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Moulting behaviour responses of Bay lobster, *Thenus orientalis*, to environmental manipulation

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Abstract Bay lobster, *Thenus orientalis*, locally known as Moreton Bay bug, is being investigated for aquaculture, and controlling the moulting process is critical to success in the production of softshell animals. Identification of moult stages is possible because of the existence of exoskeleton ecdysial sutures (crack lines) on the gill chambers. The timing of moulting is synchronised and occurs around sunset when animals are under a natural day-night condition. The timing and synchrony of moulting can be manipulated by altering the day-night cycle, whereas the length of the actual moult stage can be shortened/prolonged by manipulating temperature. During the intermoult stage for juveniles (average weight 79 g), body weight increases only by 9%, whereas the majority of weight gain (an additional 44%) occurs within the short period just before and after the actual moult stage. These findings have application for the development of "softshell" products, which can be harvested at around the actual moult stage.

Keywords Bay lobster; *Thenus*; photoperiod; moult stage; moult increment

INTRODUCTION

Crustaceans moult regularly for growth to increase (or sometimes decrease) their size and to change their morphology. Moulting (or ecdysis) is a unique and complicated process. One moult cycle is set off by the shedding of the old exoskeleton, and dramatic changes to the physiology and biochemistry of animals may occur both before and after this event. Though the actual act of shedding the old exoskeleton is the most obvious phenomenon of the moult cycle (this comprises only a short period within a cycle that may take up to a year or more), the vast majority of events during the cycle occur internally. Many factors—environmental, nutritional, physiological, behavioural, and reproductive—are thought to influence the moult processes/phases, and these are regulated by combinations of hormones, such as moult-inhibiting hormone (MIH) and ecdysone (see Chang 1995). Little is known about the mechanism and regulation of hormonal activities during the moult cycle, because of the complexity of the hormone regulatory systems.

Bay lobsters, *Thenus orientalis* (Lund) and *Thenus indicus* (Lund), locally known as Moreton Bay bugs (or sand bug for *T. orientalis* and mud bug for *T. indicus*), are found along the entire northern coast of Australia from Shark Bay in Western Australia to Coffs Harbour in northern New South Wales (Kailola et al. 1993). Mass rearing techniques for these species have been established, and commercialisation of aquaculture development is currently being undertaken (AFFA 2001). Understanding the moult cycle is the key to improving culturing techniques. Laboratory observations on the process of moulting in *T. orientalis* were made in this study to improve the knowledge and understanding of the moult cycle.

MATERIALS AND METHODS

General rearing methods

All animals used in this study were raised in the hatchery between 1998 and 2001. Ovigerous *T. orientalis* females were caught off Hervey Bay,

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south-eastern Queensland and transported to the QDPI Bribie Island Aquaculture Research Centre in aerated sea water in a fish transporter (0.5 m^3). Berried females were kept in a 1 m^3 holding tank with running filtered (1.0 µm) sea water. Temperature was maintained at between 24 and 27°C and animals were fed once daily with fresh flesh of the bivalve *Donax deltoids*. When embryos became visibly amber-brown in colour, females were transferred to individual 0.2 m^3 hatching tanks, with 500 µm mesh covering the outlet, for larval hatching. Newly hatched phyllosomas were immediately harvested and transferred from the hatching tanks to prepared raceway larval rearing systems. These rearing systems had three major components: pre-water treatment, endless raceway (rearing vessel), and recirculation. The new incoming water was filtered through a 0.5 µm cartridge filter, and then sterilised through a 60 W UV unit at the pre-water treatment component. The treated water was pooled in a sump (0.38 m^3) of the recirculation component; ozone (O_3) was constantly injected through a snorkel equipped power head, and temperature was maintained at 27°C by a 3 kW heater. Because maintenance of O_3 level in the rearing water was extremely difficult owing to constant changes in organic matter and biomass, the maximum dose of O_3 into rearing water was preset before the start of larval rearing at 0.05 mg $litre^{-1}$, and this preset amount of O_3 was constantly injected throughout the larval rearing period. The water in the sump was sent through the 19 mm polyethylene pipe surrounding the raceway and was injected to the raceway (0.3 m width, 0.3 mm depth, 12 m length) by 4 mm injectors to create one-way circulation. The rearing water in the raceway was drained to the sump, where new water was added (1–4 litres min^{-1}) and O_3 was injected constantly, and was recirculated back to the raceway. Finely chopped fresh flesh (1–4 mm in size) of the bivalve *D. deltoids* was fed to the phyllosomas twice daily. The survival of phyllosomas to the nisto stage was generally >80%, with >1500 nistos produced from each trial. Nistos were reared in 0.38 m^3 tanks, with running 1 µm pre-filtered sea water at 25–27°C. The duration of the nisto stage was generally 7 days (>90% survival). No food was given to nistos. The juveniles of *T. orientalis* were reared either in 0.3 m wide or 1.1 m wide raceways with sea water 0.15 m deep. The rearing water was pre-filtered through 20 µm multimedia filters and was maintained at a temperature range of between 24 and 28°C. Early stage juveniles (<20 g body weight) were fed on freshly chopped *D. deltoids*, and older juveniles were fed on defrosted flesh of squid. There was no artificial illumination. The survival of juveniles (up to 45 g body weight) was generally >80%.

