JOURNAL OF CRUSTACEAN BIOLOGY, 22(2): 398–410, 2002

SYNCHRONISATION OF MOLTING AND OOGENIC CYCLES IN A CONTINUOUSLY BREEDING POPULATION OF THE SAND CRAB *EMERITA ASIATICA* ON THE MADRAS COAST, SOUTH INDIA

V. Gunamalai and T. Subramoniam

Unit of Invertebrate Reproduction, Department of Zoology,
University of Madras (Guindy Campus), Chennai (Madras) 600 025, India
(corresponding author (TS) e-mail: tsbl71@hotmail.com)

A B S T R A C T

In the mole crab *Emerita asiatica,* the female reproductive cycle is repetitive; when the pleopodal embryos undergo development, there is a concurrent maturation of oocytes within the ovary making it ready for the next spawning. However, molting occurs after hatching of the larvae from the pleopods and before spawning. In *E. asiatica,* the developmental changes of setae on the pleopods and the extent of epidermal retraction were used to define the molt cycle stages. Contrary to earlier reports on embryo-carrying decapods, in *E. asiatica* the first sign of molting, viz., the retraction of epidermis, is evident even before the hatching of the embryos. The premolt stages advance further up to D_1, at a time when the pleopodal embryos hatch. No female at the time of embryo hatching is in the intermolt. Both ovarian index and total ovarian proteins gradually increase from the intermolt stage C_1 to C_3, thereupon maintaining the same level up to spawning. In continuously reproducing females (size class 23–33-mm CL), the hemolymph protein level is also high during the entire intermolt period, but increases sharply during premolt stage with a drastic decline just before ecdysis. Furthermore, hemolymph protein of both immature females (size class 10–17-mm CL) as well as females in the first maturation (18–22-mm CL) sharply rises during premolt stages (D_0–D_2), with an ensuing decline in stage D_{3-4}. Evidently, the changes in the total hemolymph protein reflect on its role in vitellogenesis as well as new cuticle synthesis. Whereas the protein rise during the intermolt stage is coincident to the active vitellogenic phase, the second ramp in the increase of hemolymph protein during premolt stage may be related to new cuticle synthesis. A common endocrine basis of such a synchronous molting and ovarian cycle in *Emerita* is evident, but a substantial nutritional status, owing to filter-feeding habit of the sand crab, is attributed to the year-round reproduction and molting.

In Crustacea, as in other arthropods, molting facilitates continued body growth by periodic shedding of the old cuticle and secretion of a new cuticle. A characteristic feature, which is uncommon among other arthropod groups, is the continuation of molting even after attaining sexual maturity in many crustacean species. The resultant relationship between molting and reproduction is more marked in females, as active vitellogenesis during the reproductive cycle, as well as secretion of a new cuticle during molting, could affect the physiology of the organism by their competitive utilisation of reserve material from storage organs. Subramoniam (2000) recently reviewed the wide range of interrelationship existing between molting and reproduction in different crustacean species. In general, molting and reproductive activities are temporally separated in large-bodied crustaceans such as lobsters and brachyuran crabs. On the contrary, crustaceans with high fecundity and faster body growth exhibit closeness in their molting and oogenic cycles. Furthermore, many malacostracan crustaceans carry their broods, extending their reproductive cycle beyond spawning. In them, the onset of molting is perforce delayed until hatching and release of the young ones, thereby placing constraints on the continued somatic growth. Although the environmental factors controlling the reproductive cycle of marine crustaceans have been delineated (Giese and Pearse, 1974), specific interrelationship between molting and female reproductive processes is not adequately understood.

Species belonging to the sand crab genus *Emerita*, occurring in the intertidal zone, extend in distribution from tropical to temperate seas. Their ability to colonize this precarious habitat is mainly due to their burrowing habits

in the shifting sands, together with heightened growth rate and fecundity. The tropical species such as *Emerita portoricensis* and *E. asiatica*, breed continuously throughout the year (Goodbody, 1965; Subramoniam, 1977). On the Madras Coast, *E. asiatica* has also been seen to molt continuously throughout the year (Subramoniam, 1977; Gunamalai, unpublished observation). The occurrence of continuous molting and reproduction in female *Emerita* suggests common environmental conditions conducive to controlling these two processes. These facts, coupled with the ready availability of adult *Emerita* in different size classes could facilitate a study on the interrelationship between molting and reproduction.

The present study reports both field data and laboratory observation on the various phases of molt and reproductive stages of *Emerita asiatica*. The main emphasis of this study is to find out the degree of overlap in the reproductive and molting processes so as to gather information on the metabolic compatibility in achieving synchronous growth and reproduction which are primary requisites in the successful colonization of these anomuran crabs in the sandy intertidal zone.

Materials and Methods

Experimental Animal.—Female sand crabs ranging in size from 10 to 33 mm carapace length, were collected from the intertidal region of Elliots beach at Besant Nagar, Chennai, India. The size (CL) was measured from the posterior margin along the mid-dorsal line to the tip of the rostrum. Collections were made during daylight on the sandy beach. More than fifty crabs per collection were hand-picked and brought to the laboratory. They were kept in a 90 × 50 × 42 cm plastic tank with sufficient aeration, at a water depth of 6 cm with clean sand spread in slanting position. Sea water was changed every day, and the sand was changed once each week. These crabs were maintained at least one month for observation and study. The female crabs were identified by the occurrence of three pairs of pleopods.

Molt Cycle Stages.—Molt stages of the sand crab *E. asiatica* were determined based on microscopic observations of morphogenesis of setae and epidermal retraction following the methods of Drach (1939), Reaka (1975), Aiken (1980), Lyle and MacDonald (1983), and Waddy *et al.* (1995). The pleopods of the crabs under observation were plucked and wet-mounted on glass slides in clean filtered sea water and photographed on a Carl Zeiss ACE Axioplan microscope (Carl Zeiss, Germany) at a magnification of 200×.

