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The effect of temperature on the incubation of eggs of the tropical rock lobster *Panulirus ornatus*

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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 × Temperature (°C) for incubation temperatures between 24 and 30 °C. Incubation temperatures ≥ 32 °C 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.

1. Introduction

The tropical rock lobster, *Panulirus ornatus*, is a large (>4 kg), 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), while 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, 2009; Jones et al., 2006). 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, 2009; Jones et al., 2006).

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 (Aiken and Waddy, 1985; Perkins, 1972; Phillips and Sastry, 1980; Smith et al., 2002; Tong et al., 2000). 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, 1985; 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. Incubating eggs at lower temperatures may take longer, however the larvae produced may be of superior quality and result in increased overall culture success.

Egg incubation at a relatively high temperature in *Jasus edwardsii*, although expediting development, produced smaller larvae (Smith et al., 2002; Tong et al., 2000) 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).

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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 (Aiken and Waddy, 1985; Helluy and Beltz, 1991; Moss et al., 2004; Perkins, 1972; Phillips and Sastry, 1980; Smith et al., 2002; Tong et al., 2000).

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

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.

2. Materials and methods

2.1. 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 28 °C sea water. Broodstock lobster size was consistent between treatments and female size (100–130 mm carapace length) was smaller than male (>130 mm 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 h of egg extrusion.

2.2. 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 seasonally at known breeding grounds in north-eastern Australia and the Gulf of Papua New Guinea (Bell et al., 1987; MacFarlane and Moore, 1986) (http://www.metoc.gov.au/ims/website/woa01/viewer.htm). 15 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-heat chamber, a 200 L broodstock container (in which each experimental lobster was housed) and finally a larval collector (Fig. 1). Supply water was flow through and was first cooled to 24 °C in a 500 L 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 min^{-1} providing a 200% water exchange per tank day^{-1}. Used water was put to waste. Hatched larvae were collected in the larval collection chamber post hatch (Fig. 1).

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 3 pm 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 (MacDairmid and Sainte-Marie, 2006).

2.3. Hatch times and record keeping

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.

2.4. 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 0.01 mm) 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 h at 80 °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, 30 m × 0.25 mm, with a 0.25 µm coating (Agilent Technologies, USA). Column oven temperature was held at 140 °C for 5 min and then elevated at 3 °C/min 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