JOURNAL OF CRUSTACEAN BIOLOGY, 27(2): 304–308, 2007

ELEVATED ECDYSTEROID TITER AND PRECOCIOUS MOLT AND VITELLOGENESIS INDUCED BY EYESTALK ABLATION IN THE ESTUARINE CRAB, *METOPOGRAPSUS MESSOR* (BRACHYURA: DECAPODA)

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A B S T R A C T

Premolt and vitellogenesis are mutually exclusive events in the wild population of the brachyuran crab, *Metopograpsus messor*; active vitellogenesis occurs only in intermolt females, at a relatively low ecdysteroid profile, judged from radioimmunoassay. Bilateral eyestalk ablation, however, has resulted in simultaneous precipitation of premolt and ovarian growth in the same crab, thus severing the normal antagonistic programming of these two high-energy demanding processes. This result argues for the necessity of eyestalk principles in maintaining the normal (antagonistic) programming of growth and reproduction in the species. Ovaries of the eyestalk ablated crabs precociously accumulate yolk and the oöcytes could attain the size of a fully grown egg within 10 days post-ablation. The eyestalk ablated females have also shown dramatic rise in haemolymph ecdysteroid levels, even surpassing the levels ($P < 0.05$) of the normal premolt individuals, revealing that ovarian growth can occur under a high ecdysteroid titre in *M. messor*. It remains to be seen whether the precociously grown ovaries were influenced by the elevated ecdysteroid titer.

Introduction

The up- and down-regulation of growth and reproduction in crustaceans is accomplished by the interaction between stimulatory and inhibitory principles. The role of crustacean eyestalks as source of inhibitory hormones for growth (moult-inhibiting hormone, MIH) and reproduction (gonad-inhibiting hormone, GIH) has been revealed by previous investigations (Adiyodi, 1988; Van Herp and Soyez, 1997; Okumura, 2004; Okumura and Sakiyama, 2004). Removal of eyestalks, however, has produced varying results, apparently based on the phylogeny and physiological state of the species. In brachyuran crabs and lobsters, where growth and reproduction are essentially antagonistic events, eyestalk ablation is shown to accelerate either moult or reproduction. In the field crab *Paratelphusa hydrodromous* (Herbst, 1794), eyestalk ablation, if conducted during pre-breeding or breeding season, resulted in accelerated ovarian growth; none of the eyestalkless experimentals showed any tendency for premolt initiation (Anilkumar and Adiyodi, 1980, 1985). In *Homarus americanus* (Milne Edwards, 1837) (Snyder and Chang, 1991), *Carcinus menaeus* (Linnaeus, 1758) (Skinner, 1985; Webster, 1986) and *Libinia emarginata* (Leach, 1815) (Laufer et al., 2002), on the other hand, eyestalk ablation has resulted in precocious precipitation of molt. Significantly, there are instances of differential responses from the individuals of the same species to eyestalk ablation. In the snow crab *Chionoecetes opilio* (Fabricius, 1788), eyestalk ablation resulted in commencement of premolt in small-clawed immature males, while the adult males (after undergoing the terminal molt) did not show any signs of molt initiation (Tamone et al., 2005). The present paper reports instances of simultaneous acceleration of growth and vitellogenesis under eyestalk ablation in a brachyuran crab *Metopograpsus messor* (Forskal, 1775). The study also signifies the role of eyestalk (hormones) in maintaining normal programming of growth and reproduction in this highly fecund species that releases 14-18 broods a year. Inhabiting the pebbles and crevices along the inter-tidal region of the Muzhupilangad estuary (Kannur, N. Kerala, India), *M. messor* programs its breeding and molting as follows: August-December is the period for peak breeding activity. January-May is devoted for reproduction by a sect of the population, while several other individuals would engage in molting. Like other brachyurans reported, the wild population of *M. messor* exhibits a high degree of antagonism between premolt and reproduction; in nature, premolt females do not engage in reproduction. During August-December (reproductive period), almost all the females are in intermolt and engage in repeated spawning (continuously for 8-10 broods), without being interrupted by molting. During January-May (growth-reproductive period), however, a subset of the female population undergoes molting. In such females, the ongoing vitellogenic activity would be interrupted by the commencement of premolt (Sudha and Anilkumar, 1996).

