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THROUGH THE REPRODUCTIVE STATUS OF MUD CRAB
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AT PELITA JAYA, WEST SERAM DISTRICT**

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GROWTH AND MOULTING OF CRAYFISH

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ABSTRACT: Like other crustacean, crayfish must moult or shed its hard external shell (exoskeleton) to increase in size; hence, the growth process of crayfish involves periodic moulting interspersed with intermoult periods. Some internal and external factors affecting growth and moult of crayfish such as moulting hormone (ecdysteroids), moult inhibiting hormone, temperature and photoperiod.

Keywords: Growth, moulting, crayfish

INTRODUCTION

Crayfish or fresh-water lobster has attracted considerable aquaculture interest. Three important species of crayfish are native to Australia i.e. marron (*Cherax tenuimanus*), yabbies (*C. destructor*) and redclaws (*C. quadrinatus*). Although rapid expansion farming occurred, the current production has remained relatively stable over the past decade (Lawrence 2007). The major problems in the culture of crayfish are they are hard to grow. Growth in crustacean including crayfish consists of a series of moults separated by what are known as intermoult periods. Moulting is a series of steps associated with casting of the old exoskeleton and re-generating of a new one. In healthy animal, moulting cycles are repeated several times in order to allow growth throughout the life of crayfish. Juvenile crayfish moult seven to eight times during the first year of age (Lowery, 1988). The number of moult decrease with the increase of age and it is reduced to one or two moults per year when crayfish reach maturity.

Normally, crayfish grow as fast as possible to reach the size or age of sexual maturation and later, depending on the effort required in the reproduction, allocate the resources among maintenance, growth and reproduction.

DISCUSSION

Moult cycle

The moulting cycle in crustaceans is composed of four different periods (postmoult, intermoult, premoult and ecdysis), five stages (A-E) and several sub-stages within each stage (Lowery, 1988; Aiken and Waddy, 1992). Post moult is divided into stage A and stage B. Stage A or immediate post moult starts soon after ecdysis, and takes only 2% of the entire moult cycle. The activity level in

stage A is very low and animal do not eat. The integument is quite soft as calcification is not yet complete which enable crayfish to expand its volume by absorption of water. Deposition of several inner layers of the new exoskeleton (endocuticle) occurs in this stage. Stage A is divided into A₁ and A₂ sub-stages. Stage B is the main period of calcification of the new cuticle and it takes 8% of the moult cycles. Stage B is completed when final layers of the endocuticle have been deposited. This stage is divided into B₁ and B₂ sub-stages. Stage C or intermoult has several sub-stages (C₁₋₄) and it occupies about 65% of the entire moult cycle. At this stage, animal start feeding. During stage C, the exoskeleton is mineralized and it achieves maximum rigidity.

Stage D is premoult and takes about 24% of the moult cycle. During stage D, crayfish prepares to moult by separating endocuticle of the old exoskeleton from the underlying epidermis and then synthesizing the two outer layers of the new exoskeleton, epicuticle and exocuticle. At the end of this stage, extensive re-absorption of calcium from the old exoskeleton occurs and feeding ceases. When re-absorption of calcium is completed, ecdysial sutures open to facilitate ecdysis. Stage D can be divided into five sub-stages (D₀₋₄). Stage E or ecdysis starts after sub-stages D₄ and in which animals spend a short time for shedding their old exoskeleton. The moult cycle is completed and animal enter stage A.

Moult increment and intermoult period

Unlike other organisms, growth in crustacean including crayfish is a continuous stepwise process occurring through a series of moults. Therefore, growth should be analysed in terms of the interval separating successive moults (intermoult period) and the increase in size for a given moult (moult increment) (Hartnoll, 1982).

Size increment at moult is dependent on age/size and sex, and varies among crayfish. Morrissy (1984) reported weight gain at moulting 52% of the premoult weight with the intermoult period between 15 and 45 days for marron (*Cherax tenuimanus*) weighing between 0.04 g and 120 g. For the younger marron, 0+ year old (0.03 – 7.86 g) the direct and indirect estimated of mean intermoult duration were 28 d and 32 days, respectively (Morrissy, 1990). In another study, Jussila and Evans (1998) reported an intermoult period of 42 and 58 days and a weight gain of 45% for 0+ year old marron (initial mean weight 11.5 ± 0.3 g). Jones and Evans (1996) reported a weight gain of 61% and 56% at moult for redclaws (*C. quadrinatus*) and yabbies (*C. destructor*) respectively. The corresponding intermoult period was 22 to 29d for redclaws and 24 to 30days for yabbies.

