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EFFECT OF EYESTALK ABLATION ON MOLT CYCLE AND REPRODUCTION IN THE BANDED CORAL SHRIMP, *STENOPUS HISPIDUS* (OLIVER)

DONG ZHANG,[1] JUNDA LIN,[1] AND R. LEROY CRESWELL[2]

[1]*Department of Biological Sciences*
Florida Institute of Technology
150 West University Boulevard
Melbourne, Florida 32901-6988
[2]*Aquaculture Division*
Harbor Branch Oceanographic Institution, Inc.
5600 US 1 North
Fort Pierce, Florida 34946

ABSTRACT This study examined the effect of eyestalk ablation on molt cycle and reproduction of the banded coral shrimp, *Stenopus hispidus* (Oliver), a decapod popular among marine aquaria hobbyists. Eyestalk ablation significantly shortened the molt interval in the male shrimps: 11–15 (12.3 ± 0.7, mean ± SE, n = 6) days compared with 15–27 (21.3 ± 0.9, mean ± SE, n = 6) days for the nonablated (control) individuals. The effect decreased gradually. By the third molt, the interval was not significantly different between the ablated and intact shrimps. The result was more complicated for the female shrimps. The females were divided into two groups: "stagnant" shrimps (ovary remained undeveloped for a long time) and ovigerous shrimps with eggs artificially removed. In the "stagnant" shrimps, eyestalk ablation significantly enhanced gonadal development. The ovaries developed (became green) on the fourth day after the ablation and spawned on the eighth day (8.3 ± 0.3 days, mean ± SE, n = 4). In contrast, nonablated individuals spawned in 18–25 (22.5 ± 1.5, mean ± SE, n = 5) days. Once spawned, there was no significant difference in ovarian development between the ablated and control shrimps. In the shrimps with eggs removed, neither unilateral nor bilateral eyestalk ablation stimulated ovarian development and maturation. The unilaterally ablated, bilaterally ablated, and intact females spawned in 15.5 ± 0.3, 15.4 ± 0.3, and 15.8 ± 1.1 (mean ± SE, n = 5) days, respectively. This study suggests that eyestalk ablation can be used to promote the ovary redevelopment in the banded coral shrimp.

KEY WORDS: banded coral shrimp, *Stenopus hispidus*, eyestalk ablation, molt, reproduction

INTRODUCTION

Since Zeleny's study (1905), in which he removed both stalked eyes of the fiddler crab (*Uca pugilator*) and observed a decrease in the molt interval, this simple procedure of eyestalk ablation has been used in many crustacean species, especially decapods (see a review by Chang 1989). With increasing knowledge of the endocrine activity and its control of gonad development in crustaceans, the technique is receiving greater attention as a method of inducing precocious maturation of the ovary and subsequent spawning in captivity (Browdy and Samocha 1985, Primavera 1985 for a review, Makinouchi and Primavera 1987).

Crustacean eyestalks are known to contain the neuroendocrine center, X-organ-sinus gland complex, that is responsible for storing and releasing both the molt- and gonad-inhibiting hormones (MIH, GIH). MIH has been found to inhibit the secretion of molting hormones (MH) by the Y-organs (Soumoff and O'Connor 1982, Mattson and Spaziani 1985). It is long known that molting and gonadal development in crustaceans are regulated in a complicated way by the hormones: MIH, GIH, MH, GH (gonad hormone), and JHs (juvenile hormones). The mechanisms through which these hormones control molting and gonad development have been investigated (see reviews by Passano 1960, Adiyodi and Adiyodi 1970, Cooke and Sullivan 1982, Chang 1984, Chang 1985, Kleinholz 1984, Fingerman 1987, Laufer and Landau 1991). The MH ecdysteroid is a crucial hormone that influences molting and reproduction (see Chang 1989 for a review). The concentration of MH varies during the course of the molt cycle in a number of different crustacean species (reviewed in Chang 1989). Immediately after egg extrusion, the circulating level of ecdysteroids in the female crab (*Cancer anthonyi*) is low. The concentration then steadily increases to a maximum just before hatch. Immediately after hatch, the female undergoes ovarian development without an intervening molt. As the ovaries develop, circulating levels of ecdysteriods decrease to a minimum just before subsequent egg extrusion (Chang 1991). Similar results were obtained in the shrimp, *Palaemon serratus* (Spindler et al. 1987). There is a significant delay of molt due to egg extrusion in American lobster, *Homarus americanus* (Chang 1984). Brood incubation results in longer molt cycle in the isopod (*Oniscus asellus*) (Steel 1980), and no females with developed ovaries were molting in *Penaeus canaliculatus* (Choy 1987). Molting crabs (*Carcinus maenas* and *Paratelphusa hydromous*) with developing ovaries have never been observed in nature (Adiyodi and Adiyodi 1970 for a review). These studies indicate that crustaceans can coordinate molt and reproduction.