Determination of Ovarian and Embryo Developmental Stages.—Ovarian developmental stages were determined by observing color changes in the ovary (Kerr, 1969; Wolin *et al.,* 1973) and by direct microscopical examination of the oocytes. The embryo development in the pleopods was classified according to Subramoniam (1991).

Ovarian Index (OI).—Ovarian index was calculated as ovary weight / body weight × 100.

Determination of Total Proteins.—The female crabs were washed thoroughly with filtered sea water. The hemolymph was collected by piercing the arthrodial membrane of the last appendage using a fine syringe. To quantify the hemolymph total protein, 10% trichloroacetic acid (TCA) was added and the protein precipitated. For determination of total protein in the ovary, the ovarian tissues were homogenized with 10% TCA in ice-cold condition and centrifuged at 6,000 rpm for 10 min. The protein precipitate was dissolved in 1N NaOH and quantified by the method of Lowry *et al.* (1951).

Statistical Analysis.—Data obtained on biochemical constituents of hemolymph during different molting and reproductive cycles were subjected to analysis of variance (ANOVA) following Zar (1984).

Results

The molt cycle stages have been determined in *E. asiatica* using the criteria of changes in the cuticular morphology, epidermal retraction, and setagenic events occurring in the pleopod. Four major stages—namely postmolt, intermolt, premolt, and ecdysis—have been distinguished and several substages under each category have been classified. The description of exoskeleton as well as the pleopods, along with the time duration of each molt cycle stage is summarised in Table 1.

Postmolt

Postmolt stage refers to the crab immediately after ecdysis. During this period the soft and pliable new cuticle undergoes hardening. Generally, the animal is inactive during this phase which lasts for 30 min; thereafter, it regains activity and burrows in the sand. The pleopod is soft and transparent. The setae are thin-walled, and their lumen is wide and prominent with a granular matrix filling up the space (Fig. 1a). This stage is divided into A_1, A_2 and B (Table 1).

Intermolt

As for many malacostracan crustaceans, intermolt stage is the longest of all molt cycle stages. In this stage, the exoskeleton has become progressively hard and calcified and hence further subdivision of this stage is difficult. However, the characteristic feature of intermolt stage is that the setal development in the pleopod has been completed (Fig. 1b). Nevertheless, the intermolt stage can be divided into three substages based on the hardness as

Table 1. Molt cycle characteristics of *Emerita asiatica*.

Molt stages	Duration of each stage	Characteristics of exoskeleton	Microscopical observation of the pleopodal changes
Post molt			
A_1	5–6 h	Freshly molted crabs; cuticle soft and pliable; crab not active; after 15–30 min, becomes active and burrows into sand, if molting is outside the burrow	Pleopod soft and transparent; setal shaft thin walled; setal lumen wide and filled with granular matrix; setal base evenly arranged on pleopods
A_2	24 h	Exoskeleton pliable and soft but begins to harden	No change in pleopod
B	4 d	Carapace continues to harden	Pleopod hard and rigid
Intermolt			
C_1	5 d	Exoskeleton remains hard; lateral side of the carapace depressed by finger pressure	Setal lumen becomes narrow; setal wall thickened; setal cone visible
C_2	4–5 d	Exoskeleton evenly hard throughout body surface	Setal cone prominent, a tube-like structure observed under setal articulation; epidermis condensed with setal articulation (node)
C_3	3–4 d	Carapace attains rigidity on dorsolateral sides	No changes in pleopod
Premolt			
D_0	3–4 d	No change in exoskeleton	Appearance of setal groove at base of pleopod; no epidermal retraction
D_0'		Same as stage D_0	Apolysis starts; narrow gap between old cuticle and epidermis evident; setal groove extends up to tip of pleopod
D_1	2–3 d	Exoskeleton becomes brittle	Retracted zone between old cuticle and epidermis widens; tip of new setae still within setal groove; new cuticle appears wavy
D_1'		No further changes in exoskeleton	New setae protrude into retracted zone
D_1''		Same as above	New cuticle clearly seen as a layer
D_2	3 d	Exoskeleton becomes more brittle; epidermis and secreted new cuticle appear as thick black layer on removal of old cuticle	Epidermal retraction continues; new setae clearly visible and thin walled; appearance of setal articulation at base of new seta
D_3	12–24 h	Carapace becomes thin and soft, cracks under pressure; exoskeletal color changes to pale grey from white	Setal articulation more prominent; new setae have extruded almost completely in the retracted area; setal lumen clearly seen within new setae
D_4	3–6 h	Animal inactive; appearance of suture at intersegmental membrane of carapace; ecdysis commences	Old setal exoskeleton completely separated from new setae
Ecdysis			
E_1	5–10 min	A crack occurs at intersegmental membrane of the posterior carapace; animal remains inactive and starts swelling up by absorption of water	Complete detachment of new pleopod from old exoskeleton
E_2	20–30 min	Ecdysial suture ruptures; carapace is lifted as animal swells more by ingestion of water; carapace and appendages pulled out and abdomen remains attached to old exoskeleton	New setae completely separated from old setae
Active phase I			
Active phase II	10–15 min	Entire animal comes out quickly	—

well as the rigidity of the exoskeleton both on the dorsal and lateral sides (see Table 1).