In other crayfish, weight increment at moult for juvenile (< 4 g) noble crayfish (*Astacus astacus*) under laboratory condition was between 33% and 65% with the duration of intermoult 58 d to 93 d (Ackefors *et al.*, 1995). Mean value of intermoult period for female and male noble crayfish (9 cm TL) were 40 d and 44 d respectively (Henttonen *et al.*, 1993).

Hormonal regulation of moulting

The moult cycle of crayfish is controlled by two antagonistic hormones: moult inhibiting hormone (MIH) and moulting hormone (ecdysteroids) (Aiken and Waddy, 1992). Ecdysteroids (moulting hormones) is produced by the

moulting gland or Y organ which is located in the anterior thorax (Aiken and Waddy, 1992; Lachaise *et al.*, 1993). Moulting hormones induce physiological and biochemical changes in the intermolt which leads to the moulting. The levels of ecdysteroids are low after ecdysis, then increase in late stage A and remain constant in stage B and C. In sub-stage D₀ ecdysteroids level increase again and reach its peak in sub-stage D₁ and D₂ when pre-exuvial cuticle is being formed, and then the levels drop dramatically in the sub-stage D₃.

Moult inhibit hormone is produced in the X organ-sinus gland complex located in the eyestalk (Aiken and Waddy, 1992; Keler, 1992). This hormone regulates moulting by suppressing synthesis and release of ecdysteroid from the Y organ and by regulating cholesterol uptake (D'Abramo *et al.*, 1985; Naya *et al.*, 1989). The moult inhibit hormone is affected by several environmental factors, particularly light levels, photoperiod and temperature, explaining the responsiveness of moult cycle to change in environment (Wahle and Fogarty, 2006).

Environmental factors affecting growth and moulting

Temperature

Temperature strongly affects the growth of all poikilothermic animals including crayfish. Crayfish have species-specific temperature threshold for growth to take place (Morrissy, 1990; Jones, 1995). Temperatures below the optimum normally inhibit growth while the temperatures above optimal cause stress and result in increased mortality (Morrissy, 1976; Rouse and Kartamulia, 1992). Morrissy (1990) reported 24°C as an optimal temperature for maximum growth of marron. He suggested that over 50% of maximum growth can be achieved when water temperature ranged from 17°C to 27°C for different strain of marron. He also proposed lower and upper limits of temperature for marron at 11°C and 30°C, respectively. In this study, 83% of marron died after exposure to 30°C for 50 day, indicating higher temperature can be very lethal. In a short-term lethal temperature experiment, Morrissy (1976) observed 50% mortality at 31°C ± 0.1°C after 96 h. Rouse and Kartamulia (1992) also reported negative effect of high temperature on survival of marron.

Temperature also affects moulting. Morrissy (1976) reported that no ecdysis for marron weighing 30 – 50 g over a period of 4 months when natural water temperature less than 12°C. In contrast, intermolt period for marron of 30 and 50 g under favourable condition in laboratory (16 - 20°C with regular feeding and aeration) were 2 and 3 months respectively (Morrissy, 1979). Using marron with mean weight of 0.8 g, Morrissy (1990) reported a frequency of 0.5 for ecdysis during a 50 day period of experiment at 12°C. Westin and Gydemo (1986) showed that low temperature affected the delay but not the inhibition of moulting. Intermolt period tend to decrease with increase in water temperature (Reynold, 2002). A reduction of intermolt period from greater than 90 days at 14°C to 40 days at 20-22°C was observed for New Zealand crayfish *Paranephrops zealandicus* by Hammond *et al.* (2006). Rouse and Kartamulia (1992) found that higher temperature resulted on average a higher number of moults compare to the lower one.

Light and photoperiod

Light intensity and photoperiod can significantly affect growth and determine moult cycle of freshwater crayfish. Several studies have examined the impact of light on growth and moulting of freshwater crayfish but the results still contradictive. Sáez-Royuela *et al.* (1996) reported that light intensity and photoperiod have minimal effect on growth. However, other studies showed that growth and survival improved with increase in photoperiod (Mason, 1979, Taugbølt and Skurdal, 1992). Stephens (1955) reported that no moulting occurred when freshwater crayfish, *Cambarus virilis* were maintained in constant darkness for 64 days. He also found that 54% of the animals exposed to twenty hours of light per day showed indication of moult or its successful completion. On the other hand, Quackenbush and Herrkind (1983) showed that spiny lobsters (*Panulirus argus*) exposed to short days (Light:Dark 8:16) entered premoult sooner than those exposed to long days (LD 16:8). The authors suggested that short photoperiod may block moulting inhibiting hormone, and thus stimulate the onset of premoult. Once premoult is initiated, this process generally continues without delay to ecdysis (Aiken, 1973).