Elevated ecdysteroid titer is a result of eyestalk ablation in *M. messor*. This has offered us an insight into the possible ecdysteroid influence on vitellogenesis, a question that has not yet been resolved in crustaceans (Subramoniam, 2000; Okumura, 2004; Diwan, 2005, for reviews). In insects, on the other hand, previous hormonal assays coupled with the hormone receptor gene expression studies have provided us with increasing evidences on the role of ecdysteroids in reproduction; the ecdysteroid receptor is shown to be a requisite for normal oogenesis in *Drosophila* (Carney and Bender, 2000; Koslova and Thummel, 2000; Riddiford et al., 2001). Being close phylogenic relatives of insects, crustaceans could arguably be relying on a comparable mechanism for regulating reproduction. However, the diverse patterns of programming of growth and reproduction, having been exercised by the various taxonomic groups, do not permit us to draw a common pattern for the entire class.

The antagonistic programming of growth and reproduction, in brachyuran females raises the question whether a high ecdysteroid titer could impede reproduction. The present study addresses this question through ecdysteroid assay in relation to stages of normal growth and reproduction and under eyestalk ablation.

Materials and Methods

All the adult female crabs (*Metopograpsus messor*) used in the present study belonged to the size class 20-22 mm carapace width, and were collected during January (growth-reproductive period, after Sudha and Anilkumar, 1996) from the Muzhupilangad (N.Kerala, India) estuarine region. The animals were maintained in plastic cisterns laid with wet sand at the bottom and were fed ad libitum on clam meat. Care was taken for daily removal of the left-over meat and for replenishment of the cisterns with fresh seawater as and when required.

Characterization and classification of ovarian stages (1-4) were performed using oöcyte diameter and ovarian hue as the criteria. At Stage 1, ovary of *M. messor* appears creamy with oöcyte diameter ranging between 11 and 90 μm. The ovary attains bright yellow hue at Stage 2, the oöcyte size ranging between 91 and 155 μm. As vitellogenesis progresses towards Stages 3 and 4, the ovary appears brown or brownish black, the oöcyte diameters being 156-230 μm and 231-330 μm respectively. The pleopod setagenic events were used to precisely identify the molt stages. Taking care not to inflict much stress to the animal, the pleopod tip was cut out, placed on a clean glass slide along with a drop of saline and observed through a light microscope so as to observe clearly the epidermal retraction, development of cuticular layer and the formation of new setae (Sudha and Anilkumar, 1996; Suganthi and Anilkumar, 1999).

Eyestalk Extirpation

Adult intermoult females of *M. messor* having ovaries in early vitellogenesis were subjected to bilateral eyestalk extirpation in January; both the eyestalks were excised from its base using a pair of scissors, ensuring complete removal of the Sinus Gland-X organ complex. Results of eyestalk extirpation on molt stages were assessed by microscopic examination of the setagenic events. Simultaneous with the experimentals (bilaterally eyestalk-ablated females), a set of controls with intact eyestalks was also maintained for the present study.