Premolt

This is the preparatory stage to ecdysis and includes several substages (D_0–D_4). This stage starts with apolysis, the retraction of epidermis from the cuticle, creating a molting space for the formation of new cuticle. The epidermal retraction followed by the secretion of new cuticle is easily seen in the pleopods. Hence, the pleopodal examination during premolt stage has yielded several substages characterising the extent of the epidermal retraction and the formation of new cuticle. Furthermore, there is a sequential change in the

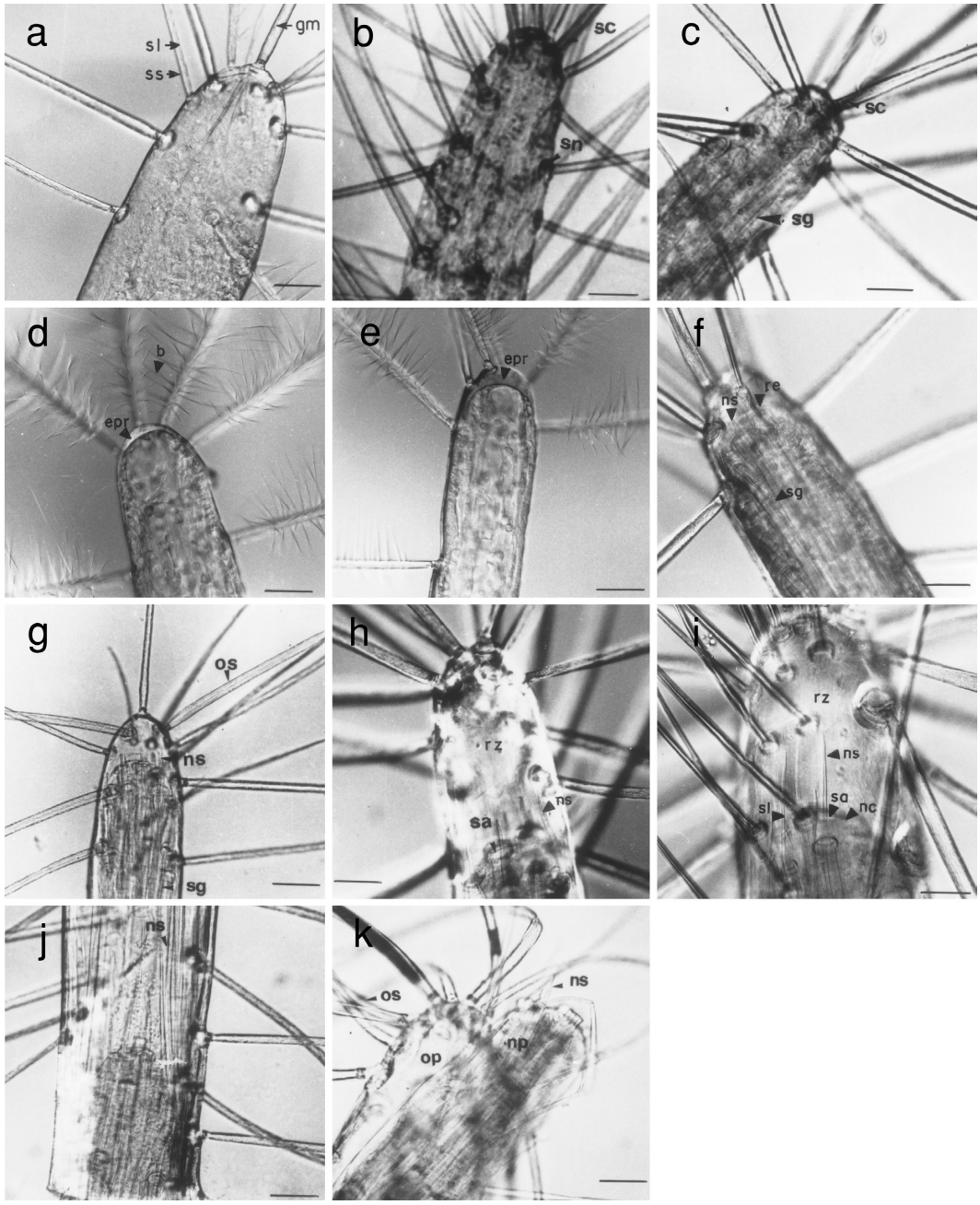

Fig. 1. Epidermal and setal changes in the pleopods of *Emerita asiatica*. a, stage AB, setal lumen (sl), granular matrix (gm); b, Stage C_3, setal cone (sc), setal node (sn); c, stage D_0, setal groove (sg); d, stage D_0', epidermal retraction (epr), barbules (b); e, stage D_1, epidermal retraction (epr); f, stage D_1', new setae (ns), retracted epidermis (re); g, stage D_1'', old setae (os), new setae (ns), setal groove (sg); h, stage D_2, setal articulation (sa); i, stage D_3, new cuticle (nc), setal articulation (sa), setal lumen (sl), new setae (ns), retracted zone (rz); j, stage D_4, new setae (ns); k, stage E, old setae (os), new setae (ns), old pleopod (op), new pleopod (np). Scale bars: a, 90 µm; b, 75 µm; c, 80 µm; d, 70 µm; e, 65 µm; f, 90 µm; g, 65 µm; h, 90 µm; i, 130 µm; j, 125 µm; k, 185 µm.

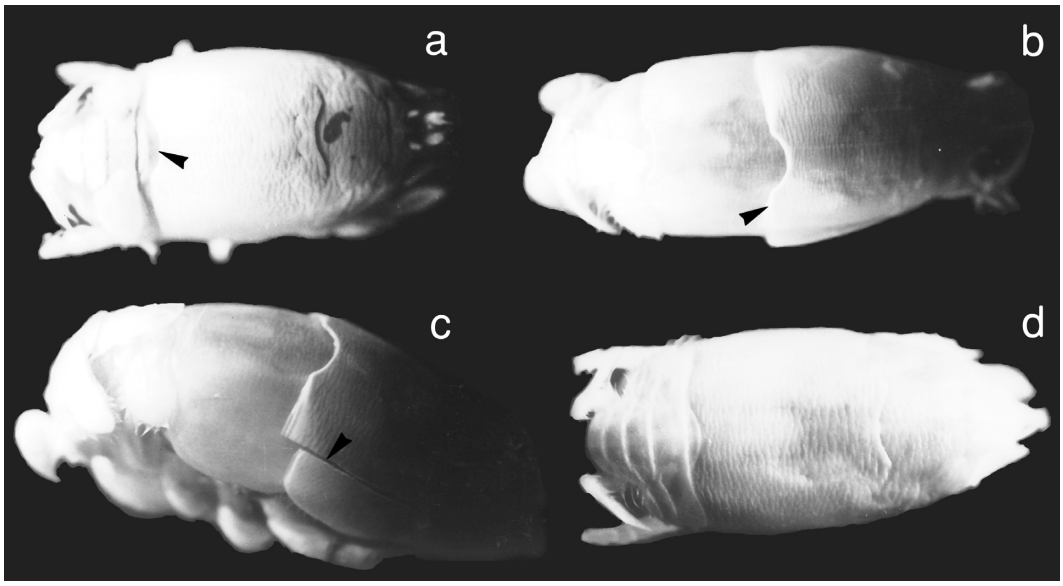

Fig. 2. Molting sequence in *E. asiatica*, a) showing the first phase of ecdysis in which the ecdysial suture is visible; b and c) animal has exposed part of its cephalothorax and abdominal region seen through the ecdysial suture (dorsal view); d) fully molted animal with soft exoskeleton.