Identification of moult stages

Fifty post-moult juveniles (79 ± 7.1 g (mean ± SD)) of *T. orientalis* from the same batch were numbered on the surface of their carapaces by liquid paper, and then the sutures on the ventral surface of the carapace were monitored. At the same time, body weight of individual animals was monitored throughout the intermoult period. Another group of 50 juveniles (16 ± 5.7 g (mean ± SD)) were selected from the same batch of juveniles on the morning of moulting, and changes in body weight were monitored.

Diel timing of moulting

The diel timing of moulting of *T. orientalis* reared under the natural day/night period was observed. The time of daily sunset and the time of moult (when new animals are totally detached from old exoskeleton) were recorded. To understand the mechanism of moult synchrony, the photoperiod was manipulated before moulting to: (1) standard (natural day/night); (2) continuous darkness from 1 day before moulting; (3) continuous light while moulting; (4) continuous darkness from 2 days before moulting (except cleaning and feeding in the morning/evening for 10 min); (5) illuminated for 1 h during the night for 2 days before moulting; and (6) continuous light for 2 days before moulting. Also, premoult juveniles were transferred to water 4°C warmer than rearing temperature 12 h before moulting (at 0600 h).

RESULTS

Moult stages

Four moult stages were recognised in juveniles of *T. orientalis* from visual observation—(1) postmoult: soft exoskeleton, inactive, no feeding; (2) intermoult: hard exoskeleton, active feeding, no ecdysial sutures at ventral side of carapace; (3) premoult: hard exoskeleton, inactive feeding, appearance of ecdysial sutures (Fig. 1); and (4) actual moult: swallowing body under exoskeleton, shedding of old exoskeleton.

The length of each moult stage can be manipulated since the duration of the intermoult stage depends on the size of animals and rearing conditions such as nutrition and temperature, but in general it is 7% for the postmoult stage, 74% for the intermoult stage, 17% for the premoult stage, and 2% for the actual moult stage in juvenile animals (<45 g

Fig. 1 Premoult stage of *Thenus orientalis* juveniles. Arrow indicates ecdysial sutures.

body weight). Small juveniles (<16 g) started eating from 24 h after moulting, but larger juveniles (16–45 g) did not eat until at least 2 days after moulting. Ecdysial sutures on the ventral side of the carapace started appearing 7–12 days before the actual moult stage, and cracks appeared at the beginning of the actual moult stage (c. 12 h before shedding the exoskeleton).

Figure 2 shows the change in body weight through four moult stages in *T. orientalis* (79 ± 7.1 g). At this size, the average increment in body weight between two actual moult stages was 54.5% from original weight. During intermoult and premoult stages, body weight did not change much; animals only gained 8.9% of original weight, with the majority of weight gain occurring only at the actual moult stage. Fig. 3 shows the change in body weight at the actual moult stage in *T. orientalis* juveniles (16 ± 5.7 g). At this stage, the mean increment in body weight during actual moult stage was 61%. The increase in body weight started c.12 h before the shedding of the exoskeleton, gradually increasing by 16% of body weight. From 1 h before to 2 h after the moult, there was a further 45% increase in weight, with a further gradual increase by 2% over the following 10 h.

Diel timing of moult

Under the natural day/night photoperiod, all juveniles of *T. orientalis* moulted between 0.5 h and 5 h after sunset (mean 2 h 23 min). When rearing temperature of juveniles (all sizes) was increased 4°C on the morning of the expected moult day, the

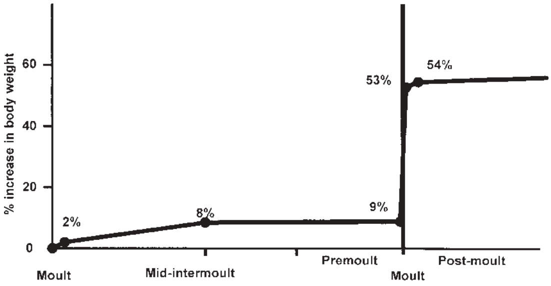

Fig. 2 Change in body weight over the moult cycle of *Thenus orientalis* juveniles (79 ± 7.1 g (mean ± SD)).