Ecdysteroid Assay

The hemolymph ecdysteroid titer was estimated by radioimmunoassay (RIA) as described in Chang and O'Connor (1979), and Suganthi and Anilkumar (1999). 10 μl fresh samples of hemolymph were collected in vials, each containing 100 μl borate buffer (pH 8.4) and the radioligand ^{3}H-ecdysone (~12,000 dpm, from DuPont/NEN). A set of standards comprising the radioligand (^{3}H-ecdysone), the (phosphate) buffer and the standard ecdysone (Sigma, USA) was also run along with the unknown samples for the purpose of plotting the standard graph. All the vials (the haemolymph samples and the standards) were vortexed to allow adequate mixing. To each vial, 100 μl antiserum (2-succinyl conjugate of ecdysone, a generous gift from Dr. W. E. Bollenbacher, Chapel Hill, USA) was added [prior to treatment with the antigen, the antiserum was subjected to titer assay to ensure optimal binding sensitivity; 1:500 was found to be the most appropriate dilution for the purpose]. The mixture was then incubated for 12 h at 4°C, after which 200 μl saturated ammonium sulfate was added to each vial and incubated at 4°C for 20 minutes to allow complete precipitation. The pellets, resultant of the antigen-antibody binding, were separated out by centrifugation at 4800 g for 20 minutes, and washed with 400 μl of 50% ammonium sulfate in borate buffer and centrifuged. The pellets were dissolved in 25 μl distilled water and mixed with 500 μl of Scintillation cocktail (0.8% PPO and 0.02% POPOP in a toluene:triton [2:1] mixture) and read using an LKB Liquid Scintillation Counter. A standard curve plotted with 50, 100, 200, 400, 800 and 1600 pg ecdysone on a semi-log paper, was used for accurate estimation of the ecdysteroid titer.

Biochemical Estimations

Quantitative estimations were carried out primarily for assessing the levels of organic reserves in the ovaries of the experimentals and the controls. Total protein in the tissue was precipitated by cold TCA (10%). The precipitate, separated out by centrifugation, was dissolved in 0.1 N NaOH, and was used as the protein extract for colorimetric analysis with BSA as standard (after Lowry et al., 1951; Anilkumar and Adiyodi, 1980). In order to extract total lipid, the tissue was homogenized with Chloroform:Methanol (2:1) mixture to a final volume 20 times that of the tissue sample. The homogenate was centrifuged; the supernatant was washed briefly with 0.9% NaCl solution (by vortexing a few seconds), and centrifuged (2000 rpm) again so as to separate the two phases. The lower chloroform phase (containing total lipid) was separated out and subjected to evaporation and gravimetric analysis (Folch et al., 1957). Carbohydrates of the tissue were separated into ethanol-soluble oligosaccharide fraction and ethanol-insoluble polysaccharide fraction (after Johnston and Davies, 1972). The tissue was homogenized with 80% ethanol and centrifuged. The supernatant was evaporated in an incubator at 60°C, subsequently dissolved in distilled water, and was used as the ethanol-soluble oligosaccharide fraction. The precipitate (ethanol-insoluble fraction) was dissolved in distilled water, and used as polysaccharide fraction. The carbohydrate content of both the fractions was estimated by phenol-sulphuric acid method (Dubois et al., 1956). A portion of the ethanol-soluble fraction (prepared as above) was also used to estimate the total free amino acids. The ethanol-soluble extract, along with ninhydrin reagent prepared in citrate buffer was heated in a boiling water bath for 12 minutes to optimize color development which in turn was read colorimetrically at 540 nm, with glutamic acid as the standard (Lee and Takahashi, 1966).

Statistical Analyses

The extent of significance in fluctuation of ecdysteroid levels and the ovarian organic reserves in response to eyestalk ablation has been estimated by assessing the *P*-values through Tukey-Kramer multiple comparisons test or through Student's *t*-test, using InStat Software (GraphPad InStat, Version 2.00, 1993) on a Pentium IV computer.

Results

Bilateral eyestalk ablation in *M. messor* resulted in hyperphagia, noticeable from the first day post-ablation; the experimentals consumed almost 3-4 fold quantities of food and the tendency prevailed up to five days post-ablation. As the experiment proceeded towards 5-10 days post-ablation, however, the hyperphagia showed a declining tendency.