development of new setae from the epidermal grooves (Fig. 1c–k, Table 1). It may be seen from the figures that a setal groove originates as a deep depression in the retracted epidermis in the pleopod. As the epidermal retraction continues with the formation of new cuticle, the new setae begin appearing from the base of setal grooves (Fig. 1f, g, h). In the following stages of premolt, the setal grooves get elevated pushing the internal setae to the outside of the groove. When the setal groove reaches the periphery of the epidermis, the new setae will be completely protruded out into the retracted zone (Fig.1i). The raised epidermis and the cuticle surrounding the new setae form the basis for setal articulation (Fig. 1h, i). Correlated to the internal changes in the setal development, the resorption of old cuticle occurs simultaneously. When the process is complete, the old cuticle becomes brittle, and at stage D_3 a gentle depression will result in the cracking of old cuticle (Fig. 2a). As the crack widens exposing the inner soft cuticle, water absorption through the soft cuticle begins resulting in the swelling up of the body cavity.

Ecdysis

During this stage the crab emerges through the ecdysial sutures that have been formed in the old cuticle (Fig. 2, Table 1). Due to endocuticular resorption, the old cuticle is thin and friable. The first ecdysial suture appears in the intersegmental membrane connecting cephalothorax and the abdomen. When flexed ventrally (Fig. 2a) the suture ruptures in a transverse direction allowing the animal to emerge (Fig. 2b, c). When the crab emerges, the old exoskeleton along with all appendages is intact. After emergence, the animal remains inactive for about 5–10 min (Fig. 2d). On no occasion was the molted crab found to consume the exuvium. The new cuticle continues to expand by water absorption, thereby increasing the body volume.

Relationship Between Reproductive and Molt Cycle

Emerita asiatica, on the East Coast of Peninsular India, breeds continuously and repetitively (Subramoniam, 1977, 1979). Like many crustaceans, *E. asiatica* broods the embryos in the pleopods. Ovarian development after spawning is concurrent with embryo development, and by the time the brood hatches, the ovary is almost ripe and ready for the next spawning. However, molting invariably occurs between hatching and spawning.

An analysis of molt cycle stages has revealed interesting data concerning the commencement of premolt stages during the reproductive cycle. The first sign of molting,

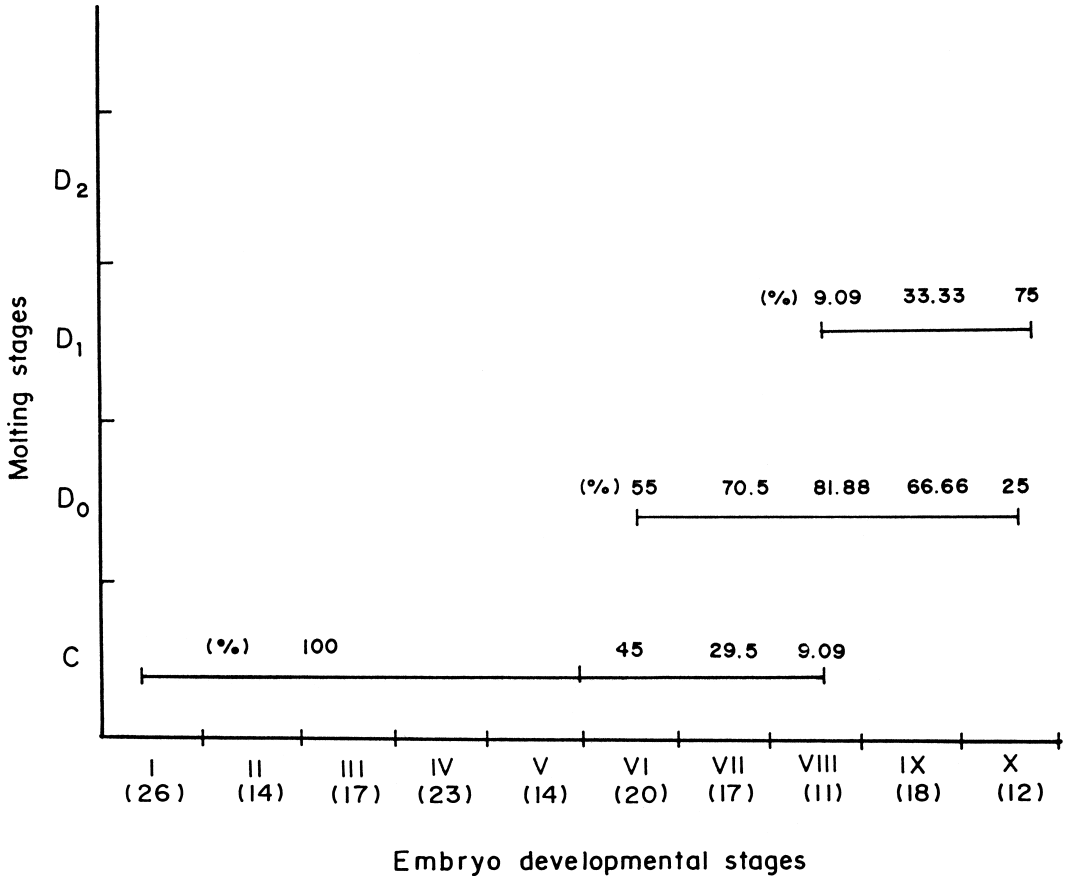

Fig. 3. Percentage occurrence of different molt cycle stages *versus* embryo developmental stages in the brooding females. Classifications of embryo developmental stages are according to Subramoniam (1991). Number of animals analysed in each stage is given in parentheses.

