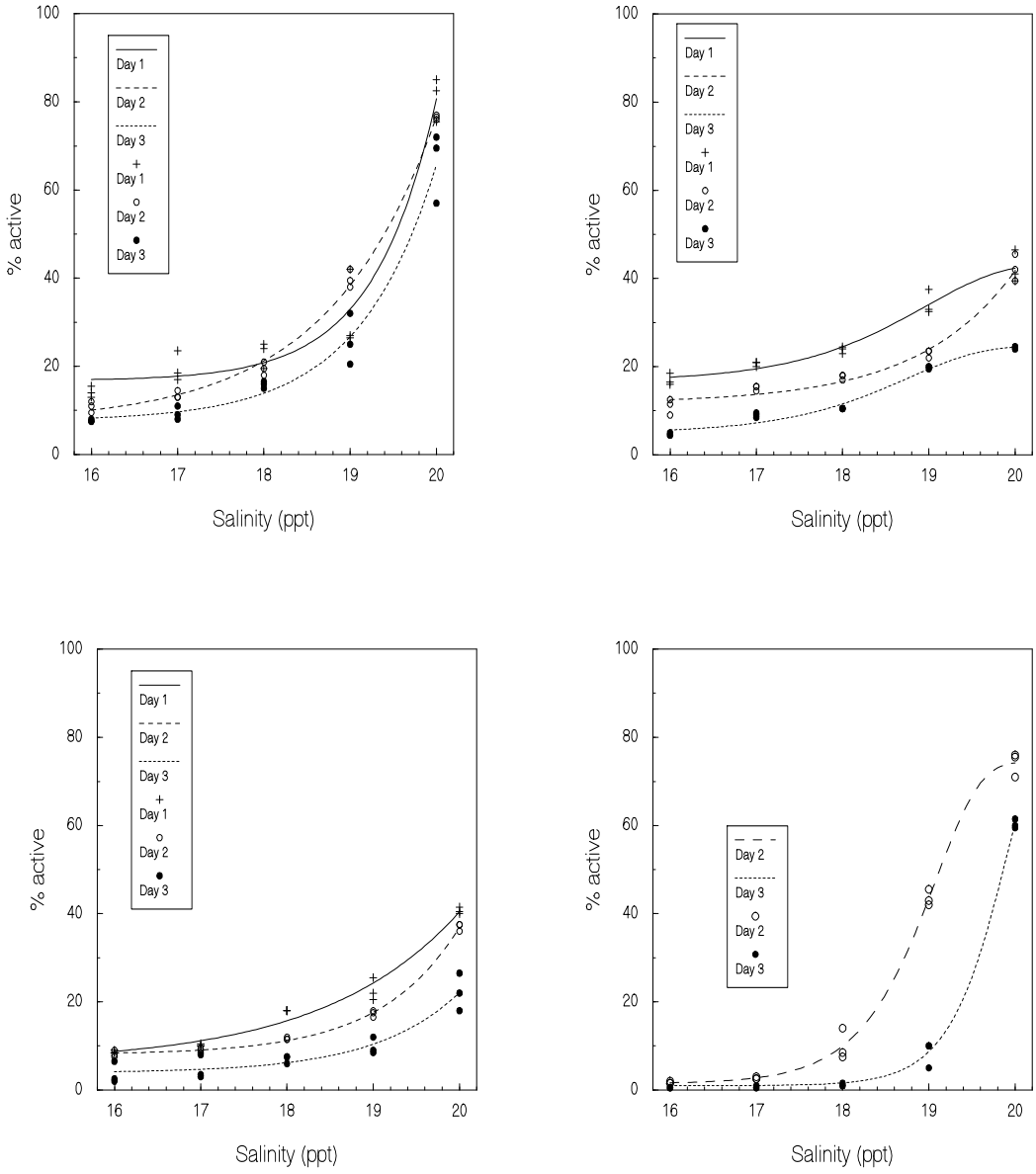

Figure 13: Salinity (ppt) vs % activity for Trial 1, days 1, 2 and 3 (including all replicates) for larvae from four different female *P. ornatus*. Larval activity under stress test conditions (competency) was significantly reduced over successive days of starvation for all broods ($P<0.01$).

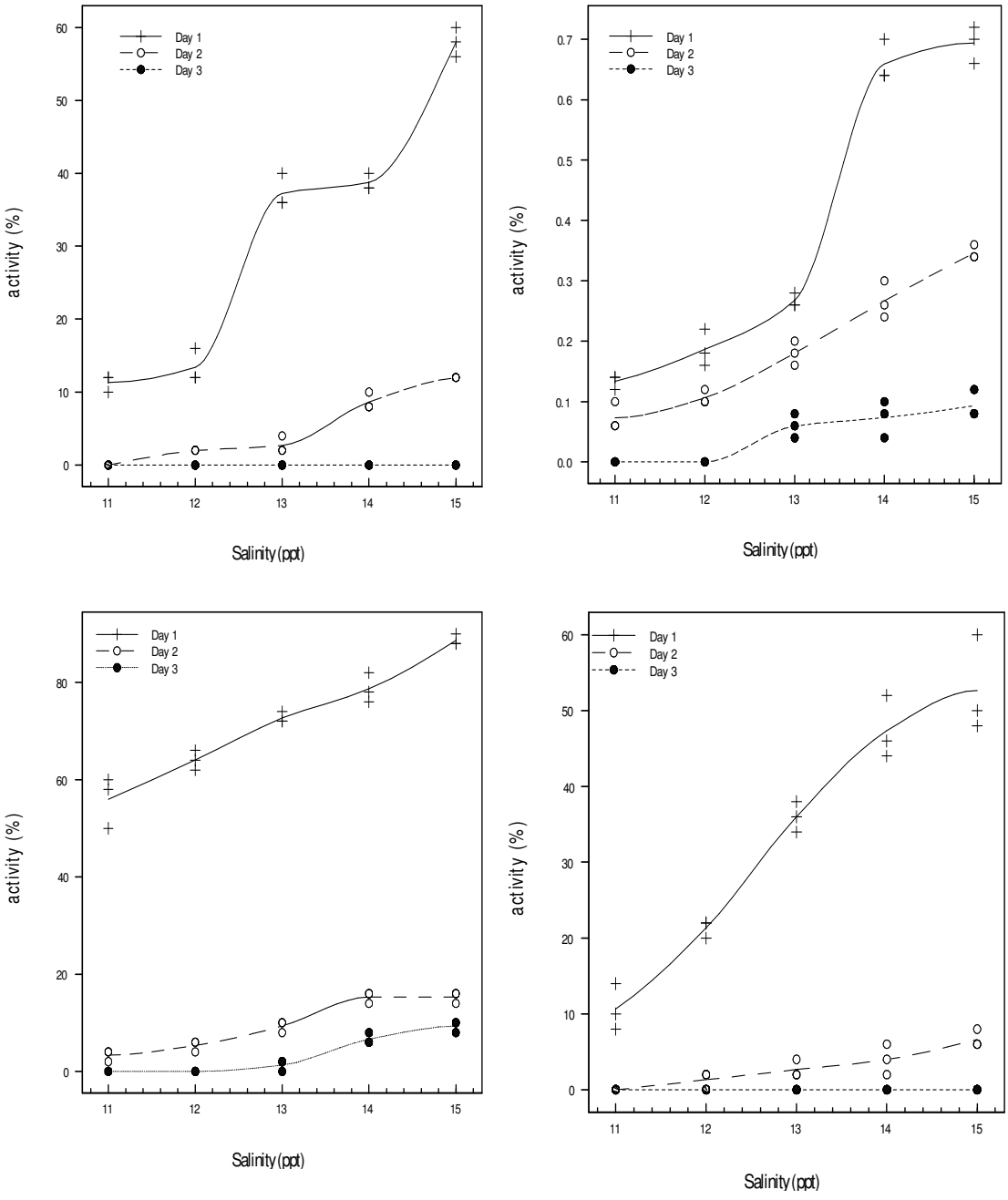

Figure 14: Experiment 3: Salinity (11-15 ppt) vs % activity after 10 minutes exposure, days 1, 2, and 3 (including all replicates) for four different female *P. ornatus*. A significant difference between stress tested larval activity from successive days of starvation for larvae from all four lobsters clutches ($P<0.01$).

Discussion

As previous research had shown that 24 hour ammonia stress tests had been successful in determining post larval quality for *M. rosenbergii* and *L. vannamei* (Cavalli *et al.*, 2000; Cavalli *et al.*, 2001; Racotta *et al.*, 2004), it was thought that a one hour ammonia stress testing procedure might also be as effective on lobster larvae at hatch. In this experiment, larvae from day 1 (stronger) tolerated significant higher median lethal concentrations of ammonia when compared to day 3 starved (weaker) larvae in both trials. This illustrates that larvae of different quality can be distinguished by using ammonia as a stressor. It could also be seen that trial 1 larvae were more competent than those used in trial 2, but the difference between broodstock can only be speculated upon, as they were not held under different conditions.

Although results showed a statistically significant relationship between test days for each trial, variability of NH_3 concentrations suggests that test repeatability is difficult. The variation experienced in pH values caused very few duplicate concentrations of NH_3 to occur, making repeatability of each concentration difficult. The USA Environmental Protection Agency states that it is extremely important to have the pH values accurately measured as a difference of ± 0.1 during the test may result in a 25% variation in the NH_3 concentration. It is also noted that pH meter electrode pairs may experience variation in the liquid junction potential causing non-systematic errors of approximately ± 0.03. The changes in pH in this experiment may have been due to the biological processes occurring in the breakdown of tissue in larvae that had succumbed to the effects of the ammonia earlier than others.

Although the test was conducted within a suitably short time frame (one hour), this test did not fall within the acute stress testing guidelines proposed by Tackaert *et al.* (1989) in terms of ease, repeatability, and simple quantification of results. These issues relating to ammonia stimulated the change of focus to salinity as a stressor.

Salinity has been successfully used as a stressor in acute stress tests for crustacean larvae (Samocha *et al.*, 1998; Smith *et al.*, 2003b; Álvarez *et al.*, 2004; Racotta *et al.*, 2004). These studies successfully use salinity stress to distinguish between larvae of various qualities prior to hatchery stocking.