Somatic Growth vs Reproductive Growth Under Eyestalk Ablation

At the commencement of the (eyestalk ablation) experiment (0-day), ovaries of both the controls (with intact eyestalks) and the eyestalk ablated crabs were in Stage 1 (oöcyte diameter, OD: 11-90 μm). Interestingly, each of the eyestalk ablated females showed clear signs of simultaneous enhancement of ovarian growth [judged by significant ($P < 0.05$) increase in oöcyte diameter and change in ovarian hue] and premolt initiation (see below for details). Within five days post-ablation, all the eyestalk ablated females were in mid-premolt (D2/D3) stage (marked by the development of new setae and the setal grove in the pleopod) (after Sudha and Anilkumar, 1996), while their ovaries reached Stage 3 (OD: 156-230 μm) of vitellogenesis; controls, however, remained in intermolt and their ovaries were in Stage 1 or Stage 2 (OD: 91-155 μm) of vitellogenesis (Table 1). Within ten days post-ablation, all the eyestalk ablated crabs were either in late premolt (D4) stage (marked by complete development of the new setae prior to ecdysis), or underwent ecdysis, and their ovaries attained Stage 4 (231-330 μm) of vitellogenesis; all the control crabs remained in intermolt throughout the entire duration of the experiment, and their ovaries were in either Stage 2 or early Stage 3 of vitellogenesis (Table 1). Eyestalk ablation also resulted in significant increase in yolk components coupled with the precocious ovarian growth, throughout the experimental period. There was a progressive increase ($P < 0.05$) in the

Table 1. Effects of eyestalk ablation on molt stage and ovary growth in *Metopograpsus messor* (Ovary wet weights represented in mg/individual; ecdysteroid levels represented in ng/ml haemolymph, Mean ± SE). Oöcyte size 0 day: C = E (NS); 5 days: C < E**; 10 days: C < E***. Wet weight 0 day: E = C (NS); 5 days: C < E***; 10 days: C < E***. Ecdysteroid levels 0 day: E = C (NS); 5 days: C < E***; 10 days: C < E***; 0 day E < 5 days E***; 5 days E < 10 days E***. [a]Five ablated crabs that molted within eight days postablation were not included in this sample; see text for details. Sample size in parentheses. ** denotes $P < 0.001$, and *** denotes $P < 0.0001$.

	Experimental (Ablated) (E)					Control (With intact eyestalks) (C)				
Period in days	Molt stage	Oöcyte size (μm)	Ovary stage	Ovary wet weight (mg)	Ecdysteroid[a] levels	Molt stage	Oöcyte size (μm)	Ovarian stage	Wet weight (mg)	Ecdysteroid levels
0	C4 (18)	63.94 ± 2.55 (18)	1 (18)	12.22 ± 1.08 (18)	9.85 ± 1.3 (18)	C4 (10)	65.30 ± 3.56 (10)	1 (10)	13.70 ± 1.35 (10)	10.35 ± 2.04 (10)
5	D2/D3 (15)	169.07 ± 4.45 (15)	3 (15)	37.53 ± 1.76 (15)	103.47 ± 7.41 (15)	C4 (7)	110 ± 12.29 (7)	1/2 (7)	18.29 ± 1.73 (7)	27.12 ± 4.56 (7)
10	D4 (18)	262.78 ± 7.34 (18)	4 (18)	72.89 ± 2.44 (18)	233.89 ± 14.38 (18)	C4 (8)	130.0 ± 8.19 (8)	2/3 (8)	23.25 ± 2.21 (8)	33.88 ± 6.89 (8)

profiles of the major yolk components such as total proteins and total lipids (Table 2). Nevertheless, the biochemical reserves of the precociously grown ovaries of ablated crabs were found to be impoverished when it was compared with those of individuals from the wild having respective stages of ovarian growth (Table 3).