namely the retraction of the epidermis in the pleopod, is evident during stage VI of embryo development. Thereupon, the premolt advances up to D_1 stage at the time of hatching (stage X of embryo development). The percentage occurrence of premolt stages during the embryo developmental period is given in Fig. 3. It is clear that as the embryo development approaches hatching, no female is in the intermolt stage; but up to 75% of them have advanced to stage D_1, when the animal has passed through almost 50% of their premolt changes. After the release of the brood, the female crab quickly completes the remaining premolt stages and undergoes ecdysis. The next spawning invariably occurs during postmolt stage (B). The calculated percentage occurrence of premolt stages in the late embryo developmental stage facilitated the formulation of additional criteria for staging different molt cycle stages by simply observing the developing embryos in the pleopod using the criteria given by Subramoniam (1991). Thus, progressive changes in embryo development serve as a useful index to subdivide the intermolt stage into C_1–C_3.

Ovarian Index

The lowest ovarian index occurs soon after spawning (stage C_1)($1.06 \pm 0.26\%$). It increases sharply in the following stages of the intermolt, reaching the maximum value in stage D_0 ($4.12 \pm 0.59\%$) ($P < 0.001$). Thereafter, the ovarian index almost maintains the same level throughout the premolt stage. Microscopic observation of oocytes also reveals the completion of ovarian maturation within the intermolt stages. There seems to be a slight reduction in the ovarian index after ecdysis. However, the absolute weight of the ovary during the postmolt stage remains the same, suggesting that a decreased gonad in-

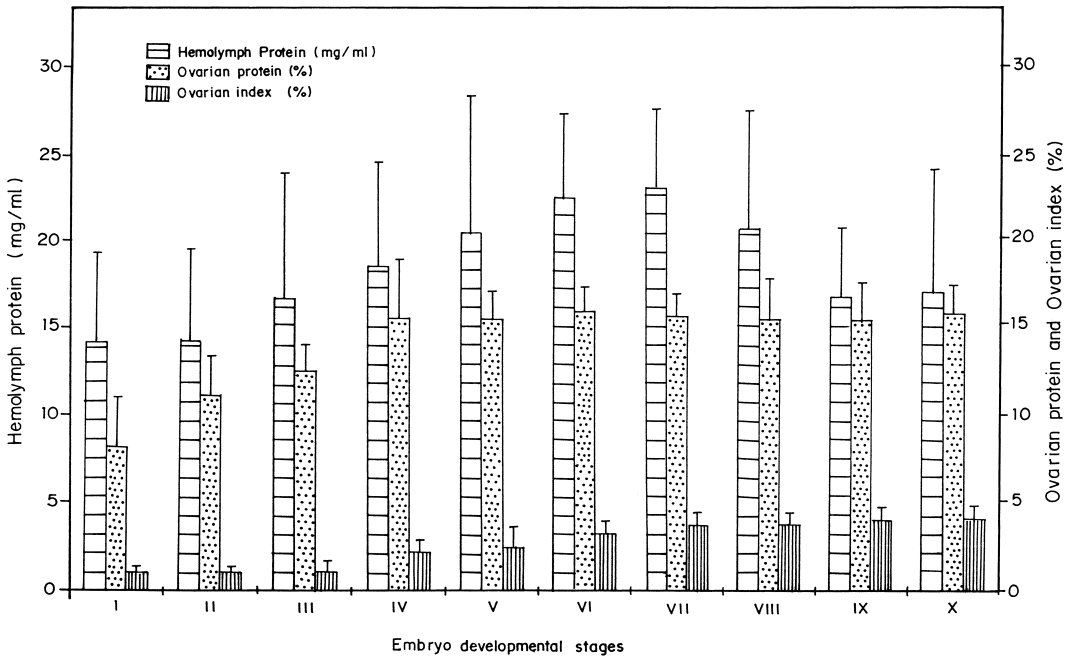

Fig. 4. Changes in the ovarian index (OI), percentage of ovarian protein, and hemolymph protein (mg/ml) in relation to different embryo developmental stages.

dex may be due to overall increase of body weight caused by enormous water intake during ecdysis. This data reveals a close synchrony between ovarian development and embryo development. Apparently, the process of ecdysis alone gives respite to the ovarian cycle. These results are given in Figs. 4 and 5.

Total Protein Changes in the Ovary During the Brooding and Molting Cycle

Protein, being the chief constituent of the ovary and the new cuticle, is expected to show variation during the reproductive and molting cycles. The protein level of the ovary is minimum in stage C_1 (8.18 ± 2.85%), but increases steadily during the subsequent intermolt stages, reaching highest value in stage D_0 (15.75 ± 1.44%) ($P < 0.001$). Thereupon, the protein level of the ovary is unchanged until next spawning (Fig. 5). Thereafter, the value fluctuates minimally without further increase within the premolt as well as postmolt stage. The non-linear increase of ovarian protein during the premolt and postmolt stage suggests the completion of yolk protein accumulation within the ovary by stage D_0. This trend in the ovarian protein during the intermolt, premolt, and postmolt is similar to that of the ovarian index. This is not unexpected, for the protein forms the major organic component of vitellogenic oocytes in crustaceans.

