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Effect of photoperiod on the culture of early-stage phyllosoma and metamorphosis of spiny lobster (*Sagmariasus verreauxi*)

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ABSTRACT

This study examined the effects of photoperiod on the survival, growth, feeding and development of early and late-stage spiny lobster, *Sagmariasus verreauxi*. The effects of five photoperiods (0, 6, 12, 18 and 24 h photophase) were examined in larvae from hatch to instar 5. Phyllosoma at 6 h photophase grew significantly larger, however, significantly fewer survived. They also ingested fewer *Artemia* which suggested that the observed inverse relationship between survival and growth may have been a result of cannibalism. Phyllosoma cultured in continuous light were significantly smaller, and moult-stage development was slower, possibly due to an imbalance in energy uptake and consumption due to prolonged activity. Development of phyllosoma was also slower in the dark. The results suggested that a light: dark regime is important for early-stage phyllosoma and a photophase of 12 to 18 h is recommended for culture. The effects of four photoperiods (6, 12, 18 and 24 h photophase) were examined in late-stage larvae (instar 15 to17) to metamorphosis. Significantly more phyllosoma attempted metamorphosis at 24 h photophase. Greatest survival at metamorphosis and overall best pueruli survival, size and mass occurred at either 18 or 24 h photophase. The results provide further evidence that a long, or a shift to a longer photoperiod, promotes more and improved metamorphosis in culture. Long photophase during late-stage development can increase pueruli production by 20% which is important for improving culture efficiency given the long and resource intensive phyllosoma rearing phase.

1. Introduction

The high value and demand of several spiny lobster species (Palinuridae) coupled with fully or over exploited wild fisheries make them extremely attractive candidates for closed-life cycle aquaculture. Substantial research effort has focused on developing technologies for intensive spiny lobster aquaculture, particularly in the culture of the intricate phyllosoma larvae. Although propagation research on spiny lobsters began in Japan more than a century ago, the last 15 years has witnessed the most rapid development (Matsuda and Takenouchi, 2007). Since Kittaka (1988) cultured the first spiny lobster from egg to puerulus in 1987, several laboratories have successfully closed the larval cycle of other spiny lobster species (Kittaka, 1997; Matsuda and Takenouchi, 2007; Ritar et al., 2006; Smith et al., 2009). In Tasmania, Australia, at the Institute for Marine & Antarctic Studies (IMAS), research originally focused on the southern rock lobster, *Jasus edwardsii*, which was first cultured through to juvenile in 2004 followed by the eastern rock lobster, *Sagmariasus verreauxi* in 2006. The latter species has a number of attributes that make it easier to culture than *J. edwardsii*, including a shorter larval duration, faster growth and better survival (Fitzgibbon and Battaglene, 2012; Kittaka et al., 1997). Research on *S. verreauxi* now focuses on improving production efficiency by understanding abiotic requirements of larvae in culture.

The relationship between the daily light (photophase) and dark phase (scotophase) is a critical environmental factor influencing the performance of aquatic organisms. Many organisms have evolved light-sensitive circadian clocks which provide internal synchronization for rhythmic behavioral and physiological functions (Bromage et al., 2001; Villamizar et al., 2009). Photoperiod has been shown to be an important factor affecting daily activities such as behavior and feeding of juvenile and adult spiny lobsters (Crear et al., 2003) and is the dominant environmental factor affecting seasonal events such as reproduction (Matsuda et al., 2002; Sachlikidis et al., 2005). Light is also an important factor affecting the physiology and behavior of larval decapods and is probably the most influential environmental variable affecting diurnal rhythmic activities such as feeding and swimming behaviors of larvae in the wild (Rimmer and Phillips, 1979; Sulkin, 1984). In culture, photoperiod regimes also have a profound influence on decapod larval performance, affecting growth (Aiken et al., 1981), feeding (Bermudes and Ritar, 2008; Minagawa, 1994), activity (Mikami and Greenwood, 1997), cannibalism (Gardner and Maguire, 1998), ecdysis (Eagles et al., 1986), development and metamorphosis (Matsuda et al., 2012; Minagawa, 1994). Few studies have examined the influence of photoperiod in spiny lobster larval culture (Bermudes and Ritar, 2008; Matsuda et al., 2012). There is

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no obvious inter-species consistency in the response of decapod larvae to photoperiod (Bermudes and Ritar, 2008). Therefore, defining optimal lighting regimes for a species and development stage is important to maximize larval culture success.