Ecdysteroid Titer in Normal vs Ablated Females

In normal females, ecdysteroid titers were found to be correlated with molt stages; the profiles registered significant ($P < 0.05$) increase until late premolt (D4), but declined thereafter, following exuviation (Table 4). We have also compared the ecdysteroid levels in relation to vitellogenic stages. It is worth recalling in this context that the females releasing successive broods (''berried females'') would remain in intermolt throughout the vitellogenic cycle. Ecdysteroid titre of these females did not fluctuate significantly (NS), while their ovaries underwent vitellogenesis (Table 5). Another sub-set of the female population, however, would commence premolt changes subsequent to spawning (Sudha and Anilkumar, 1996 for details). Vitellogenesis in this set of females would set in only when they undergo postmolt changes, while it (still) maintains a significantly higher haemolymph ecdysteroid titer ($P < 0.001$), when compared to that of the 'berried females' (Table 5). In response to eyestalk ablation, the haemolymph ecdysteroid levels of all the females registered a substantial increase, coupled with premolt acceleration; the controls, however, remained in intermoult, and did not show a comparable change in its ecdysteroid levels (Table 1). Five of the total 56 eyestalk ablated females, molted within 8 days postablation, and ecdysteroid titer (100.66 ± 35.56) of these postmolt crabs declined perceptibly ($P < 0.05$).

Discussion

Premolt and vitellogenesis are mutually exclusive events in *M. messor* (Sudha and Anilkumar, 1996), akin to other brachyuran crabs studied so far (Adiyodi, 1988; Van Herp and Soyez, 1997; Lopez Greco and Rodriguez, 1999; Zapata et al., 2003). The present study demonstrates that eyestalk ablation (conducted in January, the growth-reproductive period), resulted in simultaneous enhancement of growth and reproduction in all the crabs, thus severing the growth-reproduction antagonism existing in the wild population. In this respect, we also considered the question that the physiological condition of the animal could vary with

Table 2. Profiles of organic reserves of ovary of *Metopograpsus messor* under eyestalk ablation (Weights represented in mg/100 g body weight; Mean ± SE). 0 day: E = C (NS); 5 days: C < E**; 10 days: C < E***. Sample size is 6 in all the instances.

Reserves	0 days	5 days	10 days
Protein			
Ablated	25.78 ± 1.77	151.00 ± 29.96	410.44 ± 73.01
Control	20.14 ± 2.75	30.14 ± 4.84	50.38 ± 8.26
Lipid			
Ablated	36.14 ± 1.41	200.17 ± 34.56	283.14 ± 49.23
Control	34.38 ± 0.90	46.38 ± 6.0	60.14 ± 5.46
Polysaccharide fraction			
Ablated	1.13 ± 0.08	20.34 ± 4.15	40.68 ± 1.28
Control	1.03 ± 0.04	2.41 ± 0.35	4.1 ± 0.55
Oligosaccharide fraction			
Ablated	1.20 ± 0.03	24.00 ± 3.74	27.98 ± 4.22
Control	1.31 ± 0.03	2.36 ± 0.05	5.11 ± 0.40
FAA			
Ablated	2.14 ± 0.06	26.13 ± 3.09	34.14 ± 4.98
Control	3.00 ± 0.37	4.11 ± 0.36	8.11 ± 1.28

Table 3. Comparison of the ovarian biochemical reserves of the ablated crabs with those of the normal individuals with respective ovarian stages collected from the wild (Weights represented in mg/100 g body weight; Mean ± SE). Stage 1:E = C (NS); Stage 3: E < C***; Stage 4: E < C***. Sample size is 6 in all the instances.