Results from this study suggest that larvae of *P. ornatus* are also sensitive to low salinities and that larvae of different qualities can be determined using salinity based stress testing. One method is to expose newly hatched *P. ornatus* larvae to salinities between 16 and 20 ppt for one hour whilst scoring larval mortality cumulatively at six minute intervals. At the end of the one hour period the cumulative in-activity total is added and larvae with the lowest score determine which larvae are of the highest quality.

A faster, alternative method is an acute test. For this test, *P. ornatus* larvae are exposed to salinities of between 11 to 15 ppt for ten minutes after which time in-activity is estimated. At the end of the ten minute test period, larvae with the lowest inactivity score are deemed the best quality. The difference here is that because larvae are only exposed to the stressor for ten minutes, a lower, more stressful salinity is used. In effect, instead of assessing activity over varied time like in the longer cumulative test, the salinity is able to be varied over a set period of time. This gives the user the ability to vary the salinity of the test should larval quality

improve into the future as well as a more rapid assessment of larval quality.

All methods trialled in this study highlight differences between larvae of good and poor (starved) quality. For all three tests (ammonia, salinity and acute salinity), larvae which were of known poor quality scored significantly lower under test conditions than those of better quality. The acute salinity stress test however, was the most suitable in terms of ease, speed and repeatability.

Larval stress test scores varied between broods from different females, with larvae from some females scoring much higher than others at the same salinity. This highlights the difference in larval quality between batches which, ultimately, is where this test will be most useful. Over many hatches, a database of larval quality may be built up allowing the end user testable criteria to define acceptable larval quality for hatchery stocking. Regular testing will help to ensure that better quality larvae are used as well as giving a relative gauge on larval quality differences between seasons of individual females.

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Chapter VI: An assessment of density effects on the larvae of the tropical rock lobster *Panulirus ornatus* in culture

Abstract

Larval stocking density is an important factor affecting larval growth and survival for many aquaculture species. This study examined the effect of larval culture density on the growth and survival of early stage tropical rock lobster (*Panulirus ornatus*) phyllosoma. Larvae were stocked into 50.5L upwelling tanks at densities of 5, 10, 20 or 40 larvae/L (n=4) and fed daily with 2-3 mm *Artemia*. Measurements of survival and growth were taken over a four-week period post hatch. Density had a significant effect on survival with the 20 and 40 larvae/L treatments having significantly lower survival than both the 5 and 10 larvae/L treatments. Larval growth was not significantly different between at any of the densities trialled. To maximise both survival and overall culture output, it is recommended that culture of early stage *P. ornatus* be at densities less than 20 larvae/L.

Keywords: **Larval density; lobster; *Panulirus ornatus*; lobster larval culture.**

Introduction

The tropical rock lobster, *Panulirus ornatus,* is a large, high value lobster fished commercially in north-eastern Australia (Holthuis, 1991; Pitcher *et al.*, 1996; Pitcher *et al.*, 1997). Well established markets (Hart, 2009) and limited capacity for increased fishery production (Ye *et al.*, 2008) have prompted interest in development of aquaculture for this species.

Over the past decade, research effort in Australia has examined the various life history phases of this species, in particular the complex and protracted larval phase, with the ultimate goal of commercial hatchery production (Grove-Jones *et al.*, 2002; Jones *et al.*, 2003; Jones *et al.*, 2006; Sachlikidis *et al.*, 2009; Smith *et al.*, 2009a; Smith *et al.*, 2009b).

Although captive culture of spiny lobster species has been achieved for many species (See reviews: Kittaka, 2000; Phillips and Melville-Smith, 2006), many technical and biological issues still stand in the way of commercial culture of lobster larvae (Kittaka, 1997; 2000). A review by Grove-Jones *et al.*(2002) identified knowledge gaps for the culture of *P. ornatus* larvae, which included basic rearing techniques and conditions. Many of these gaps have now been resolved through research, culminating in the captive culture of the first juvenile *P. ornatus* from egg. One such component was an examination of larval stocking density and this research is reported here.

Stocking density is one of the most important factors affecting growth and survival in larval culture of aquaculture species.

Economically, density is important as the balance between survival and total output effects the viability of commercial larval culture (Zmora *et al.*, 2005; Anon., 2006). Biologically, density is critical as it effects the number of interactions between larvae, impacts on incidence of stress caused by energetic response, disease transfer, feed and space competition and incidence of cannibalism (Pillay, 1990; Taylor *et al.*, 1998; Smith and Ritar, 2006).

Survival is generally negatively correlated with stocking density in larval and juvenile culture for most species. However, there are some exceptions. For some fish species, larval density positively correlated with survival and growth rates up to a critical point (Kotani *et al.*, 2009). One possible explanation is that the aggressive tendencies are initially suppressed at increased stocking density, decreasing the incidence of aggressive behaviour and in turn cannibalism (Sakakura and Tsukamoto, 1998; Kotani *et al.*, 2009). The critical point is where the behavioural benefits are exceeded by the higher incidence of cannibalism which may occur in cultures at excessively high densities (Kotani *et al.*, 2009). Additionally, high stocking density has resulted in the production of significantly smaller fish larvae even at densities where survival remains unaffected (King *et al.*, 2000; Ludwig and Lochmann, 2007).

For decapod crustaceans, there has been no such competitive behaviour reported. Increases in larval culture density for most species are generally reported as negatively correlated with survival and growth (Keenan, 1997; Kittaka *et al.*, 1997; Andres *et al.*, 2007; Mikami and Kabullla, 2007; Robson *et al.*, 2008). Excessive larval density can potentially result in increased inter-moult period, increased time to metamorphosis and reduced metamorphosis rates in crustacean species (Kittaka *et al.*, 1997; Calado *et al.*, 2005;

Zmora *et al.*, 2005; Figueiredo and Narciso, 2006; Mikami and Kabullla, 2007).

Studies on larval spiny lobsters have suggested similar patterns. Smith and Ritar (2006) concluded that phyllosoma growth and survival decreased significantly at higher stocking densities and recommended early stage *Jasus edwardsii* larvae be cultured at densities of no higher than 40 larvae/L^{-1}. Matsuda and Takenouchi (2005) also found that survival decreased with increasing stocking density for the culture of *Panulirus japonicus* larvae. However, at the densities trialled, there was no significant effect on phyllosoma growth (Matsuda and Takenouchi, 2005).

This study assesses the effect of density on the growth and survival of early stage *P. ornatus* phyllosoma under culture conditions.

Methodology

Larval production

The larvae used in this experiment were produced from broodstock lobsters commercially caught at Cockburn Reef (11°49'E 143°21'S), Northeast Queensland, Australia. These lobsters were held at the Queensland Primary Industries and Fisheries, Northern Fisheries Centre (QPI&F, NFC), Cairns.

Captive breeding was achieved using methodology described in Sachlikidis *et al.*(2005). In brief, adult lobsters were bred under a 14 light:10 dark photoperiod in 28ºC water. Broodstock lobster size was consistent between treatments with females (100-130mm carapace length) being slightly smaller than males (>130mm

carapace length). Broodstock diet consisted of frozen pipis (*Plebidonax deltoids*), green mussels (*Perna canaliculus*) and squid (*Loligo sp.*) provided once per day after 3pm at around 3% bodyweight adjusted according to observation.

Berried females were removed from their tanks two days before egg hatch and transferred to a flow-through larval hatching system, comprising one 100L and two 50L polyethylene rectangular bins interlinked with hosing. The inflowing water passed through a Titan-Series™ polyester cartridge filter and ultra-violet sterilisation before flowing into the first bin (50L); the water then flowed through to the second bin (100L) where the berried female was placed; and flow continued through to the third bin (50L) where a 500µm banjo screen covered the outlet to prevent loss of the newly hatched larvae. Water was delivered to each tank at the rate of 16.6 L/minute^{-1}. Used water was not recycled through the system but was put to waste. Air stones were used to provide oxygen within each chamber.

Larvae rearing

The recirculation system used for the larval rearing experiment was described in Jones *et al.* (2003). In brief, recycled water was first filtered through mechanical filtration to 1µm before being sterilised by exposure to ozone and ultraviolet light.

The experimental system comprised of 16 cylindrical upweller tanks (Jones *et al.,* 2003) that were modified versions of the New Zealand upweller tank (Illingworth *et al.* (1997). Each upweller had a volume of 50.5L and was manufactured locally. Larvae remained in the same larval rearing tank for the duration of the experiment.

Environmental conditions consisted of a 12hr dark: 12hr red light regime and the water temperature was maintained at 26^{o}C. Moss *et al.* (1999), demonstrated that larvae reared at lower light intensities moulted to a larger size due to lower activity levels using less energy than counterparts reared at higher light intensity. Light levels were maintained at less than 100 lux.

Treatments and sampling

Immediately post hatch, larvae were stocked into an upweller at densities of either 40, 20, 10 or 5 larvae per litre. Sampling was undertaken immediately following stocking of larval upweller tanks, then weekly until completion of the experiment. Sampling was carried out using a specially designed sampling device (Sachlikidis and Guy, 2010). This non-invasive method of sampling allows an accurate, uniform sample of larvae throughout the entire water column whilst reducing the physical stress of handling on the delicate phyllosoma which may reduce survival.

Husbandry

Artemia have been accepted as the most suitable feed for early stage lobster phyllosoma in terms of nutrition and ease of use (Illingworth *et al.*, 1997). The *P. ornatus* larvae in this trial were fed once daily at midday. They were presented with 2-3 mm *Artemia* at a density of 4 per ml. This feed density is prescribed to be in excess of the amount required as a minimum for the survival and growth of early stage *J. edwardsii* larvae (Tong *et al.*, 1997) and in this experiment, *Artemia* were never seen to be completely depleted through feeding of the phyllosoma.

The outlet screens were changed daily. Feed screens (250µm) were placed on the outlets prior to feeding at 12:00 noon to retain the *Artemia* overnight. Cleaning screens were placed on the outlets at 9:00am and remained in place for 3 hours to retain larvae while permitting the uneaten *Artemia* to be washed from the tank. Initially a screen size of 500µm was used as a clean screen and when larvae moulted to instar 3 the screen size was changed to 850µm.