One of the most profound factors that distinguish spiny lobsters from most other decapods species is the long duration of their larval phase. The larval phyllosoma phase duration of *S. verreauxi* is approximately 12 month in the wild (Montgomery and Craig, 2005) but can be reduced to 6 to 12 months in the laboratory (Phillips et al., 2006). They moult through 17 instars, increasing in size from 2 to 40 mm as they develop more complex anatomy and physiology (Kittaka et al., 1997). The completion of the phyllosoma phase is characterized by metamorphosis into necktonic pueruli. Metamorphosis represents a critical life stage because pueruli are the transitional stage between pelagic larvae and benthic juveniles (Phillips et al., 2006). Where and when metamorphosis occurs could potentially influence juvenile recruitment because the lecithotrophic pueruli have finite energy reserves to complete the journey to the coast (McWilliam and Phillips, 1997). This has led to suggestions that metamorphosis is controlled by endogenous cues, but to date, no definitive evidence has been found to demonstrate that spiny lobster metamorphosis is triggered by any direct, abiotic or biotic factor (McWilliam and Phillips, 1997, 2007). However, settlement and thus likely metamorphosis of spiny lobsters show clear seasonal trends (Montgomery and Craig, 2005; Sekiguchi and Inoue, 2002), and it is possible that photoperiod plays a role in regulating metamorphosis, similar to other seasonal events such as reproduction. Recently, Matsuda et al. (2012) demonstrated that photoperiod effected larval size and pleopod development of Japanese spiny lobster, *Panulirus japonicus*, at metamorphosis and concluded that longer photoperiods may promote a smooth and regular metamorphosis. However, that study only examined *P. japonicus* phyllosoma which were developmentally delayed and photoperiod treatments used differing light sources (Matsuda et al., 2012).

The current study examines the effects of photoperiod on survival, growth, feeding and development of early and late-stage phyllosoma, including the effects of photoperiod on metamorphosis from phyllosoma to pueruli. The early and late-stages, particularly at metamorphosis, were examined because they represent the critical periods in culture where mortality is often high (Fitzgibbon and Battaglene, 2012; Kittaka et al., 1997).

2. Materials and methods

2.1. Broodstock and larvae

Broodstock were collected from the wild as recently settled pueruli/juveniles and held in 4000 L fibreglass tanks for nine years, under a regime of simulated ambient photoperiod and 15 °C. They were fed a combination of fresh blue mussel (*Mytilus galloprovincialis*) and commercial prawn pellet (Higashimaru, Vital No. 12, http://www.k-higashimaru.co.jp/), and weighed approximately 2.5 kg at spawning. Newly-hatched phyllosoma larvae were either used immediately from hatch (early-stage experiment) or mass-cultured in 200 L tanks until they reached the appropriate instar for the late-stage experiment. In mass culture, phyllosoma were maintained at 21–23 °C under a 12:12 light:dark photoperiod supplied by fluorescent lamps which provided a light intensity at the water surface between 0.08 and 0.1 μmols s^{-1} m^{-2}. From hatch larvae were fed 3–6 mm juvenile *Artemia* daily. *Artemia* were cultured in 800 L cones, fed live microalgae (*Chaetoceros mulleri*, *Isochrysis galbana* and *Pavlova lutheri*), baker's yeast, *Dunaliella salina* paste and rice bran, as described by Ritar et al. (2006). Before feeding to phyllosoma, *Artemia* were purged with concentrated *C. mulleri* (Tolomei et al., 2004) and 400 ppm formaldehyde for 30 min in order to reduce bacterial load. From instar 13, the phyllosoma diet was supplemented with blue mussel gonad at a rate of 0.025 mL L^{-1} daily. Tanks were cleaned once a fortnight.