Dissolved oxygen

Dissolved oxygen saturation level should be over 70% to ensure uninhibited growth, while saturation level under 60% could inhibit growth as has been reported in juvenile spiny lobster (Chittleborough, 1975). In high temperature, short period of low dissolved oxygen can cause delay in moulting (Jussila, 1995), and thus decreased growth rates. In laboratory experiments, Morrissy (1979) found that moult increment was reduced when oxygen levels fell below 70% at 20°C.

Calcium

Calcium is essential for recalcification of the exoskeleton after moulting (Aiken and Waddy, 1992), and low concentration may therefore slow down growth by delaying moulting or reducing the moult increment. Lower limits of 5 mg Ca/L have been suggested for freshwater crayfish to ensure proper exoskeleton mineralization (Greenway, 1974; Lowery, 1988). A large proportion of the calcium required to harden the new exoskeleton is potentially assimilated from the water rather than from the food, as only 10 %-20% of the required calcium is stored in the form of gastrolith in the anterior wall of the stomach (Lowery, 1988; Taugbølt *et al.*, 1996). Calcium in gastrolith primarily is used to harden mouth parts enabling resumption of feeding, the gastric ossicles and the dactyls of the walking legs (Aiken and Waddy, 1992). Ionic calcium in water aids hardening of new exoskeleton (Malley 1980, Taugbølt *et al.*, 1996). Rapid hardening of new exoskeleton is a key to limit vulnerability to cannibalism (Aiken and Waddy, 1992). In the New Zealand freshwater crayfish *P. zealandicus*, survival increased with increase in water calcium, partly through a decrease in the number of moult-related death (Hammond, 2006).

pH

pH has important role in the uptake of calcium, and thus affects calcification. The uptake of calcium was reduced by 60% at pH of 5.2 (Zanotto

and Wheatly, 1993) and totally inhibited at pH 4.0 (Malley, 1980). Low pH conditions have been reported to inhibit carapace mineralization and growth in freshwater crayfish (Aiken and Waddy, 1992). In extreme situation (low pH), population collapse can be expected from acute change in water pH (Davies, 1989).

Ammonia

Ammonia is toxic to freshwater crayfish in the gaseous form. To minimize inhibition on growth of freshwater crayfish in the intensive system, unionized ammonia should be less than 0.01 mg L^{-1} (Lourey and Mitchel 1995). Freshwater prawn *Macrobracium rosenbergii* seems to show no sublethal growth inhibition at 10 mg L^{-1} total ammonia but levels above 32 mg L^{-1} at pH 7.6 inhibit growth (Armstrong *et al.*, 1978).

Density

The increase in stocking density decreased the growth of freshwater crayfish including marron (Morrissy, 1975; 1992). Difference in density ($0.2\text{-}2.16 \text{ crayfish m}^{-2}$) affected the final length of marron grown in different environment with the higher density resulting in slower growth (Morrissy, 1975). Jussila and Evans (1998) observed higher growth in marron juvenile reared in the communal tanks with $5 \text{ individuals m}^{-2}$ compared with marron reared in the intensive crayfish culture system with a density of $25 \text{ individuals m}^{-2}$. This may be due to the increased competition for food and space, population hierarchical structure or stress caused by more frequent agonistic interaction. The provision of shelter/hide may reduce the effect of growth inhibition by the more dominant individual and increase the surface area, thereby reducing the effective density (Sokol, 1988).

Shelter

Using hides or shelter are common practices in crayfish farming which are used for various reason such as reducing interaction among animals, minimizing aggressive encounters, providing refuge for moulting animal and increasing available substrate in the water column (Jones and Roscoe, 2001; Lawrence *et al.*, 2007). Various studies had evaluated effect of shelter on growth, survival and production of crayfish but the results are still ambiguous. Jones and Ruscoe (2001) using 5 types of shelter showed that mesh bundles was the most effective shelter for redclaw. Shelters have increased survival of yabbies and redclaw but not growth (Geddes *et al.*, 1993; Jones and Ruscoe, 2001). The addition of shelters did not increase growth of both species but they did increase pond biomass primarily through improving survival (Geddes *et al.*, 1993; Jones and Ruscoe, 2001). However, Verhoef and Austin (1999) showed that under laboratory condition, the addition of shelters did not improve growth, survival and yield of yabbies concluding use of shelter is unnecessary for the intensive indoor rearing of juvenile yabbies. Furthermore, Lawrence *et al.* (2007) showed that the extra shelter provided by doubling the number of hide did not improve growth and survival of marron.