Reserves	Ovarian stages	Ablated (E)	Normal (wild population) (C)
Protein	Stage 1	23.78 ± 2.51	25.64 ± 2.19
	Stage 3	151.00 ± 29.96	410.21 ± 19.44
	Stage 4	410.44 ± 73.0	959.76 ± 75.75
Lipid	Stage 1	36.14 ± 1.41	38.78 ± 5.27
	Stage 3	200.17 ± 34.56	336.60 ± 44.48
	Stage 4	283.14 ± 49.23	764.35 ± 118.39
Polysaccharide fraction	Stage 1	1.13 ± 0.08	1.45 ± 0.12
	Stage 3	20.34 ± 4.15	9.15 ± 0.79
	Stage 4	40.68 ± 1.28	31.93 ± 3.34
Oligosaccharide fraction	Stage 1	1.20 ± 0.03	1.38 ± 0.08
	Stage 3	24.00 ± 3.74	6.06 ± 1.02
	Stage 4	27.98 ± 4.22	21.17 ± 1.69
FAA	Stage 1	1.14 ± 0.06	1.15 ± 0.02
	Stage 3	26.13 ± 3.09	17.09 ± 3.25
	Stage 4	34.14 ± 4.98	23.91 ± 1.79

Table 4. Haemolymph ecdysteroid levels of *M. messor* (with intact eyestalks) in relation to molt stages (Weights represented in ng/ml haemolymph; Mean ± SE). D1 < D2***; D2 < D3***; D3 < D4***; A < D4*** Sample size in parentheses.

Premolt stages				Postmolt stage
D1	D2	D3	D4	A
42.58 ± 3.49 (19)	79.75 ± 3.91 (12)	117.25 ± 10.06 (8)	170.83 ± 12.52 (12)	36.29 ± 2.51 (7)

season-dependent factors which in turn could influence the results of the experiment. We have hence compared the present results with those of the eyestalk ablation experiments conducted in our laboratory during other seasons i.e., August-December (reproductive period) and June-July (inactive period) (Sudha and Anilkumar, unpublished observations). The comparison unequivocally reveals that the removal of eyestalks would result in acceleration of premolt and vitellogenesis simultaneously, irrespective of the season. We are thus encouraged to suggest that the eyestalk inhibitory principles are necessary for maintenance of the antagonism existing between growth and reproduction, which in turn could be responsible for a balanced energy budget to accommodate successful breeding and somatic growth in the species. At this juncture, it would be worth comparing the results of our present eyestalk ablation experiments (in *M. messor*) with that of the palaemonid shrimp, *Macrobrachium rosenbergii* (De Man, 1879) wherein somatic growth and vitellogenesis are synergistic events and ecdysteroid build-up parallels with yolk deposition (Wilder and Aida, 1995). Interestingly, eyestalk ablation in *M. rosenbergii* resulted in simultaneous acceleration of growth and reproduction, but without significantly altering the normal relation between these two metabolic events, implying that eyestalk hormones are not necessary for integrating molt and reproduction (Okumura et al., 1992; Wilder et al., 1994; Okumura, 2004). Apparently, the innate, synergistic programming of molt and vitellogenesis existing in the wild population, has led to the precocious, but unbiased acceleration of both somatic and reproductive growths following eyestalk ablation in this palaemonid shrimp.

Although our experiments resulted in precocious ovarian growth, the yolk thus incorporated was found to be impoverished in terms of its biochemical reserves, when compared with that of the individuals from the wild with respective ovarian stages (Table 3). Importantly, none of the deeyestalked females oviposited. Similar situations of abstinence from spawning by eyestalkless females are reported in the field crab, *Paratelphusa hydrodromous* (Anilkumar and Adiyodi, 1985) and the estuarine grapsid crab *Chasmagnathus granulata* (Dana, 1851) (Stella et al., 2000). Biochemical impoverishment of the precociously incorporated yolk (after bilateral eyestalk ablation), as also reported in *P. hydrodromous* (Anilkumar and Adiyodi, 1980, 1985; Adiyodi, 1988), could be the factor responsible for the inability of spawning by these crabs, suggesting that the eyestalk hormones are necessary for normal programming of vitellogenesis. Discrepancy in brood biochemistry between the eggs produced through eyestalk ablation has been reported in penaeid shrimps as well (Palacios et al., 1999). There are further reports from other sources that demonstrate the inadequacy of eyestalk extirpation as an optimized technique for induced breeding (Quackenbush, 1986; Choy, 1987; Fingerman, 1997; Reddy et al., 2005), thus rendering additional support to our contention on the relevance of eyestalk(hormone)s.