Changes in the Hemolymph Total Protein During Molting and Reproductive Cycles

Fluctuations in the total hemolymph protein during reproductive and molt cycles are summarised in Fig. 5 and Table 2. The hemolymph protein fluctuation has been determined for the female crabs in the continuously reproducing size-class (23–33-mm CL). In these crabs the hemolymph protein is at a low level soon after spawning in postmolt stage (AB) (5.23 ± 1.58 mg/ml). This is expected, for the animal has completed both vitellogenesis and molting. The hemolymph protein level steadily increases from stage C_1 (14.22 ± 5.20 mg/ml) to D_0 (22.47 ± 5.31 mg/ml) ($P < 0.001$). This increasing trend corresponds to the increase in the ovarian protein. However, the hemolymph protein exhibits a significant decline in stage D_1 (16.88 ± 4.83 mg/ml) ($P < 0.001$), which coincides with the last stage of pleopodal embryo development (IX and X) leading to the hatching of the larvae. Following this decline, the hemolymph protein level rises sharply to reach a significant maximum in stage D_2 (25.42 ± 3.26 mg/ml) ($P < 0.001$). However,

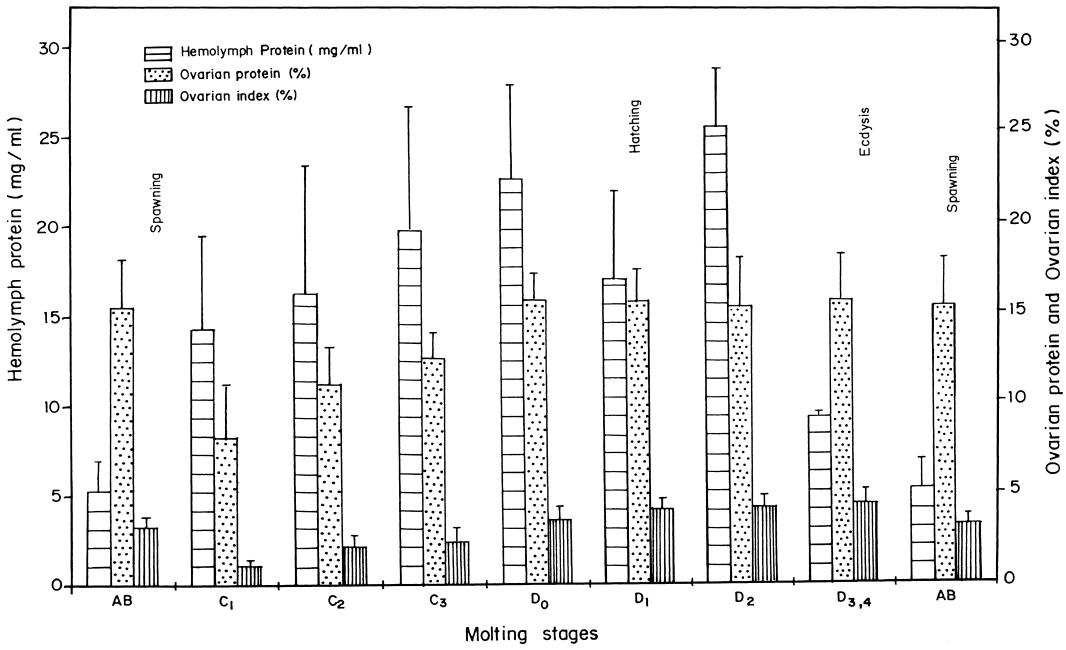

Fig. 5. Changes in the ovarian index (OI), percentage of ovarian protein and hemolymph protein (mg/ml) in relation to entire molt cycle stages (size class 23–33-mm carapace length (CL)).

the hemolymph protein once again declined very sharply in stage D_{3-4} (9.18 ± 0.322 mg/ml) as well as the postmolt stages (AB) ($P < 0.001$). The rise and fall of hemolymph protein during intermolt and premolt stages may be correlated to their use both in vitellogenesis and new cuticle formation.

The fluctuations of hemolymph protein in the size class of reproducing females may reflect their role in molting or vitellogenesis, as they occur synchronously. In order to determine the involvement of hemolymph protein in molting or ovarian development, two size classes, CL 10–17 mm and CL 18–22 mm, of females were used. These two groups correspond to completely immature and maturing for the first time, respectively (Fig. 6; Table 2). The data reveals that in the size class 10–17-mm CL, the hemolymph protein level shows a rising trend from postmolt stage to premolt stage. As there is no ovarian development in these animals, a rise in the hemolymph protein level during premolt stage is genuine and could be solely correlated to

Table 2. Hemolymph protein levels during the molt cycle stages *versus* different size classes (10–17-mm CL (carapace length), immature females; 18–22-mm CL, first maturing females; 23–33-mm CL, continuously reproducing females). Numbers within parentheses indicate the total number of crabs analysed within each stage.

		Reproductive stages Hemolymph Total Protein (mg/ml)		
Molt stages		10–17-mm CL Immature females	18–22-mm CL First maturation of the females	23–33-mm CL Continuously reproducing females
AB		0.5418 ± 0.2783 (3)	2.9865 ± 0.8726 (11)	5.2388 ± 1.5801 (5)
C	C_1			14.2266 ± 5.2020 (35)
	C_2	1.1451 ± 0.246 (3)	8.9593 ± 4.1198 (11)	16.2619 ± 7.0891 (14)
	C_3			19.6398 ± 6.9638 (18)
D_0		2.0705 ± 0.4949 (4)	16.6802 ± 1.1542 (19)	22.4768 ± 5.3111 (42)
D_1		2.2545 ± 0.6899 (6)	17.8527 ± 3.0275 (8)	16.8872 ± 4.8338 (13)
D_2		3.0418 ± 1.0626 (6)	18.7403 ± 4.0847 (16)	25.4245 ± 3.2675 (14)
D_{3-4}		0.7438 ± 0.1425 (4)	3.0367 ± 2.0376 (6)	9.1816 ± 0.3221 (3)

*Mean ± SD.