Our study assessed the effect of eyestalk ablation on the ovarian development and the molt cycle of the banded coral shrimp, *Stenopus hispidus*. The banded coral shrimp is a popular aquarium species. Its low abundance and delicate nature make its collection challenging. Efforts have been made to develop the larval rearing method for the shrimp (e.g., Young 1979, De Castro and Jory 1983, Fletcher et al. 1995). Generally, a mature female (>3.5 cm in total length) has a molt/reproduction cycle interval of about 16 days at temperatures of 26–30°C. Immediately after hatch, the female molts, mates with a male, and undergoes ovarian development without an intervening molt (Zhang et al. in press). However, ovaries of females collected from the wild or purchased from a pet store are usually undeveloped ("stagnant"). It takes a long time (about 30 days) for the ovary to redevelop. Shortening this duration will benefit the production of the shrimps.

Removal of the eyestalk results in precocious gonadal development and decrease in molt interval, but the effect is different among the life stages. Chang (1989) found that eyestalk ablation cannot shorten molt cycle and promote gonadal development in lobsters once the female enters the sexual maturation cycle. It is not known whether this is a pervasive phenomenon in crustaceans. In the isopod (*O. asellus*), appearance of premolt is greatly delayed if one or two eggs remained stuck in the pouch (Steel 1980).

In this study, we tested the response of the banded coral shrimp (in both males and females) to eyestalk ablation. We tested the hypothesis that the effect of eyestalk ablation is different for animals of different reproduction stages, specifically before and after ovarian development. We also investigated whether the embryo and gonad affect the molting cycle regulation.

MATERIALS AND METHODS

The shrimps (total length: 3.0–4.2 cm for male and 4.0–4.8 cm for female) were purchased from local pet shops that had them collected from the wild (1–2 days before our purchase). They were maintained in a recirculating seawater system under 14 h light: 10 h dark. Each pair (one female and one male) was cultured in a 25-L plastic tank in a greenhouse. Water temperature was kept at 26–31°C. The shrimps were acclimated for a week before the study and fed in excess with frozen *Artemia* or squid once a day.

For each experimental shrimp, the eyestalk was ablated at the base with forceps. The same number of nonablated shrimps was used as controls. Eyestalks of six males were unilaterally ablated 20 h after molting. The first three molt cycle intervals after the ablation were recorded. The females in two states, "stagnant" and with developing gonad, were used. Four "stagnant" females were used to test the effect of unilateral eyestalk ablation on ovarian development. The first two molt cycle intervals after the ablation were recorded. Five females with developing gonads were used for unilateral and bilateral eyestalk ablation, respectively. This experiment was to test whether removal of the X-organ through eyestalk ablation is effective in shortening the molt cycle and promoting gonad development of the shrimps during the reproductive period. The embryos were removed with forceps on the day of egg extrusion, and the eyestalks were ablated 20 h later. Because the oviposition occurs just after the ovigerous females molt and mate, molt cycle can be used to indicate the ovarian development cycle. Student's *t*-test was used to compare molt interval between ablated and intact shrimps.

RESULTS

Male

For the males, the molt interval after eyestalk ablation was 11–15 days (12.3 ± 0.7, mean ± SE) compared with 15–27 days (21.3 ± 0.9, mean ± SE) for nonablated individuals ($p < 0.001$, Table 1). The difference decreased gradually over time and became not significant by the third molt ($p > 0.05$, Table 1).

Female

Eyestalk ablation significantly shortened the molt cycle of "stagnant" females ($p < 0.001$, Table 1). The ovaries of ablated "stagnant" females developed (ovary became green) on the fourth day after the ablation, and spawning/molt occurred on the eighth day (8.3 ± 0.3, mean ± SE). In contrast, spawning of intact individuals occurred in 18–25 days (22.5 ± 1.5, mean ± SE). The second molt cycle interval of ablated "stagnant" females was 15.3 ± 0.3 days (mean ± SE), not significantly different ($p > 0.05$) from that of the intact individuals (15.4 ± 0.3 days, mean ± SE). For ovigerous females with eggs (embryos) removed, eyestalk ablation (both unilateral and bilateral) was not effective in shortening the molt cycle interval (Table 1), or in promoting gonadal development.