Measurements

Larvae were counted in the inter-moult period to avoid unnecessary stress on larvae over the moult. Moulting occurs every 7-9 days for the early stages of this species and survival counts were adapted accordingly and carried out every week. Numbers of live phyllosoma were recorded at each interval.

Ten larvae were measured from each tank at the termination of the trial to determine the impact of the treatments on larval growth. Larvae were photographed using a Leica digital camera mounted on a Leica MZ6 stereomicroscope. Measurements were made using the Image Tool© program *(Wilcox et al., 1995)*.

The experimental data was analysed as a Latin square block using an ANOVA with additional Pearson's LSD analysis performed post-hoc on significant results. Arcsine√ transformations was used on percentage data. Data was analysed using Genstat (11^{th} edition) statistical software (Genstat, 2008).

Results

Larval survival for each week is presented in Figure 15. Treatment effects became apparent after three weeks of culture

with the highest density treatments of 20 and 40 larvae/L producing significantly lower survival than the 5 and 10 larvae/L treatments ($p=<0.001$)(Figure 15). At week four, survival rates in the 5 and 10 larvae/L treatments were still significantly higher than the 40 larvae/L treatments ($p<0.05$). However, the 20 larvae/L treatment showed some overlap with the 5 and 40 larvae/L treatment (Figure 15). The least significant difference (LSD) values for weeks three and four are denoted by superscript.

Although the percentage larval survival was significantly greater in the 5 and 10 larvae/L treatments after four weeks of culture (Figure 15), overall numbers of larvae were significantly greater in the 10, 20 and 40 larvae/L than the 5 larvae/L (Figure 16).

Larval size for each treatment at four weeks indicated that there were no significant differences in growth between treatments at that stage (Figure 17). This was true for total length ($p=0.353$), carapace length ($p=0.33$), carapace width ($p=0.431$), thorax width ($p=.297$) and abdomen length ($p=0.504$) in each of the four stocking density treatments.

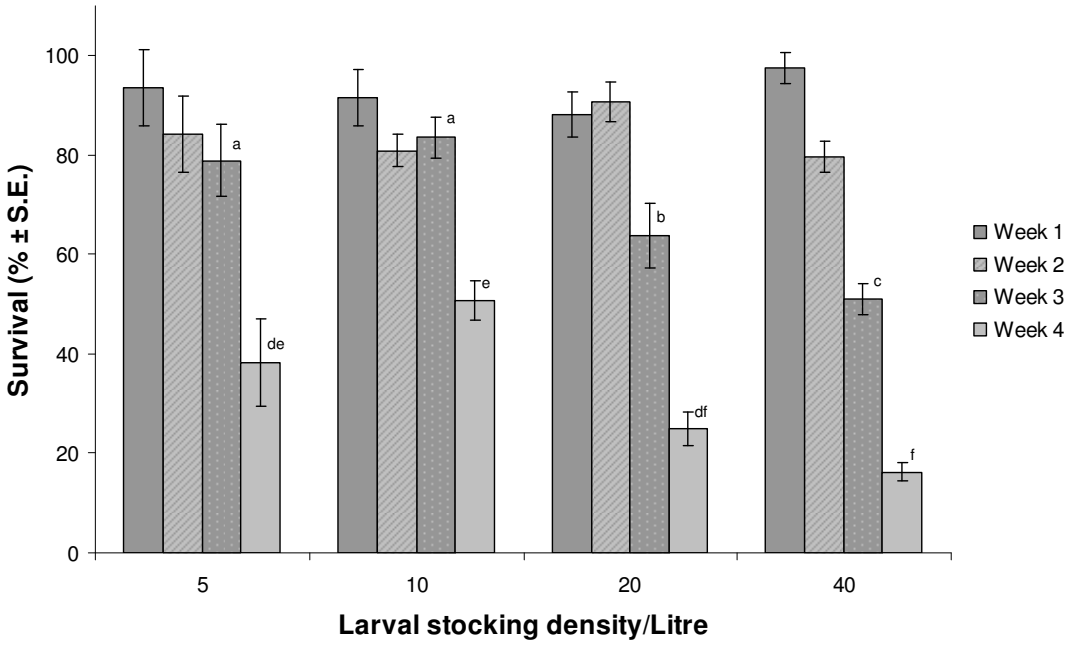

Figure 15: Average larval survival (±SE)over four weeks for larvae initially stocked at densities of 5, 10, 20 and 40 per litre. LSD values for survival in larval stocking density treatments at week 3 and 4. Values with the same superscript are not significantly different ($P < 0.05$).

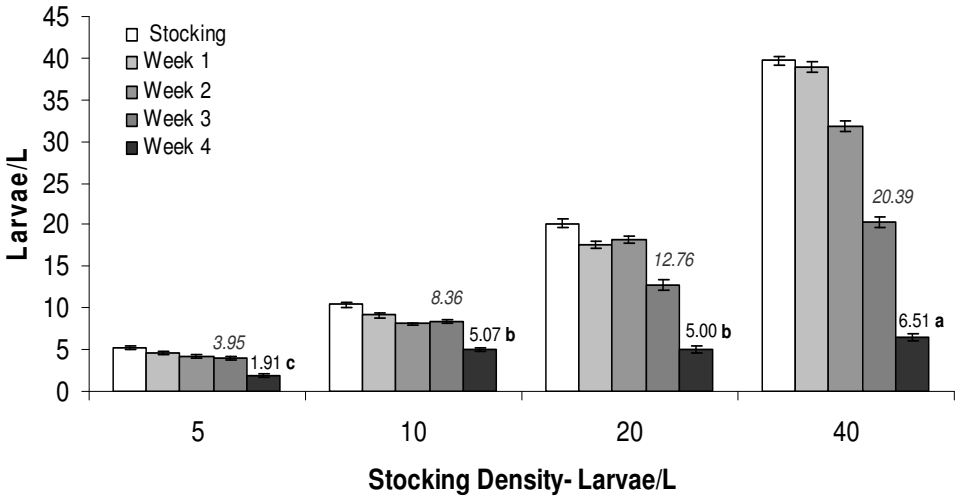

Figure 16: The average number of larvae/L (±SE) for each treatment from initial stocking to four weeks of culture. Superscripts denote statistical differences in week four.

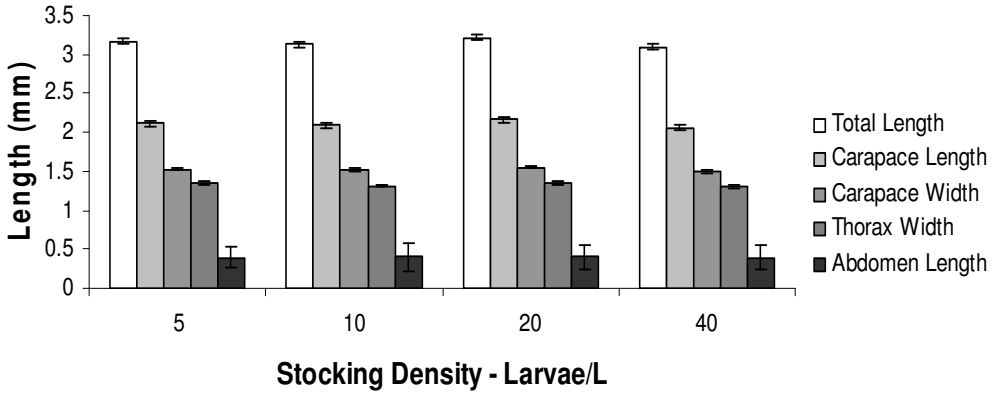

Figure 17: Average larval size (±SE)at week 4 for larvae initially stocked at densities of 5, 10, 20 and 40 larvae per litre.

Discussion

In this study, larval density significantly effected the survival of early stage *P. ornatus* larvae under culture conditions. Larval survival after three weeks of culture was significantly lower in treatments stocked at densities of 20 larvae/L or higher (Figure 15), suggesting that stocking levels above this are unsuitable to achieve high survivorship under the experimental culture conditions. Additionally, survival was not significantly increased at culture densities below 10 larvae/L. This suggests that stocking densities below this point are not advantageous for successful culture of early stage *P. ornatus* under these culture conditions.

Previous studies have shown that it is possible to rear lobster phyllosoma successfully at varying densities (Kittaka, 2000). It is apparent that the upper limits of stocking density may vary between culture systems, tank designs and because of biological factors in culture conditions.

Matsuda and Takenoushi (2005), described how changing tank designs improved the survival of *P. japonicus*. Kittaka (1997) began to successfully rear *Jasus verreauxi* through to puerulus only after larval stocking density was reduced from 50 to 10 per litre and larger culture tanks were used. Though survival in this study is comparable to other similar works (Smith and Ritar, 2006), increased survival and stocking density may be possible through future system improvements as has been achieved for other decapod larval species (Matsuda and Takenouchi, 2005; Calado *et al.*, 2008).

Although survival was improved at lower densities, overall numbers of larvae produced remained higher in treatments stocked at higher densities after four weeks of culture (Figure 16). The lowest total output in terms of larval numbers was from the lowest density treatment (1.19 larvae/L), significantly less than the average produced from the highest density treatment (6.51 larvae/L)(Figure 16). Zmora *et al.* (2005) and Andres *et al.* (2007) highlighted the importance of the balance between larval survival and overall output from larval culture. Both studies recognise the balance between high survivorship and the adequate initial culture stocking density to allow for the output of appropriate numbers of juveniles through intensive culture.

Economically, it would appear that running high larval culture densities will produce a greater economic benefit overall. However, poor survivorship does suggest a detriment to the health of the remaining larvae in the high density treatments. For other species of spiny lobster, the chance of successful metamorphosis is greatly reduced when phyllosoma are compromised (Kittaka, 2000). The viability of the remaining larvae grown from high initial density, for on-growing through to successful metamorphosis may be reduced.

A density of 10 larvae/L achieved the best balance between survivorship and total output for *P.* ornatus larvae in this study. After four weeks of culture, survivorship was highest in this treatment and overall larval numbers were only slightly less than in the 40 larvae/L treatment (5.07 to 6.51 respectively). Additionally, there was no difference in the total output between the 10 and 20 larvae/L treatments (5.07 and 5.00 respectively). This data shows that there is little economy in stocking at >20 larvae/L under the current culture regime and given the implications for further culture, densities higher than this may have long term negative effects on culture success.