2.2. Experimental larval culture systems

Flow-through water was filtered and treated as described by Ritar et al. (2006). The early-stage experiment was conducted in replicated opaque 2 L flat-bottom cylindrical vessels with the total water volume maintained at a constant 1.6 L and water flow rate at five exchanges h^{-1}. The late-stage experiment was conducted in red 13 L flat-bottom cylindrical vessels with the total water volume maintained at 10 L and three exchanges h^{-1}.

In all experiments, feeding, cleaning and abiotic factors were as conducted for mass culture conditions. Water temperatures were logged hourly (iButton, Maxim, http://www.maxim-ic.com/products/ibutton/) in spare replicate containers for each treatment. Dissolved oxygen (HQ10, Hach, http://www.hach.com/) and total ammonia (Test kit, Aquasonic, http://www.aquasonic.com.au/) were measured twice weekly. Dissolved oxygen remained between 105 and 110% saturation (slightly super-saturated due to ozonation water pre-treatment) and total ammonia below 0.5 mg L^{-1}. Maintenance of systems in dark treatments was conducted under the illumination of red light (36 W fluorescent globe) and the anatomy of the spiny lobster eye suggests that it is incapable of perceiving red light (Mills et al., 2005).

2.3. Early-stage experiment

In the early-stage experiment, five photoperiod treatments were examined: 0, 6, 12, 18 and 24 h photophase. For each photoperiod, six replicate vessels ($n = 6$) were stocked with newly hatched phyllosoma at a rate of 40 phyllosoma L^{-1} (104 phyllosoma replicate $^{-1}$). Replicated vessels were contained in five identical light-proof cubicles, one for each photoperiod treatment. Light was supplied to each cubical by a single cool white florescent globe positioned 75 cm above the replicate containers. Phyllosoma were cultured from hatch until they reached the 5th instar.

2.4. Late-stage experiment

In the late-stage experiment, four photoperiod treatments were examined: 6, 12, 18 and 24 h photophase. For each treatment, four replicate vessels ($n = 4$) were stocked with 223 day old phyllosoma (instar 15 to17, mean instar ± SD of eighteen random phyllosoma = 15.4 ± 0.6) at a rate of 1 L^{-1} (10 replicate^{-1}). Treatments were contained in four identical light-proof cubicles. Phyllosoma were cultured for 57 days. The experiment was terminated when most of the animals (>90%) in the most advanced treatment had either died or attempted metamorphosis.

2.5. Survival and development

In the early-stage experiment phyllosoma survival was assessed by counting live phyllosoma during the transfer between two illuminated 1000 mL glass beakers once during each inter-moult period for each instar. Instar development was assessed through the observation of moults within replicates which were typically synchronized over a few days within treatments. Phyllosoma growth was quantified according to length and dry mass for six randomly selected phyllosoma from each replicate at the end of the experiment. Length was measured on a profile projector (Nikon 6C, Japan) from the anterior tip of the cephalic shield between the eyestalks to the posterior tip of the abdomen. Dry mass was determined by rinsing phyllosoma with 0.5 M ammonium formate and freeze drying at −55 °C for 48 h (Ilshin, freeze dryer, http:www.ilshineurope.com) before weighing to the nearest 10 μg on a precision balance (AT261 DeltaRange, Mettler-Toledo, Switzerland). Synchronicity of the moult from instar 4 to 5 was determined by counting the number of instar 5 larvae within each replicate at between 1 and 3 day intervals around the moult.