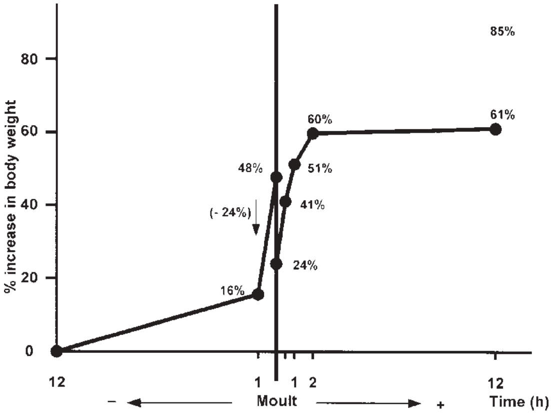

Fig. 3 Change in body weight over the moult cycle of *Thenus orientalis* juveniles (16 ± 5.7 g (mean ± SD)).

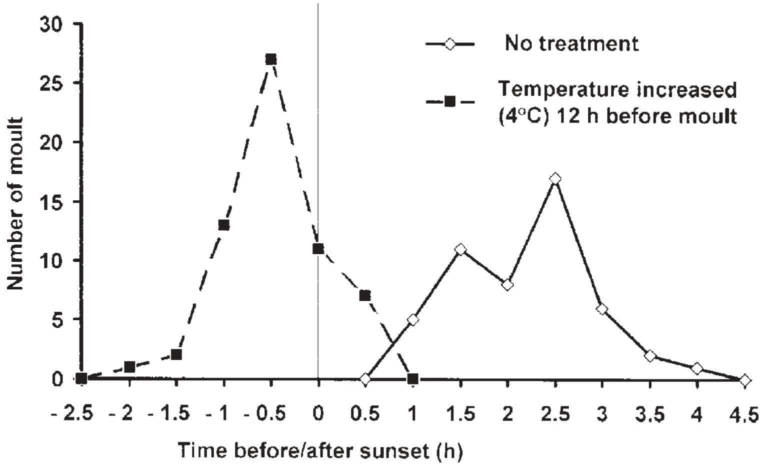

Fig. 4 Effect of temperature on diel timing of moult in *Thenus orientalis* juveniles.

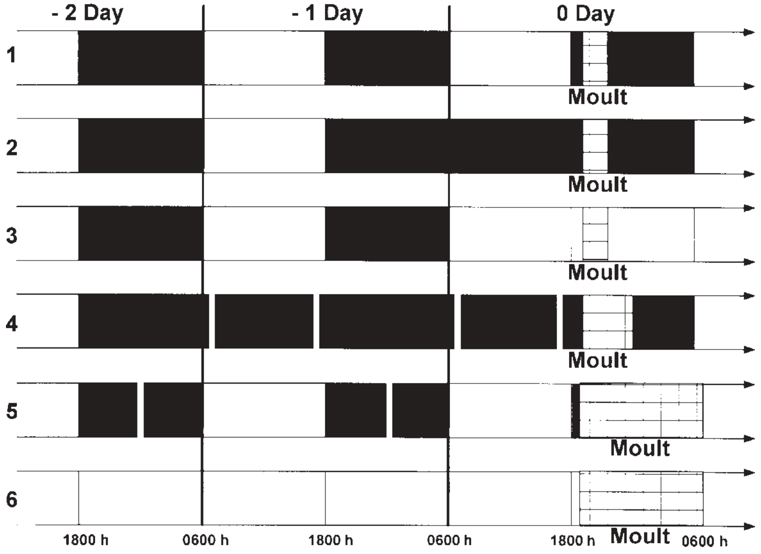

Fig. 5 Effects of changing of photoperiod on the timing of moulting in *Thenus orientalis* juveniles. (1, natural light; 2, continuous dark at the day of moult; 3, continuous light at the day of moult; 4, continuous dark from 2-day before moult (0.5 h light on at morning and evening for husbandry); 5, short illumination (0.5 h) during the dark period; and 6, continuous light from 2-day before moult.) Black area indicates dark period; white area indicates light period; mesh area indicates period animal moulted.

time of moult was advanced on average 3 h 38 min more than that of the no treatment group (Fig. 4). The diel time of moult lost synchrony when the day/night cycle was modified 24 h before the actual moult (Fig. 5). However, synchrony of moulting was not affected if the day/night cycle was changed within 24 h of the actual moult.