Growth was not significantly affected by stocking density in this study. Treatment effects of stocking density on larval growth may become evident at later stages in larval development but this experiment was terminated when larvae had moulted to stage 4 (week 4).

This is consistent with a study by Matsuda and Takenouchi (2005) who found that larval density made no difference to the growth of middle to late stage *P. japonicus* phyllosoma rear at between 1-2.25 larvae to the litre. The densities trialled however, were low when compared to Smith and Ritar (2006) who found reductions in growth for early stage *J. edwardsii* phyllosoma at densities greater than 40 larvae/L. The current study did not test levels higher than 40 larvae/L above which there is the potential for density to significantly affect larval growth for this species.

Additionally, owing to poor survivorship in the more heavily stocked treatments early in the trial, densities in the final two weeks of the trial were similar between treatments. This error

could also account for the lack of difference in the growth rate in this experiment.

To culture large numbers of *P. ornatus* larvae through to puerulus there is a need to maintain high numbers of larvae throughout the entire larval life. This will necessitate re-evaluation of stocking densities at later larval stages as they become larger and change behaviour. Optimum stocking densities will also need to be evaluated for different tank designs and methods of culture to maximise the return from larval culture for this species.

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Chapter VII: Discussion and Conclusion

Discussion

The experiments carried out in this study address phase I of the Grove-Jones review (2002) to most effectively achieve the commercialisation of spiny lobsters in Australia. It closes important knowledge gaps towards the commercial culture of this species and uncovers aspects of the biology previously unknown for *P. ornatus*. In summary, the major findings are:

I - The control of gonad maturation, breeding and timing of egg extrusion.

Results from these studies (see chapter III) implicate photoperiod as a significant cue for breeding in *P. ornatus*. Photoperiod is used to cue processes such as diapause, hibernation, breeding, and migration in many species of animals including marine invertebrates resulting most probably from annual reliability (Herrnkind, 1980; Gwinner, 1981; Olive, 1995).

Findings from this study compare with other similar studies, which have also determined photoperiod to be an important environmental cue to breeding in other palinurid species (Lipcius and Herrnkind, 1985; 1987; Matsuda *et al.*, 2002).

The reasons that photoperiod is used as a primary cue and not temperature may be related to the synchronisation of mass spawning migrations for *P. ornatus*. General trends in water temperature, although most likely important to moulting and gonad

maturation, are subject to significant special and/or temporal variation within season or local geographic area. Although mass spawning populations of *P. ornatus* are restricted within the species distribution, local variations in water temperature across this range (especially at small isolated reefs) may not allow for synchronised timing of the migratory and spawning event if temperature was used a a breeding cue.

In contrast, photoperiod is a feature of latitude and would be expected to vary little over the range of this species, and as such may be a more reliable cue, allowing for more accurate breeding synchrony across the population (Olive, 1995).

Although the summer photoperiod cue may be appropriate to stimulate spawning, it may only work for lobsters physiologically prepared. An important part of this may be a necessity to moult before breeding. The dramatic increase in moulting of lobsters after the sea-water temperature was increased suggests that the increase in temperature may be a cue for pre-reproductive moulting (see chapter III).

Twelve weeks after the sudden increase in moulting, spawning activity was still not evident and the photoperiod of the winter photoperiod treatment (13 L : 11 D) was increased to 18 L : 6 D to provide a further cue to trigger onset of reproduction. This appeared to have an effect with breeding occurring in subsequent weeks.

It may be possible to somewhat hasten breeding through the induction of a moult and subsequent increase in photoperiod post moult.

The speed with which these treatments had their desired effect has important implications to the establishment of management protocols for year-round breeding. Although it may be possible to shift the reproductive season, using controlled conditions, to enable breeding out of phase with wild populations, it may not be possible to significantly compress the reproductive cycle into a period less than 12 months.

Additionally, condensation of the breeding season may have consequences in terms of larval quality. Although several other tropical Palinurid species have been shown to breed year round (Juinio, 1987; Briones-Fourzan and Lozano-Alvarez, 1992), continuous breeding of the same captive lobsters, many successive times, may not generate high quality eggs and larvae. For example, it is possible that lobsters that breed for two or more seasons in one year will hatch larvae of lesser quality than those that undergo the usual one breeding season annually potentially negatively affecting larval survival.

The establishment of multiple breeding populations that are out of phase with wild season is likely to be the most effective way to generate high quality larvae year round.

Physiological rest and possibly a moult may be required between breeding seasons before spawning can occur. Temperature may also be an important cue for pre-reproduction moulting.

II- The control of the embryonic developmental period by incubation temperature manipulation.

The period over which spiny lobster eggs develop varies widely and is related to incubation temperature. The developmental rate of *P.* ornatus embryos can be controlled by manipulating the incubation temperature of the developing embryos.

These results are in line with previously reported findings for other clawed and spiny lobster species where incubation temperature has been shown to govern the embryonic developmental rate (Perkins, 1972; Phillips and Sastry, 1980; Aiken and Waddy, 1985a; Tong *et al.*, 2000; Smith *et al.*, 2002; Moss *et al.*, 2004).

For the first time, an understanding of the effect of temperature on egg incubation period makes the management of the incubation process possible for *P. ornatus*. From a larval culture perspective, this information allows for the deliberate timing of larval cultures and planning for provision of live prey items, system setup and preparation prior to larval hatch when the eggs are incubated at temperatures of between 24ºC and 30ºC.

Incubation temperatures ≥32ºC are unsuitable for this species and result in the termination of egg clutches. Additionally, egg clutches incubated at lower temperatures hatch over more nights than those at higher temperatures.

The number of days over which a single brood hatches highlights subtle differences between temperate and tropical spiny rock lobster species. Temperate species such as *J. edwardsii* have been shown to hatch larvae from the same brood of eggs over a possible eight consecutive nights (Smith *et al.*, 2002) as compared to a maximum of two nights for *P. ornatus* in this study.

For temperate species, this gives greater flexibility in choosing either suitable larvae or a suitable stocking date when compared to tropical species. This also places greater importance on the accuracy of hatch modelling for tropical species as there are relatively fewer opportunities for larval collection when compared to temperate species.

The predictive hatch model presented in this dissemination is a practical tool for the management of the incubation period of *P. ornatus*. The consistent production of larvae at predictable times has resulted in an increased ability to forward plan and prepare for larval culture, ultimately resulting in significant time saving, materials and labour cost when culturing the larvae of *P.ornatus*.

III - Development of the tools to assess phyllosoma quality

Acute stress tests, using either ammonia or salinity stressors, have previously been used for other crustacean species, such as penaeid prawns, to distinguish between strong and weak larvae prior to continued larval culture (Samocha *et al.*, 1998; Cavalli *et al.*, 2001; Smith *et al.*, 2003b; Álvarez *et al.*, 2004; Racotta *et al.*, 2004). For freshly hatched *P. ornatus* phyllosoma, both stressors are technically suitable for the determination of the physiological condition of early stage *P. ornatus* larvae. However, salinity is more practical as it not impacted with fluctuations in water pH.

An acute (10 minute) test suitable for testing the competency of early stage *P. ornatus* phyllosoma was also developed, which makes competency testing quicker and easier than previous studies.

All methods trialled in this study highlight differences between larvae of good and poor (starved) quality. For all three tests (ammonia, salinity and acute salinity), larvae which were of known poor quality scored significantly lower under test conditions than those of better quality. The acute salinity stress test however, was the most suitable in terms of ease, speed and repeatability.

Larval stress test scores varied between broods from different females, with larvae from some females scoring much higher than others at the same salinity. This highlights the difference in larval quality between batches which, ultimately, is where this test will be most useful. Over many hatches, a database of larval quality may be built-up allowing the end-user testable criteria to define acceptable larval quality for hatchery stocking.

Regular testing will help to ensure that better quality larvae are used as well as giving a relative gauge on larval quality differences between seasons of individual females. Additionally, this test is a practical tool for maximising the quality of larvae used in culture of *P. ornatus*.

IV - Improvement of larval husbandry to maximise growth and survivorship through to phyllosoma stage V.

Larval density significantly affected the survival of early stage *P. ornatus* larvae under culture conditions. Larval survival after three weeks of culture was significantly lower in treatments stocked at densities of 20 larvae/L or higher (Figure 15) suggesting that stocking levels above this are unsuitable to achieve high survivorship under the experimental culture conditions. Survival was not significantly increased at culture densities below 10

larvae/L. This suggests that stocking densities below this point are not advantageous for successful culture of early stage *P. ornatus* under the culture conditions.

Previous studies have shown that it is possible to rear lobster phyllosoma successfully at varying densities (Kittaka, 2000). The upper limits of stocking density may however vary between culture systems, tank designs and because of biological factors in culture conditions (Kittaka, 2000).

Matsuda and Takenoushi (2005), described how changing tank designs improved the survival of *P. japonicus*. Kittaka (1997) began to successfully rear *J. verreauxi* through to puerulus only after larval stocking density was reduced from 50 to 10 per litre and larger culture tanks were used. Though survival in this study is comparable to other similar works (Smith and Ritar, 2006), increased survival and stocking density may be possible through future system improvements (Matsuda and Takenouchi, 2005; Calado *et al.*, 2008).

Growth was not affected at any of the densities trialled through the first four weeks of culture. This is consistent with a study by Matsuda and Takenouchi (2005) who found that larval density made no difference to the growth of *P. japonicus* phyllosoma.

The densities trialled in both this and the abovementioned study were however low when compared to Smith and Ritar (2006) who found reductions in growth for early stage *J. edwardsii* phyllosoma at densities greater than 40 larvae/L. This may possibly explain the lack of density-growth effect show in this study. The current study did not test levels higher than 40 larvae/L, above

which there is the potential for larval density to significantly affect growth in cultured early stage *P. ornatus* phyllosoma.

Culture of early stage *P. ornatus* should be at densities less than 20 larvae/L to maximise both survival and overall culture output. Larval tank and system design may also be important in determining the maximum density at which *P. ornatus* can be cultured, with the potential of increases with future improvements in design.