Table 5. Haemolymph ecdysteroid levels in relation to vitellogenic stages in berried and nonberried females of *M. messor* (crabs with intact eyestalks; weights are represented in ng/ml haemolymph) (Mean ± SE). [a]ES = LS (NS); [b]LS < ES***; [a]ES < [b]ES*** [a]LS = [b]LS (NS). Sample size in parentheses.

	Early stages (ES) (Stages 1 & 2)	Late stages (LS) (Stages 3 & 4)
Berried females[a]	9.25 ± 1.18 (23)	9.43 ± 1.63 (7)
Nonberried females[b]	21.2 ± 1.83 (9)	11.88 ± 1.41 (8)

The present study also draws our attention to the relation between ovarian growth and ecdysteroids in decapods. Although there is ample evidence to demonstrate that ecdysteroids enhance growth in crustaceans (Snyder and Chang, 1991; Van Herp and Soyez, 1997; Laufer et al., 2002), information on its involvement in reproduction is still scanty (Subramoniam, 2000; Okumura, 2004; Diwan, 2005, for reviews). The ambiguity existing in respect of ecdysteroid's role in crustacean reproduction could essentially be attributed to the diverse patterns of integration between growth and reproduction in various taxonomic groups, for instance, the synergistic and antagonistic relations in shrimps and brachyuran crabs respectively (Okumura et al., 1992; Wilder et al., 1994; Wilder and Aida, 1995; Sudha and Anilkumar, 1996).

The antagonistic programming of growth and reproduction, as exhibited by the brachyuran females, implicates that vitellogenesis in this group of crustaceans occurs at low ecdysteroid titer. Such a contention is further strengthened by the ecdysteroid assay performed on *M. messor* (present study), revealing the presence of relatively low ecdysteroid level during intermoult when active vitellogenesis occurs (Table 5). Nevertheless, that this low ecdysteroid titer is a requisite for successful female reproduction, is another question that deserves to be addressed.

To conclude, the occurrence of vitellogenesis exclusively in intermoult crabs, as seen in *M. messor* (present study), poses a question whether high ecdysteroid levels are antagonistic to reproduction in brachyurans. Firstly, to address this question, we would draw our attention to Table 5 which reveals that early vitellogenesis (Stages 1 and 2) in 'Nonberried females', occurs at a significantly high ecdysteroid titer compared to the levels in 'Berried females'. Secondly, bilateral eyestalk extirpation has not only enhanced precocious vitellogenesis and premolt in all the females of *M. messor*, but also resulted in elevated ecdysteroid levels in all the experimentals (Table 1), clearly demonstrating that vitellogenesis can occur under a high ecdysteroid titer in

this brachyuran crab, much higher than the premolt levels. Further investigations are, however, necessary to examine whether the high ecdysteroid titer consequent to eyestalk ablation has exerted any influence on the precocious vitellogenesis in the experimentals. Recent investigations on hormone receptor mechanisms have shown that the ovary of the fiddler crab *Uca pugilator* (Bosc, 1802) is a potential target for ecdysteroid receptor gene *EcR* (Durica et al., 2002; Wu et al., 2004), signifying that receptor-oriented studies could be effective tools to address the question of ecdysteroid influence on ovarian development in crustaceans.

Acknowledgements

The authors gratefully acknowledge the financial support from the International Foundation for Science (Stockholm, Sweden) (RGA: A/3520-1) and the Department of Atomic Energy (Government of India). Thanks are also due to Dr.W.E.Bollenbacher (Chapel Hill, USA) for his generous gift of the Ecdysteroid antiserum, and M/s. Amala Cancer Research Centre for the provision of Liquid Scintillation Counter.

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Received: 21 April 2006.
Accepted: 19 October 2006.