Food

The type and amount of food affect the production of crayfish (Lowery, 1988). In a study carried out by Jussila and Evans (1998) on marron, growth rate was affected by type of pellet. The authors reported that marron fed with stable pellets showed larger weight increment at moult and shorter intermoult period compared to the marron fed with unstable (disintegrate within minutes after immersion in the water) pellets. Slower growth could be a result of lower ingested food because marron tended to ignore the remains of disintegrated pellets. Jones *et al.* (1996) found that growth performance of redclaws and yabbies was higher when fed with high protein diets. The high protein diet (300g kg⁻¹) resulted in shorter intermoult period and higher weight gain for both species, relative to the animals fed with low protein (150g kg⁻¹). Weight gain of freshwater crayfish was also affected by feeding level (Gu *et al.*, 1996). Using five feeding levels (1%, 5%, 10%, 15% and 20% of wet body weight) they showed that juveniles (mean weight 130.9 mg) fed at 15% or 20% levels gained the largest increase in weight and had the highest percentage of body protein content compared with those fed at lower levels.

Induction of moulting

Moulting in crustacean is regulated by two antagonistic hormones i.e. moulting hormones (ecdysteroids) produced by Y organ found in the anterior thorax and moult-inhibiting hormone (MIH) produced by sinus gland X organ located in the eyestalks. MIH regulates synthesis and release of ecdysteroids namely ecdysone and its secretory product, 20-hydroxyecdysone which is considered as the major moulting hormone in crustacean (Chang and O'Connor, 1978; Chang, 1995). Seumoff and O'Connor (1982) reported that in vitro, ecdysteroid secretion by Y organ was inhibited when cultured with conditioned medium that has previously been incubated with explanted sinus gland.

Artificial induction of moulting using endocrine manipulations through the removal of X organ (eyestalk ablation) or the administration of exogenous ecdysteroids (Gilgan and Burn, 1976; Snyder and Chang, 1991; Aiken and Waddy, 1992; Shechter *et al.*, 2005) and environment manipulations via the regulation of photoperiod and temperature (Stephens, 1955; Quackenbush and Herrkind, 1983; Morris, 1990; Rousse and Kartamulia, 1992) persuaded by researchers to study the moulting process.

Removal of the X organ through eyestalk ablation is likely to reduce inhibition on the Y organ to produce ecdysteroids (Aiken and Waddy, 1992; Chang *et al.*, 2001). Concentration of ecdysteroids produced by Y organ is low during postmoult and intermoult and rises rapidly at early premoult, then declines prior to ecdysis. The initial rise and decline of ecdysteroids are necessary for successful moulting (Chang and Bruce, 1980). In the experiment using juvenile American lobster Chang and Bruce (1980) found that ecdysteroids concentration elevated sooner in the eyestalk ablated animals and accelerated moulting interval about three times faster than intact animals. Eyestalk removal is also effective to shorten the moulting cycle in shrimp (Chan *et al.*, 1990), and the effect is more pronounced in bilateral ablation than unilateral ablation.

Like eyestalk removal, ecdysteroids injection is also effective to shorten moult cycle in crustacean. However, this type of induction has produced various

results ranging from acceleration of premoult, death during premoult and at ecdysis or viable ecdysis (Gilgan and Burns, 1976; Chan *et al.*, 1990; Aiken and Waddy, 1992; Shechter *et al.*, 2005). Those results depend on the type, time and dosage of ecdysteroids injection. Madhavan (1981) reported that multiple injections of 20-hydroxyecdysone is more effective to accelerate moulting than a single injection. Shechter *et al.* (2007) successfully induce viable moulting of redclaws using multiple injections of 20-hydroxyecdysone. The authors suspected that abnormality which caused mortality during premoult or at ecdysis might be due to incorrect dosage of injected 20-hydroxyecdysone.

A number of environmental factors affect moult cycle. Among those, temperature and photoperiod are considered as the major factors in regulating moult cycle in crustacean (Stephens 1955; Aiken 1969; Armitage *et al.*, 1973; Aiken and Waddy, 1992). Increasing water temperature tends to induce moulting by reducing intermoult period (Hammond *et al.*, 2006; Morrissy, 1990). The authors suggest that rise in temperature increases metabolic rate and food intake of the animals, making them ready to moult.

Photoperiod controls moult cycle in crustacean, although its affect less pronounced than temperature. Some researchers show that long photoperiod induce moulting (Stephens, 1955; Aiken, 1969; Armitage *et al.*, 1973), while other reported that short photoperiod accelerate moulting (Quackenbush and Herrkind 1983). Aiken (1969) suggested that effect of photoperiod on moulting depend on previous history of animals and concluded that long photoperiod will induces moulting during the season (winter) when moulting would be disadvantageous and *visé versa*.

CONCLUSION

Moulting of crayfish is affected by internal and external factors. Artificially, moulting can be induced to accelerate growth of crayfish by endocrine manipulation such as eyestalk ablation and moulting hormone (ecdysteroids) injection or by environmental manipulation through increasing of water temperature. These practices tend to shorten intermolt period and thus accelerate moulting cycle of crayfish.

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