Conclusion

Results from these experiments significantly add to the knowledge of the biology of this species and lay the platform for subsequent work adapting larval culture techniques for the successful culture of this species from egg to puerulus. Independently, these findings are important in understanding and controlling reproduction and larval production for *P. ornatus*. Researchers and hatchery managers can now reliably control breeding and egg production, estimate larval hatch date close to one month in advance, evaluate larval quality prior to stocking for culture and have an understanding of basic husbandry techniques for *P. ornatus* larvae.

These results represent significant steps forward towards the development of hatchery technology for *P. ornatus*. Combined, these results also form a practical platform for future research towards the commercial culture of this species.

Since the completion of the research reported here, the culture of this species has moved forward considerably. This rapid advancement was aided by the adaptation of the research results

discussed in this dissemination. The completion of the larval stages of this species under laboratory conditions has now been achieved, though significant research input is still required to take this technology to the commercial phase.

Research is now focus on the improvement of the understanding of the larval biology of this species and the adaptation of hatchery techniques and methodologies to increase phyllosoma survival rates under culture conditions. Focal points of further research include:

1) Research on the other husbandry requirements of *P. ornatus* phyllosoma including the effects of photoperiod on moulting and growth, feed density and density effects on later stage phyllosoma.

2) Research on larval nutrition including investigations into the nutritional requirements of phyllosoma of *P. ornatus* and how or if they change throughout development, especially through metamorphosis to the puerulus stage.

3) Research on system technology suitable for the rearing of *P. ornatus* phyllosoma. This includes a focus on technologies that will enable the up-scaling of phyllosoma cultures to commercial levels and the control of bacteria in culture systems throughout the relatively long (current shortest time from egg to puerulus 115 days. Sachlikidis pers. obs.) larval cycle.

4) Development of inert diets to reduce the dependence on expensive and unreliable live feeds in phyllosoma culture stages. Reductions in the cost of phyllosoma rearing will

be important to ensuring the economic viability of the long (relative to other cultured species) and expensive hatchery component.

5) The development of fundamental economic production models for hatchery and grow out.

Although each point above represents an entire specialist field, the combination of these areas will be required to yield meaningful commercial results. The successful commercial culture of *P. ornatus* will require the understanding of aspects of each of these areas, highlighting the complexity of future work.

As well as the biological challenges associated with the culture of this species, technical and operational issues must first be overcome before the building blocks of commercial success are a reality. The up-scaling of tanks and systems to provide consistently high survivorship of larvae through to juvenile at a commercially viable scale are the reality of shifting from laboratory to commercial hatchery operations.

Economics will also be pivotal, meaning that the process of larval production and grow-out must become economically viable if commercialisation is to become a reality. Although *P. ornatus* is already a much sought after seafood product, considerable effort will still be required to ensure that commercial production is economically feasible. This will involve significant effort aimed at stream lining the production process and reducing the cost of larval, juvenile and market size production.

Despite these challenges, this species is still one of the most likely lobster species to become a commercial reality. Significant

commercial interest combined with very high market prices look set to drive interest in the culture of this species in the next five to ten years.

Although there are as yet many critical questions to be answered, the research contained in this dissemination adds to the foundation of information required for the successful culture of this species. Ultimately, this work gives researchers and hatchery managers' practical techniques to control the successful production of *P. ornatus* phyllosoma as well as optimising pre-stocking larval quality and early stage larval production. It is for this reason that much of the data presented in this thesis has now been incorporated into production manuals for TRL hatcheries.

These factors are critical to any successful hatchery and are especially important towards the commercialisation for emerging species such as *P. ornatus*.

References

Aiken, D. E. & Waddy, S. (1980). Reproductive biology. In *Biology and Management of Lobsters. Volume1: Physiology and behaviour.* (Cobb, J. S. & Phillips, B. F., eds.). New York
Academic Press.

Aiken, D. E. & Waddy, S. L. (1985a). Production of seed stock for lobster culture. *Aquaculture* **44**, 103-114.

Aiken, D. E. & Waddy, S. L. (1985b). The uncertain influence of spring photoperiod on spawning in the American lobster, *Homarus americanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **42**, 194-197.

Aiken, D. E. & Waddy, S. L. (1989). Interation of temperature and photoperiod in the regulation of spawning by american lobsters (*Homarus americanus*). *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 145-148.

Álvarez, A. L., Racotta, I. S., Arjona, O. & Palacios, E. (2004). Salinity stress test as a predictor of survival during growout in pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture* **237**, 237-249.

Andres, M., Estevez, A. & Rotllant, G. (2007). Growth, survival and biochemical composition of spider crab *Maja brachydactyla* (Balss, 1922) (Decapoda : Majidae) larvae reared under different stocking densities, prey : larva ratios and diets. *Aquaculture* **273**, 494-502.

Annala, J. H. & Bycroft, B. W. (1987). Fecundity of the New Zealand red rock lobster, *Jasus edwardsii*. *New Zealand Journal of Marine and Freshwater Research* **21**, 591-597.

Anon. (2002a). Estimated daylength data for Cockburn Reef, Australia. Geoscience Australia. 2002.

Anon. (2002b). Sea temperature data for Cockburn Reef, Australia. "Reynolds" blended satellite/observations data set. International Research Institute for Climate Prediction.

Anon. (2006). Kailis' babies make three. *Fisheries R&D News* **14**.

Anon. (2008a). Australian Fisheries Statistics. Australian Bureau of Agricultural and Resource Econimics (ABARE), Canberra, ACT, Australia.

Anon. (2008b). Wester Rock Lobster Stock Assessment and Harvest Workshop Report. Western Australian Department of Fisheries. Fisheries occasional publication No. 50.

Ansell, A. D. & Robb, L. (1977). The spiny lobster *Palinurus elephas* in Scottish waters. *Marine Biology* **43**, 63-70.

Bell, J. D. (2004). Key issues for sustaining aquaculture production of the spiny lobster, Panulirus ornatus, in Vietnam. *Spiny lobster ecology and exploitation in the South China Sea region. Proceedings of a workshop, Nha Trang, Vietnam, July 2004*, 59-62.

Bell, R. S., Channells, P. W., MacFarlane, J. W., Moore, R. & Phillips, B. F. (1987). Movements and breeding of the Ornate Rock Lobster, *Panulirus ornatus*, in the Torres Strait and on the North-East coast of Queensland. *Australian Journal of Marine and Freshwater Research* **38**, 197-210.

Beltz, B. S., Helluy, S. M., Ruchhoeft, M. L. & Gammill, L. S. (1992). Aspects of the embyology and neural development on the American lobster. *Journal of Experimental Zoology* **261**, 288-297.

Berry, P. (1970a). Mating behaviour, oviposition and fertilization in the spiny lobster *Panulirus homarus* (Linnaeus). *Oceanographic Research Institute Reports* **24**, 1-16.

Berry, P. & Heydorn, A. (1970). A comparison of the spermatophoric masses and mechanisms of fertilization in Southern African Spiny Lobsters (Palinuridae). *Oceanographic Research Institute Reports* **25**, 1-18.

Berry, P. F. (1970b). Mating behaviour, oviposition and fertilization in the spiny lobster Panulirus homarus (Linnaeus). *South African Ocenographic Research Institute, Investigation Report* **No.24**, 1-16.

Berry, P. F. (1971). The biology of the spiny lobster *Panulirus homarus* (Linaeus) off the East Coast of Southern Africa. *Oceanographic Research Institute Reports* **28**, 1-29.

Bower, C. & Bidwell, J. (1978). Ionisation of Ammonia in seawater: effects of temperature, pH and salinity. *Journal of the Fisheries Research Board of Canada* **35**, 1012-1016.

Briones-Fourzan, P. & Lozano-Alvarez, E. (1992). Aspects of the reproduction of *Panulirus inflatus* and *P. gracilis* from the Pacific coast of Mexico. *Journal of Crustacean Biology* **12**, 41-50.

Calado, R., Figueiredo, J., Rosa, R., Nunes, M. L. & Narciso, L. (2005). Effects of temperature, density, and diet on development,

survival, settlement synchronism, and fatty acid profile of the ornamental shrimp *Lysmata seticaudata*. *Aquaculture* **245**, 221-237.

Calado, R., Pimentel, T., Vitorino, A., Dionisio, G. & Dinis, M. T. (2008). Technical improvements of a rearing system for the culture of decapod crustacean larvae, with emphasis on marine ornamental species. *Aquaculture* **285**, 264-269.

Cavalli, R. O., Berghe, E. V., Lavens, P., Thuy, N. T. T., Wille, M. & Sorgeloos, P. (2000). Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* **125**, 333-343.

Cavalli, R. O., Hernandez-Herrera, R., Racotta, I. S., Lavens, P. & Sorgeloos, P. (2001). The use of an ammonia stress test as a tool to evaluate larval quality. In *Larvi '01- Fish and shellfish larviculture symposium* (Hendry, C. I., Van Stappen, G., Wille, M. & Sorgeloos, P., eds.). Oostende, Belgium: European Aquaculture Society.

Chang, Y. J., Sun, C. L., Chen, Y., Yeh, S. Z. & Chiang, W. C. (2007). Reproductive biology of the spiny lobster, *Panulirus Penicillatus*, in the southeastern coastal waters off Taiwan. *Marine Biology* **151**, 553-564.

Chittleborough, R. G. (1976). Breeding of *Panulirus longipes cygnus* George Under Natural and Controlled Conditions. *Australian Journal of Marine and Freshwater Research* **27**, 499-516.

Crear, B. J., Thomas, C. W., Hart, P. R. & 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.

de Lestang, S. & Melville-Smith, R. (2006). Interannual variation in the moult cycle and size at double breeding of mature female western rock lobster (*Panulirus cygnus*). *Ices Journal of Marine Science* **63**, 1631-1639.

Dennis, D. M., Pitcher, C. R. & Skewes, T. D. (2001). Distribution and transport pathways of Panulirus ornatus (Fabricius, 1776) and Panulirus spp. larvae in the Coral Sea, Australia. *Marine and Freshwater Research* **52**, 1175-1185.