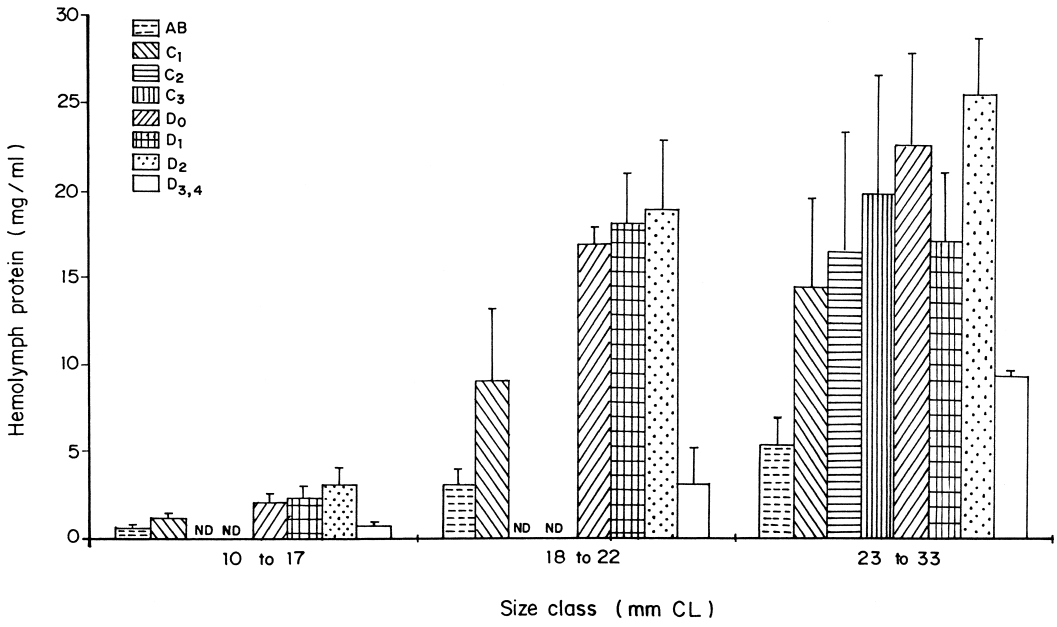

Fig. 6. Changes in the hemolymph protein level during the molt cycle stages of three groups of animals, immature 10–17-mm CL; first maturation females 18–22-mm CL; continuously reproducing females 23–33-mm CL to indicate specific rise in the hemolymph protein during the premolt stage. ND, Not determined due to absence of stages in the size classes 10–17-mm and 18–22-mm CL.

molting. A similar trend in the hemolymph protein is also observed for the females in the size range 18–22-mm CL. In these animals, four stages in the ovarian development occur sequentially during their growth from 18-mm to 22-mm CL. In this group of crabs, the lowest value is obtained in the postmolt stage. From this, the hemolymph protein level increases sharply in the intermolt stage followed by a further stepwise rise in the premolt stage. Among the premolt substages, there is a gradual and steady increase from D_0 to D_2 stages; however, this value declines precipitously in the D_{3-4} stage. This trend is almost similar to that of the fully mature females belonging to the size class of 23–33-mm CL, excepting that there is no decline in the hemolymph protein value in stage D_1.

Discussion

Molting is an integral process in the life cycle of all arthropods. In many crustaceans, it occurs continuously all through the developmental cycle. The success of crustaceans to carry on molting process even in adult life when reproduction becomes the primary physiological activity results in enormous body growth especially in decapods such as lobsters, crabs, and crayfishes. Nevertheless, molting in crustaceans imposes enormous physical stress to the animals inasmuch as their exoskeleton is highly mineralised. Furthermore, the formation of new cuticle at each molt is a highly energy-demanding process, entailing mobilisation of cuticular precursor molecules from the storage organ such as hepatopancreas. A similar phenomenon of energy extraction from the storage organs in the adult crustacean is vitellogenesis, which involves translocation of specific precursor molecules during egg maturation. Crustacea have evolved to accomplish these two processes by either temporally separating the two events or linking up the metabolic machinery to cope with the simultaneous synthesis and utilisation of organic reserves (Subramoniam, 2000).

Species of the sand crab *Emerita* enjoy world-wide distribution. They are typical burrowing crustaceans in the sandy intertidal zone. *Emerita* species exhibit remarkable adaptive features to colonise this precarious marine environment by virtue of their fast body growth and high fecundity. The present data demonstrate how closely the reproductive and molting processes are synchronised

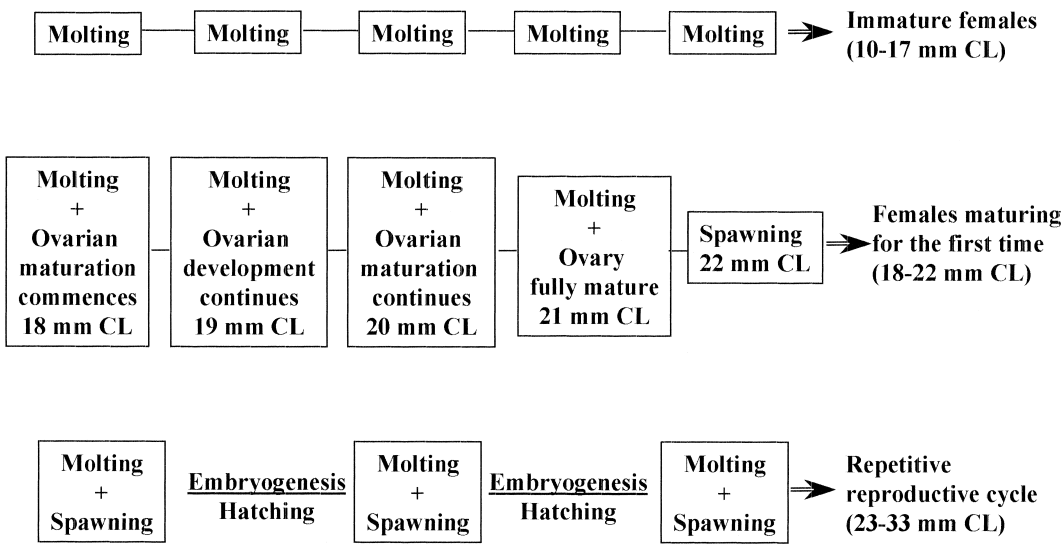

Fig. 7. Molting and reproductive cycle of *Emerita asiatica.*

in *E. asiatica.* For a clear understanding of the relationship between molting and vitellogenic cycle in *E. asiatica*, determination of molt cycle stages with their respective substages becomes an important prerequisite. Observed pleopodal changes characterising molt cycle stages were found to be profitable for many crustacean species (Aiken, 1980; Skinner, 1985). In the present study, we have combined three major criteria—namely, changes in the texture of the exoskeleton, retraction of epidermal layer, and the setagenesis in the pleopod—for precisely resolving different premolt stages. The setal groove formation along with epidermal retraction is the first sign of premolt initiation in *E. asiatica*. Various stages in the setal development exemplify the process of new cuticle formation during the premolt stage in this crab. The effective delineation of different molt cycle stages is useful in the determination of premolt stages in the brooding females. These observations have yielded interesting data on the interrelationship between molting and reproductive processes.