DISCUSSION

The characteristics of moult sequences of crustaceans have been well documented (see Phillips et al. 1980; Skinner 1985). In general there are four moult stages during one moult cycle: metecdysis (postmoult), ancecdysis (intermoult), proecdysis (premoult), and ecdysis (actual moult). In addition to these four stages, further sub-stages have been reported on the basis of morphological changes (Dall et al. 1990). Rahman & Subramonian (1989) described morphological and behavioural characteristics of the moult cycle in *T. orientalis*, and this study identified four clear moult stages (postmoult, intermoult, premoult, and actual moult) based on the external morphological characteristics of *Thenus* juveniles. Previously, the separation between intermoult and premoult stages was unclear, but in *Thenus* the separation of the two stages is obvious; the premoult stage starts when ecdysial sutures appear on the abdominal surface of the carapace.

When these ecdysial sutures appear, the length of time to actual moult can be estimated according to the progression of the ecdysial sutures. Although, of course, the estimated time to the actual moult can alter depending on the environmental and nutritional condition of the animals, this method for predicting the timing of the moult is a useful tool from an aquaculture point of view, especially with respect to the harvesting of "softshell" animals. Softshells are harvested during the actual moult/postmoult period immediately after the shedding of the exoskeleton. In the United States, softshell crabs are already well known and have achieved premium prices (Oesterling 1998). Because softshell animals have to be harvested immediately after the shedding of the exoskeleton, the prediction of moult timing is crucial. Together with synchronised moult time after sunset shown in the present study, the *Thenus* species may have great softshell potential because prediction of exact harvesting time (both date and time) is both possible and easy.

It is known that crustacean species accumulate reserves such as glycogen and lipids in the haemolymph and midgut from feeds during the intermoult stage, and that morphological and physiological preparations are made for the final stage of ecdysis in the premoult stage (Dall et al. 1990). An interesting finding in this study is that there was little body weight gain in *Thenus* during the intermoult and premoult stages, even though they feed continuously throughout these stages. The majority of weight gain occurs only a few hours before and after the actual moult stage, as a result of water absorption. Animals accumulate feed reserves and reduce water content in their body during the intermoult stage. They then use these reserves to prepare for the next moult at the premoult stage. Finally, they increase their body size by absorbing water from the environment at the actual moult stage. This pattern of body weight increase agrees with the report of the moult cycle of rock lobster *Panulirus argus* (Travis, 1954), though *P. argus* also tended to increase weight during the intermoult stage.

DeCoursey (1983) reviewed the biological timing of crustaceans, describing endogenous rhythms associated with environmental factors. Light is probably the most important single factor influencing endogenous activities, affecting the time of rhythmic moulting in *Thenus* phyllosomas, nisto, and juveniles (Mikami & Greenwood 1997). In *Thenus*, synchronised moulting can be observed just before sunrise during the planktonic phyllosoma stages (Mikami & Greenwood 1997) and just after sunset in subsequent benthic juvenile/adult stages (present study). It appears that the biological timing which regulates synchronised diel moult time in *Thenus* is influenced by the circadian day/night rhythm during the moult cycle, and that the signal for moult time is set at least 24 h before the moult. The mechanism of switching moult time from sunrise (phyllosomas) to sunset (nistos/juveniles/adults) is still unknown. Synchronised moult time can be changed quickly by altering rearing temperature on the day of actual moult. This method of altering moult time can be useful since animals tend to lose synchrony of moult time when light regimes are changed.

Though the body weight does not change much during the intermoult and postmoult stages, it is obvious that the physiology and biochemistry of the animals change dramatically during the moult cycle (see reviews by Quackenbush 1986; Chang 1995). The hormonal scheme regulating the moult cycle is very complex and little is known about it. It is generally believed that ecdysteroid secretion from the Y-organs is negatively regulated by the neuropeptide MIH, secreted from the X-organ sinus gland system located in the eyestalk. The MIH inhibits activities of the Y-organ during the intermoult stage, then the Y-organ is activated when freed from the inhibitory regulation of MIH when entering the premoult stage (Chang 1995). However, the regulatory mechanism of hormonal activities is complicated by other factors; the environment, nutrition, maturation, and growth are all involved in the process of moulting. The present study may suggest two different (but related) regulatory systems of moulting hormones; one regulates commencement of moulting (length of intermoult), and the other regulates the diel time of moulting, which is also regulated by day-night cycles. The details of these regulatory systems are still unknown, and further studies in the biochemistry of moulting are necessary for a full understanding.

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