Dhert, P., Lavens, P. & Sorgeloos, P. (1992). Stress evaluation: a tool for quality control of hatchery produced shrimp and fish fry. *Aquaculture Europe* **17**, 6-10.

Emerson, K., Russo, R., Lund, R. & Thurston, R. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. *Journal of the Fisheries Research Board of Canada* **32**, 2379-2383.

Evans, C. R. & Lockwood, A. P. M. (1994). Population field studies of the Guinea chick Lobster (*Panulirus guttatus* LATREILLE) at Bermuda. Abundance, catchability and behaviour. *Journal of Shellfish Research* **13**, 393-415.

FAO (2007). World fisheries production: estimated value by groups of species. *FAO Fisheries and Aquaculture Report*.

FAO (2009). State of the world fisheries and aquaculture. Fisheries and aquaculture department, Food and agriculture organisation (FAO) of the united nations, Rome.

Figueiredo, J. & Narciso, L. (2006). Productivity improvement of *Lysmata seticaudata* (Risso, 1816) larval rearing protocol through modelling. *Aquaculture* **261**, 1249-1258.

Folch, J., Lees, M. & Sloane Stanley, G. M. (1957). A simple method for the isolation of lipids from animla tissue. *Journal of Biological Chemistry* **226**, 497.

Follesa, M. C., Cuccu, D., Cannas, R., Sabatini, A., Deiana, A. M. & Cau, A. (2009). Movement patterns of the spiny lobster *Palinurus elephas* (Fabricius, 1787) from a central western Mediterranean protected area. *Scientia Marina* **73**, 499-506.

Fonseca-Larios, M. E. & Briones-Fourzan, P. (1998). Fecundity of the spiny lobster *Panulirus argus* (Latreille, 1804) in the Caribbean coast of Mexico. *Bulletin of Marine Science* **63**, 21-32.

Freitas, R., Medina, A., Correia, S. & Castro, M. (2007). Reproductive biology of spiny lobster *Panulirus regius* from the northwestern Cape Verde Islands. *African Journal of Marine Science* **29**, 201-208.

Gardner, C., Frusher, S., Barrett, N., Haddon, M. & Buxton, C. (2006). Spatial variation in size at onset of maturity of female southern rock lobster Jasus edwardsii around Tasmania, Australia. *Scientia Marina* **70**, 423-430.

Gardner, C., Frusher, S., Haddon, M. & Buxton, C. (2003). Movements of the southern rock lobster *Jasus edwardshi* in Tasmania, Australia. *Bulletin of Marine Science* **73**, 653-671.

Genstat (2008). Genstat 11th edition. VSN International Ltd.

George, R. W. (2005). Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **39**, 503-514.

George, R. W. & Main, A. R. (1967). The evolution of spiny lobsters (*Palinuridae*): a study of evolution in the marine environment. *Evolution* **21**, 803-820.

Gomez, E. D., Juinio, M. A. R. & Bermas, N. A. (1994). Reproduction of *Panulirus longipes* longipes in Calatagan, Bantagas, Phillipines. *Crustaceana* **67**, 110-120.

Grano-Maldonado, M. & Alvarez-Cadena, J. (2010). In Vitro Cultivation of Cymatocarpus solearis (Brachycoeliidae) Metacercariae to Obtain the Adult Stage without the Marine Turtle Definitive Host. *Korean Journal of Parasitology* **48**, 49-55.

Groeneveld, J. C. & Branch, G. M. (2001). Development of a pleopod index to assess the moulting season of rock lobster, *Palinurus gilchristi* (Decapoda, Palinuridae) off South Africa. *Crustaceana* **74**, 1083-1100.

Groeneveld, J. C. & Branch, G. M. (2002). Long-distance migration of South African deep-water rock lobster *Palinurus gilchristi*. *Marine Ecology-Progress Series* **232**, 225-238.

Grove-Jones, R., Kokovski, S. & van Barneveld, R. (2002). Phyllosoma propagation research review (FRDC 200/214 and 200/263) within the Rock Lobster Enhancement and Aquaculture Subprogram. In *Rock Lobster Enhancement and Aquaculture Subprogram review*: Fisheries research and Development Corperation (FRDC), Canberra.

Gwinner, E. (1981). Annual rhythms: perspective. In *Handbook of behavioural neurobiology, vol. 4, biological rhythms.* (Ashcroft, J., ed.), pp. 381-389. New York: Plenum press.

Hampson, B. L. (1977). Relationship between the total ammonia and free ammonia in terrestrial and ocean waters. *Ices Journal of Marine Science* **37**, 117-122.

Hart, G. (2009). Assessing the South-East Asian tropical lobster supply and major market demands. In *Final Report for the Australian- Indonesia Partnership.*: Austrlian Centre for International Agricultural Research (ACIAR), Canberra, ACT, Australia.

Hearn, A. & Toral-Granda, M. V. (2007). Reproductive biology of the red spiny lobster, *Panulirus penicillatus* and the galapagos slipper

lobster, *Scyllarides astori* in the Galapagos Islands. *Crustaceana* **80**, 297-312.

Helluy, S. M. & Beltz, B. S. (1991). Embroyonic Development of the American Lobster *(Homarus americanus*): Quantitative stageing and characterisation of an Embroyonic Molt Cycle. *Biological Bulletin* **180**, 355-371.

Herrnkind, W. & McLean, R. (1971). Field studies of homing, mass emigration and orientation in spiny lobster, Panulirus Argus. *Annals of the New York Academy of Sciences* **188**, 359-&.

Herrnkind, W. F. (1980). Spiny Lobsters: Patterns of movements. In *The biology and management of lobsters* (Cobb, J. S. & Phillips, B. F., eds.), pp. 349-408. New York: Academic Press.

Holthuis, L. B. (1991). FAO species catalogue. Vol. 13. Marine lobsters of the world: An annotated and illustrated catalogue of species of interest to fisheries known to date. In *Fisheries synopsis No. 125*, p. 292. : Food and Agriculture Organisation of the United Nations (FAO), Rome.

Hunter, E. (1999). Biology of the European spiny lobster, *Palinurus elephas* (Fabricius, 1787) (Decapoda, Palinuridae). *Crustaceana* **72**, 545-565.

Illingworth, J., Tong, L. J., Moss, G. A. & Pickering, T. D. (1997). Upwelling tank for culturing rock lobster (*Jasus edwardsii*) phyllosomas. *Marine and Freshwater Research* **48**, 911-914.

Ino, S. (1950). Observations of the spawning cycle of Ise-ebi (*Panulirus Japonicus)*. *Bulletin of the Japanese Society of Scientific Fisheries* **15**, 725-727.

Isaacs, G., Cockcroft, A. C., Gibbons, M. J. & De Villiers, C. J. (2000). Moult stage determination in the south African West Coast rock lobster *Jasus lalandii* (H. Milne Edwards) (Crustacea : Decapoda). *South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap* **22**, 177-183.

Jones, C., Linton, L., Horton, D. & Bowman, W. (2003). Final Report for Research Activity in 2000 and 2001 on *Panulirus ornatus* Aquaculture. In *Developing Tropical Rock Lobster Aquaculture*: A Collaboration between MG Kailis Pty Lty and the Department of Primary Industries, Queensland.

Jones, C. M. (2009a). Advances in the culture of lobsters. In *New technologies in aquaculture: Improving production effiency, quality*

and environmental managment. (Burnell, G. & Allen, G. L., eds.), pp. 822-844: Cambridge: Woodhead Publishing Ltd and CRC Press.

Jones, C. M. (2009b). Report on overseas travel to Indonesia and Vietnam, July/August, 2009. In *ACIAR Project SMAR/2008/021: Spiny lobster aquaculture development in indonesia, Vietman and Australia.*: Queensland Primary Industres anf Fisheries (QPIF) and the Australian Centre for International Agricultural Research (ACIAR).

Jones, C. M., Hall, M., Sachlikidis, N. G., Kenway, M., Barnard, R. & Fogarty, J. (2006). Rock lobster enhancement subprogram: Advancing hatchery propagation of tropical rock lobsters (*Panulirus ornatus*). In *Project 2003/211 final report*: Fisheries Research and Development Corperation (FRDC).

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

Jory, D. & Cabrera, T. (2003). Marine Shirmp. In *Aquaculture: Farming aquatic animals and plants* (Lucas, J. & Southgate, P. C., eds.), pp. 382-419: Blackwell publishing.

Juinio-Menez, M. A. & Ruinata, J. (1996). Survival, growth and food conversion efficiency of *Panulirus ornatus* following eyestalk ablation. *Aquaculture* **146**, 225-235.

Juinio-Menez, M. A. R. & Estrella, M. S. S. (1995). Notes on the breeding of *Panulirus ornatus* Fabricius (Decopoda: Palinuridae) in captivity. *The Phillippine scientist Special issue: Proceedings of the third national symposium of marine science*, 58-65.

Juinio, M. A. R. (1987). Some aspects of the reproduction of *Panulirus penicillatus* (Decapoda:Palinuridae). *Bulletin of Marine Science* **41**, 242-252.

Kagwade, P. V. (1988). Reproduction in the spiny lobster *Panulirus polyphagus* (Herbst). *Journal of the Marine Biological Association of India* **30**, 37-46.

Kanciruk, P. & Herrnkind, W. (1976). Autumnal reproduction in *Panulirus argus* at Bimini, Bahamas. *Bulletin of Marine Science* **26**, 417-432.

Keenan, C. P. (1997). Aquaculture of the mudcrab, Genus *Scylla* - Past, Present and Future. In *Mud crab aquaculture and biology*

(Keenan, C. P. & Blackshaw, A., eds.), pp. 9-15: ACIAR Proceedings.

Kensler, R. N. (1967). Fecundity in the marine spiny lobster *Jasus verreauxi* (H. Milne Edwards)(Crustacea. Decopoda: Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **1**, 145-155.

King, N. J., Howell, W. H., Huber, M. & Bengtson, D. A. (2000). Effects of larval stocking density on laboratory-scale and commercial-scale production of summer flounder *Paralichthys dentatus*. *Journal of the World Aquaculture Society* **31**, 436-445.

Kittaka, J. (1997). Culture of larval spiny lobsters: a review of work done in northern Japan. *Marine and Freshwater Research* **48**, 923-930.