DISCUSSION

The most successful and common application of eyestalk ablation is in *Penaeus* shrimp broodstock culture (Browdy and Samocha 1985, Primavera 1985 for a review). This study shows that eyestalk ablation can also promote both molting and gonadal development of the banded coral shrimp. Unilateral eyestalk ablation significantly and substantially reduces the molt interval in the "stagnant" females. However, once a female enters the gonadal developing period, eyestalk ablation has little effect on the

TABLE 1.

The effect of eyestalk ablation on molt interval (day) in the banded coral shrimp, *S. hispidus* (mean ± SE).

Gender		Molt After Ablation 1st	2nd	3rd
Male	UESA	12.3 ± 0.7*	16.5 ± 1.1†	19.8 ± 2.1
(n = 6)	Intact	21.3 ± 0.9	18.8 ± 1.7	18.2 ± 1.3
Female I	UESA	8.3 ± 0.3*	15.3 ± 0.3	
(n = 4)	Intact	22.5 ± 1.5	15.4 ± 0.3	
Female II	UESA	15.5 ± 0.3	15.8 ± 0.5	
(n = 5)	BESA	15.4 ± 0.3	15.4 ± 0.3	
	Intact	15.8 ± 1.1	15.4 ± 0.4	

UESA, unilateral eyestalk ablation; BESA, bilateral eyestalk ablation; Female I, "stagnant" (gonad undeveloped); Female II, ovigerous female, with the eggs (embryos) removed.

* $p < 0.001$.

† $p < 0.05$

molt cycle and gonadal development. For the female shrimps with their embryos removed, there was no significant difference in molt cycle interval between ablated and control individuals. The results show that the banded coral shrimp has an ability to coordinate molting and reproduction (possibly by regulating secretion of hormones) during the reproductive period. Because the ablated eyestalk cannot be regenerated, the hormones produced by the Y-organs would be expected to remain uninhibited, and the molt cycle would remain shorter. The results in this study do not support these findings. Similarly, after the fourth molt, the molt interval of the ablated American lobster is not significantly different from that of the intact controls (Chang 1989). In the isopod (*O. asellus*), the molt cycle of females is influenced by reproduction. Molt cycles of brood females are apparently longer than those of nonbreeding animals (Steel 1980). Obviously, this coordination is to allow sufficient time for embryo development. Endocrinology studies also support this. Eastman-Reks and Fingerman (1984) found that ovarian inhibiting hormone (OIH) secreted by the X-organ has no effect on the ovaries already begun in yolk accumulation. This means that eyestalk ablation does not stimulate ovarian development once the maturation process has been triggered. In this study, the results showed that whether or not the X-organ is intact in females with developed ovary, the molt cycle remained unchanged. There are two possible explanations for this: the activity of the X-organ is lower during the reproduction cycle (Han and Kim 1993) or MIH has the same action mechanism as OIH.

Even if eyestalk ablation does not affect the molt and reproduction cycle of the females with developed gonad or embryos, it is still possible that gonad and embryo play a role in the regulation of the molt cycle. Steel (1980) found that one or two eggs remained stuck in the pouch can greatly delay the appearance of premolt in the isopod (*O. asellus*). Chang (1984) hypothesized that embryos communicate their presence to the mother's central nervous system (CNS) to inhibit molting. There is no evidence to support this hypothesis, however. The results presented here showed that embryos do not seem to affect the CNS. Gonads produce ecdysteroids in several crustacean species (Quackenbush 1986) and may play an important role in the complex regulation. Y-organ, a major source of MH, certainly plays a critical role. Histological studies in a crab *Hemigrapsus* sp. have shown that activity of two kinds of neurosecretory cell, A'- and B'-cell in the thoracic ganglion, are related to both X-organ and Y-organ (Matsumoto 1962). There was a different response to eyestalk ablation between mature males and females. The effect of eyestalk ablation in males decreased gradually. The cause of this difference is unknown.

The coordination between molt and reproduction is a complex process in crustaceans. Future work should focus on the change in MIH titer in the hemolymph, the action on target tissues, and histological changes in the X-organ during the molt period. On a more practical aspect, research should be done to examine whether eyestalk ablation affects fecundity and egg quality.

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