Kittaka, J. (2000). Culture of Larval Spiny Lobsters. In *Spiny lobster: fisheries and culture* (Phillips, B. & Kittaka, J., eds.), pp. 508-532: Fishing news books, Blackwell Science Ltd, Gray publishing, Kent.

Kittaka, J. & Booth, J. D. (2000). Prospectus for Aquaculture. In *Spiny lobster: fisheries and culture* (Phillips, B. & Kittaka, J., eds.), pp. 465-473. Oxford: Blackwell Science Ltd.

Kittaka, J. & MacDiarmid, A. B. (1994). Breeding. In *Spiny Lobster Management* (Phillips, Cobb & Kittaka, eds.), p. 550. Oxford: Blackwell Scientific Publications.

Kittaka, J., Ono, K. & Booth, J. D. (1997). Complete development of the green rock lobster, Jasus verreauxi from egg to juvenile. *Bulletin of Marine Science* **61**, 57-71.

Kotani, T., Wakiyama, Y., Imoto, T., Suzuki, H. & Fushimi, H. (2009). Effect of initial stocking density on larviculture performance of the ocellate puffer, *Takifugu rubripes*. *Journal of the World Aquaculture Society* **40**, 383-393.

Kulmiye, A. J. & Mavuti, K. M. (2005). Growth and moulting of captive *Panulirus homarus homarus* in Kenya, western Indian Ocean. *New Zealand Journal of Marine and Freshwater Research* **39**, 539-549.

Lipcius, R. N. (1985). Characterization and control of mating and molting in the spiny lobster *Panulirus argus*. *Dissertation Abstracts International, B (Sciences and Engineering)* **45**, 3443.

Lipcius, R. N., Edwards, M. L., Herrnkind, W. F. & Waterman, S. A. (1983). Insitu mating behaviour of the spiny lobster *Panulirus argus*. *Journal of Crustacean Biology* **3**, 217-222.

Lipcius, R. N. & Herrnkind, W. F. (1985). Photoperiodic regulation and daily timing of spiny lobster mating behavior. *Journal of experimental marine biology and ecology* **89**, 191-204.

Lipcius, R. N. & Herrnkind, W. F. (1987). Control and coordination of reproduction and molting in the spiny lobster *Panulirus argus*. *Marine Biology* **96**, 207-214.

Lozano-Alvarez, E. (1996). Ongrowing of juvenile spiny lobsters, *Panulirus argus* (Latreille, 1804) (Decapoda, Palinuridae), in portable sea enclosures. *Crustaceana* **69**, 958-973.

Ludwig, G. M. & Lochmann, S. E. (2007). Effect of tank stocking density on larval sunshine bass growth and survival to the fingerling stage. *North American Journal of Aquaculture* **69**, 407-412.

Lyle, G. & MacDonald, C. (1983). Moult stage determination in the Hawiian spiny lobster *Panulirus marginatus*. *Journal of Crustacean Biology* **3**, 208-216.

MacDiarmid, A. B. (1989). Moulting and reproduction of the spiny lobster *Jasus edwardsii* (Decopoda: Palinridae) in Northern New Zealand. *Marine Biology* **103**, 303-310.

MacDiarmid, A. B. & Butler, M. J. (1999). Sperm economy and limitation in spiny lobsters. *Behavioral Ecology and Sociobiology* **46**, 14-24.

MacDiarmid, A. B. & Kittaka, J. (2000). Breeding. In *Spiny lobsters: Fisheries and culture. Second edition.* (Phillips, B. F. & Kittaka, J., eds.), pp. 485-507. Oxford: Fishing News books. Blackwell Science.

MacDiarmid, A. B. & Sainte-Marie, B. (2006). Reproduction. In *Lobsters: Biology, Managment, Aquaculture and Fisheries* (Phillips, B., ed.), pp. 45-68. Oxford: Blackwell Publishing.

MacDonald, C. D. (1982). Catch composition and reproduction of the spiny lobster *Panulirus versicolor* at Palau. *Transactions of the American Fisheries Society* **111**, 694-699.

MacFarlane, J. W. & Moore, R. (1986). Reproduction of the Ornate Rock Lobster *Panulirus ornatus* in Papua New Guinea. *Australian Journal of Marine and Freshwater Research* **37**, 55-65.

Matsuda, H. & Takenouchi, T. (2005). New tank design for larval culture of Japanese spiny lobster, Panulirus japonicus. *New Zealand Journal of Marine and Freshwater Research* **39**, 279-285.

Matsuda, H., Tekenouchi, T. & Yamakawa, T. (2002). Effects of photoperiod and temperature on ovarian development and spawning of the Japanese spiny lobster *Panulirus japonicus*. *Aquaculture* **205**, 385-398.

McKoy, L. J. (1979). Mating behaviour and egg laying in captive rock lobster, *Jasus edwardsii* (Crustacea: Decapoda: Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **13**, 407-413.

Melville-Smith, R., de Lestang, S. & Thomson, A. W. (2009). Spatial and temporal changes in egg production in the western rock lobster (*Panulirus cygnus*) fishery. *New Zealand Journal of Marine and Freshwater Research* **43**, 151-161.

Meusy, J. J. & Payen, G. G. (1988). Femal reproduction in malacostracan crustacea. *Zoological Science* **5**, 217-265.

Mikami, S. & Kabullla, A. V. (2007). Factors important in larval and postlarval moulting,growth, and rearing. In *The Biology and fisheries of the slipper lobster* (Lavallee, K. L. & Spanier, E., eds.), pp. 88-110: CRC Press.

Minagawa, M. (1997). Reproductive cycle and size-dependent spawning of female spiny lobsters (*Panulirus japonicus*) off Oshima Island, Tokyo, Japan. *Marine and Freshwater Research* **48**, 869-874.

Minagawa, M. & Sano, M. (1997). Oogenesis and ovarian development cycle of the spiny lobster *Panulirus japonicus* (Decapoda : Palinuridae). *Marine and Freshwater Research* **48**, 875-887.

Moore, R. & MacFarlane, J. W. (1984). Migration of the Ornate Rock Lobster *Panulirus ornatus* (Fabricus), in Papua New Guinea. *Australian Journal of Marine and Freshwater Research* **35**, 197-212.

Moss, G. A., James, P. J., Allen, S. E. & Bruce, M. P. (2004). Temperature effects on the embryo development and hatching of the spiny lobster *Sagmariasus verreauxi*. *New Zealand Journal of Marine and Freshwater Research* **38**, 795-801.

Moss, G. A., Tong, L. J. & Illingworth, J. (1999). Effects of light intensity and food density on the growth and survival of early-stage

phyllosoma larvae of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research* **50**, 129-134.

Muesy, J. J. & Payen, G. G. (1988). Review: Female reproduction in malacostracan crustacea. *Zoological Science (Tokyo)* **5**, 217-265.

Murugan, T. S., Remany, M. C., Leema, T. M., Kumar, J., Santhanakumar, J., Vijayakumaran, M., Venkatesan, R. & Ravindran, M. (2005). Growth, repetitive breeding, and aquaculture potential of the spiny lobster, *Panulirus ornatus*. *New Zealand Journal of Marine and Freshwater Research* **39**, 311-315.

Nakamura (1994). Maturation. In *Spiny Lobster Management* (Phillips, Cobb & Kittaka, eds.), p. 550. Oxford: Blackwell Scientific Publications.

Negrete-Soto, F., Lozano-Alvarez, E. & Briones-Fourzan, P. (2002). Population dynamics of the spiny lobster *Panulirus guttatus* (Latreille) in a coral reef on the Mexican Caribbean. *Journal of Shellfish Research* **21**, 279-288.

Nelson, K., Hedgecock, D. & Borgeson, W. (1983). Photoperiodiodic and ecdysal control of vittellogenisis in lobsters (*Homarus*) (Decopoda: Nephropidae). *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 940-947.

Olive, P. J. W. (1995). Annual breeding cycles in marine invertebrates and environmental temperature: Probing the proximate and ultimate causes of reproductive synchrony. *Journal of Thermal Biology* **20**, 79-90.

Paterson, N. F. (1969). Fertization in the cape rock lobster, *Jasus lalandii*. (H. Milne Edwards). *South African Journal of Marine Science* **65**, 163.

Perkins, H. C. (1972). Developmental rates at various temperatures of embryos of the northern lobster *Homarus americanus* Milne-Edwards). *Fishery Bulletin* **70**, 95-99.

Phillips, B. F., Cobb, J. S. & George, R. W. (1980). General biology. In *Biology and Management of Lobsters. Volume1: Physiology and behaviour.* (Cobb, J. S. & Phillips, B. F., eds.), pp. 2-72. New York: Academic Press.

Phillips, B. F., Joll, L. M., Sandland, R. L. & Wright, D. (1983). Longevity, reproductive condition and growth of the Western Rock Lobster *Panulirus cygnus* George, reared in aquaria. *Australian Journal of Marine and Freshwater Research* **34**, 419-429.

Phillips, B. F. & Melville-Smith, R. (2006). *Panulirus* species. In *Lobsters: Biology, Managment, Aquaculture and Fisheries* (Phillips, B. F., ed.), pp. 359-378. Oxford: Blackwell publishing.

Phillips, B. F., Palmer, M. J., Cruz, R. & Trendall, J. T. (1992). Estimating the growth of the spiny lobsters *Panulirus cygnus, P. argus* and *P. ornatus. Australian Journal of Marine and Freshwater Research* **43**, 1177-1188.

Phillips, B. F. & Sastry, A. N. (1980). Larval ecology. In *The biology and management of lobsters* (Cobb, J. S. & Phillips, B. F., eds.), pp. 11-58. New York: Academic Press.

Pillay, T. V. R. (1990). *Aquaculture: Principles and practises*. Oxford: Fishing News Books. Blackwell Science Ltd.

Pitcher, R., Bishop, M., Dennis, D. & Skewes, T. (1997). *The Torres Strait Lobster Fishery 1996*. Canberra: Australian Fisheries Management Authority.

Pitcher, R., Skewes, T., Dennis, D. & Bishop, M. (1996). The Torres Strait Lobster Fishery. *Fisheries Assessment Report*, 1-22.

Plaut, I. (1993). Sexual maturity, reproductive season and fecundity of the spiny lobster *Panulirus penicillatus* from the gulf of Eilat (Aqaba), Red sea. *Australian Journal of Marine and Freshwater Research* **44**, 527-535.

Quackenbush, L. S. & Herrnkind, W. F. (1981). Regulation of molt and gonadal development in the spiny lobster, Panulirus argus (Crustacea: Palinuridae): Effect of eyestalk ablation. *Comparative Biochemistry and Physiology Part A: Physiology* **69**, 523-527.

Quackenbush, L. S. & Herrnkind, W. F. (1983). Partial characterisation of eyestalk hormones controlling moult and gonadal development in the spiny lobster *Panulirus argus*. *Journal of Crustacean Biology* **3**, 34-44.

Racotta, I. S., Palacios, E., Hernández-Herrera, R., Bonilla, A., Pérez-Rostro, C. I. & Ramírez, J. L. (2004). Criteria for assessing larval and postlarval quality of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). *Aquaculture* **233**, 181-195.

Radha, T. & Subramoniam, T. (1985). Origin and nature of spermatophoric mass of the spiny lobster *Panulirus homarus*. *Marine Biology* **86**, 13-19.

Radhakrishnan, E. V. (1977). Breeding of laboratry reared spiney lobster *Panulirus homarus* (linneaeus) under controlled conditions. *Indian Journal of Fisheries* **24**, 269-270.

Radhakrishnan, E. V. & Vijayakumaran, M. (1984). Effect of eyestalk ablation in the spiny lobster (*Panulirus homarus)* on gonad maturity. *Indian Journal of Fisheries* **31**, 209-216.

Relini, L. O., Zamboni, A., Fiorentino, F. & Massi, D. (1998). Reproductive patterns in Norway lobster Nephrops norvegicus (L.), (Crustacea Decapoda Nephropidae) of different Mediterranean areas. *Scientia Marina* **62**, 25-41.

Robson, V., Ubiratã, A. T. d. S., Gilmar, P.-N., Antonio, O., Walter, A. B. & Marcio, R. P. (2008). Larval cannibalism rates in the mangrove crab (Decapoda: Ocypodidae) under laboratory conditions. *Aquaculture Research* **39**, 263-267.

Ruppert, E. E. & Barnes, R. D. (1994). *Invertabrate Zoology. Sixth edition.* New York: Saunders College Publishing.

Sachlikidis, N. G. & Guy, B. P. (2010). A techniques and apparatus for delicately and quickly sampling early stage spiny lobster phyllosoma from large experimental culture vessels. *The lobster newsletter* **23**, 21-23.

Sachlikidis, N. G., Jones, C. M. & Guy, B. P. (2009). Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS): Commercially viable production of tropical rock lobster (*Panulirus ornatus)* puerulus from eggs. In Fisheries Research and Development Corporation (FRDC) project 2006/235.30 Final report.

Sachlikidis, N. G., Jones, C. M. & Seymour, J. E. (2005). Reproductive cues in *Panulirus ornatus*. *New Zealand Journal of Marine and Freshwater Research* **39**, 305-310.

Sakakura, Y. & Tsukamoto, K. (1998). Effects of density, starvation and size difference on aggressive behaviour in juvenile yellowtails (*Seriola quinqueradiata*). *Journal of Applied Ichthyology* **14**, 9-13.

Samocha, T. M., Guajardo, H., Lawrence, A. L., Castille, F. L., Speed, M., McKee, D. A. & Page, K. I. (1998). A simple stress test for *Penaeus vannamei* postlarvae. *Aquaculture* **165**, 233-242.

Smith, D. M., Williams, K. C., Irvin, S., Barclay, M. & Tabrett, S. (2003a). Development of a pelleted feed for juvenile tropical spiny lobster (*Panulirus ornatus*): response to dietary protein and lipid. *Aquaculture Nutrition* **9**, 231-237.

Smith, D. M., Williams, K. C. & 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. *Aquaculture Nutrition* **11**, 209-217.

Smith, G., Salmon, M., Kenway, M. & Hall, M. (2009a). Description of the larval morphology of captive reared *Panulirus ornatus* spiny lobsters, benchmarked against wild-caught specimens. *Aquaculture* **295**, 76-88.

Smith, G. G., Brown, M. R. & Ritar, A. J. (2004). Feeding juvenile *Artemia* enriched with ascorbic acid improves larval survival in the spiny lobster *Jasus edwardsii*. *Aquac Nutrition* **10**, 105-112.

Smith, G. G., Hall, M. W. & Salmon, M. (2009b). Use of microspheres, fresh and microbound diets to ascertain dietary path, component size, and digestive gland functioning in phyllosoma of the spiny lobster *Panulirus ornatus*. *New Zealand Journal of Marine and Freshwater Research* **43**, 205-215.

Smith, G. G. & Ritar, A. J. (2006). The influence of animal density and water turbulence on growth and survival of cultured spiny lobster (*Jasus edwardsii*) larvae. *Aquaculture* **258**, 404-411.

Smith, G. G. & Ritar, A. J. (2007). Sexual maturation in captive spiny lobsters, *Jasus edwardsii*, and the relationship of fecundity and larval quality with maternal size. *Invertebrate Reproduction & Development* **50**, 47-55.

Smith, G. G., Ritar, A. J. & Dunstan, G. A. (2003b). An activity test to evaluate larval competency in spiny lobsters (*Jasus edwardsii*) from wild and captive ovigerous broodstock held under different environmental conditions. *Aquaculture* **218**, 293-307.

Smith, G. G., Ritar, A. J., Thompson, P. A., Dunstan, G. A. & Brown, M. R. (2002). The effect of embryo incubation temperature on indicators of larval viability in Stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. *Aquaculture* **209**, 157-167.

Subramoniam, T. (1993). Spermatophores and sperm transfer in marine crustaceans. *Advances in Marine Biology* **29**, 129-214.

Tackaert, W., Abelin, P., Leger, P. & Sorgeloos, P. (1989). Stress resistance as a criterion to evaluate larval quality of postlarval shrimp reared under different feeding procedures. In *Third simposio Brasilerio sobre cultivo de camarao*, p. 565. Joao Pessoa Brasil.

Taylor, J. J., Southgate, P. C., Rose, R. A. & Keegan, A. J. (1998). Effects of larval set density on subsequent growth and survival of

the silver-lip pearl oyster *Pinctada maxima* (Jameson). *Journal of Shellfish Research* **17**, 281-283.

Tong, L. J., Moss, G. A., Paewai, M. M. & Pickering, T. D. (1997). Effect of brine-shrimp numbers on growth and survival of early-stage phyllosoma larvae of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research* **48**, 935-940.

Tong, L. J., Moss, G. A., Pickering, T. D. & Paewai, M. P. (2000). Temperature effects on embryo and early larval development of the spiny lobster Jasus edwardsii, and description of a method to predict larval hatch times. *Marine and Freshwater Research* **51**, 243-248.

Turnbull, C. T. (1989). Pleopod cuticular morphology as an index of moult stage in the ornate rock lobster, *Panulirus ornatus* (Fabricus 1789). *Australian Journal of Marine and Freshwater Research* **40**, 285-293.

Van der Meeren, G. I., Chandrapavan, A. & Breithaupt, T. (2008). Sexual and aggressive interactions in a mixed species group of lobsters *Homarus gammarus* and *H-americanus*. *Aquatic Biology* **2**, 191-200.

Van Wijngaarden, D. (1967). Modified rapid preparation of fatty acid esters from lipids from gas chromotographic analysis. *Analytical Chemistry* **39**, 848-849.

Velazquez, A. V. (2003). Reproductive strategies of the spiny lobster *Panulirus interruptus* related to the marine environmental variability off central Baja California, Mexico: management implications. *Fisheries Research* **65**, 123-135.

Verghese, B., Radhakrishnan, E. V. & Padhi, A. (2008). Effect of moulting, eyestalk ablation, starvation and transportation on the immune response of the Indian spiny lobster, *Panulirus homarus*. *Aquaculture Research* **39**, 1009-1013.

Vijayakumaran, M., Murugan, T. S., Remany, M. C., Leema, T. M., Kumar, J. D., Santhanakumar, J., Venkatesan, R. & Ravindran, M. (2005). Captive breeding of the spiny lobster, *Panulirus homarus*. *New Zealand Journal of Marine and Freshwater Research* **39**, 325-334.

Vijayakumaran, M., Venkatesan, R., Murugan, T. S., Kumar, T. S., Jha, D. K., Remany, M. C., Thilakam, J. M. L., Jahan, S. S., Dharani, G., Kathiroli, S. & Selvan, K. (2009). Farming of spiny lobsters in sea cages in India. *New Zealand Journal of Marine and Freshwater Research* **43**, 623-634.

Villalejo-Fuerte, M. & Velazquez-Abunader, J. I. (2007). Reproduction of the blue lobster *Panulirus inflatus* (bouvier, 1895) at "Bahia de Loreto" National Park, Gulf of California. *Journal of Shellfish Research* **26**, 1165-1168.

Waddy, S. L. & Aiken, D. E. (1991). Mating and insemination in the American lobster, *Homarus americanus*. *Crustacean Sexual Biology*, 126-144.

Wilcox, D., Dove, B., McDavid, D. & Greer, D. (1995). Image Tool. San Antonio: University of Texas Health Science Center.

Ye, Y. & Dennis, D. (2009). Assessing the impacts of trawling breeding lobsters (*Panulirus ornatus*) on the catch of the Torres Strait lobster fishery shared between Australia and Papua New Guinea. *New Zealand Journal of Marine and Freshwater Research* **43**, 419-428.

Ye, Y., Dennis, D., Skewes, T., Polon, P., Pantus, F., Brewer, D., Haywood, M. D. E., Mcleod, I., Wassenberg, T., Pillans, R. D., Chetwynd, D. & Shiels, J. (2008). 2007 Relative survey abundance and pre-season surveys, assessment of the Torres Strait lobster fishery and TAC estimation. AFMA Torres Strait research program final report. Report numbers 2006/817, 2006/827.

Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V. & Zohar, Y. (2005). Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture* **244**, 129-139.