Among the decapods, the carideans tend to synchronise the major reproductive events with those of the molt cycle, but the usual reptant does the opposite (Adiyodi and Subramoniam, 1983; Nelson, 1991). In these groups, it has been shown that the ecdysis always commences after the release of young ones, suggesting a possible antagonism between brooding and the onset of molting. However, our present observation on premolt changes in the pleopod has revealed that the initiation of molting process, such as the apolysis, invariably starts almost midway through the embryo development in the brood. The premolt changes also continue up to stage D_1, when the new cuticle is laid down over the retracted epidermal layer, at the time of larval hatching. Evidently, the time interval between larval release and the ensuing ecdysis is greatly shortened, thereby promoting quicker molting frequencies. Furthermore, this observation on the initiation of premolt even when the embryos are developing on the pleopod points to the possibility that there is no real antagonism between embryo-carrying on the pleopods and molting. Understandably, the hatching of the brood takes precedence over ecdysis, lest the brood be lost along with the exuviae.

In a recent study, we have reported that the free ecdysteroid level in the developing embryos of *E. asiatica* peaks during the prehatching stage, suggesting its role in the embryonic cuticular formation and hatching of the zoea larvae (Subramoniam *et al.,* 1999). Interestingly, molting and naupliar release from the brood occurs concurrently under a common influencing factor in the cirripedes (Crisp *et al.,* 1991). Ecdysteroids being the molting hormones both for embryonic and adult ecdyses (Chang, 1992), it is tempting to

suggest that this rise in the free ecdysteroid level might have a confounding effect on molt and reproductive cycles, as they occur synchronously in *E. asiatica*. Such a type of synchrony between the breeding and molting cycle may further suggest the compatibility of both cuticle formation and vitellogenesis by deriving precursor materials from a common storage organ such as the hepatopancreas. The hepatopancreas has been shown to be a central organ to provide precursor material for cuticular formation in *E. asiatica* (see Parvathy, 1967). Recent evidence also suggests its definitive role in providing precursor materials for yolk protein synthesis in several decapods (Rani and Subramoniam, 1997; Soroka *et al.,* 2000; Yang *et al.,* 2000).

Emerita asiatica on the Madras coast has been shown to breed continuously and repetitively (Subramoniam, 1977, 1979). Data reported in the present study adduce further evidence that the ovarian development starts soon after spawning. The ovarian index and the protein content of the ovary rise sharply from C_1 to D_0 stage, suggesting that vitellogenesis is almost completed within the intermolt stage. Both values are maintained at the same level during premolt and postmolt until next spawning. Analysis of the hemolymph protein has revealed interesting data pertaining to energy utilization from storage sites during ovarian development and molting. The hemolymph protein is low after spawning in the postmolt stage but rises steadily through the intermolt stages, when the ovary accumulates yolk materials and completes maturation. By the onset of premolt stage, there is another rise in hemolymph protein, suggesting a role in new cuticle synthesis and/or in the continued ovarian maturation. However, there is a dip in hemolymph protein level during stage D_1, corresponding to hatching of larvae. This decline is temporary, and the hemolymph protein level rises again during D_2 stage only to fall precipitously in the following $D_{3–4}$ and early postmolt stages. This fall in hemolymph protein may be a result of the enormous dilution of hemolymph due to water influx through the soft new cuticle (Skinner, 1985; Chang, 1992). Water and ionic uptake through the soft cuticle during D_4 stage have been shown to be under a coordinated release of neurohormones such as crustacean hyperglycemic hormone and crustacean cardioactive peptides in the crab *Carcinus maenas* (Chung *et al.,* 1999; Phlippen *et al.,* 2000). The animals during stages $D_{3–4}$ do not feed, contributing further to the depletion of hemolymph protein.

The rise of hemolymph protein both during intermolt and premolt has been implicated for other crustaceans, such as *Macrobrachium nipponense* and *Macrobrachium rosenbergii*, with overlapping reproductive and molting processes (Okumura *et al.,* 1992; Okumura and Aida, 2000). Here, the level of vitellogenin as measured by ELISA techniques has clearly indicated a rise in the premolt stage D_1. Although vitellogenin as measured by ELISA technique was not carried out, electrophoretic investigation by Tirumalai and Subramoniam (1992) characterised the vitellogenin fraction in *Emerita asiatica.* In the present study, we followed the occurrence of vitellogenin throughout the molting and reproductive cycle and found it to be present in all stages except in the post molt stage (A, B). The concentration of the vitellogenin fraction during the premolt stage is also high, suggesting continuous production and release of vitellogenin into the hemolymph.

Our results on the hemolymph protein of both immature females and females in the first maturation show a sharp rise during early premolt stages (D_0–D_2), similar to that of larger, actively reproducing females. In the immature females, the hemolymph protein rise is expected to be solely due to molting as the ovarian development has not yet commenced. These females do not contain any vitellogenin fraction in the hemolymph (unpublished observation), suggesting that there is no synthesis of yolk precursor protein in the extra ovarian sites. The fluctuation of hemolymph protein in the first maturing females (18–22-mm CL) is also similar to that of the actively reproducing females (23–33-mm CL) except that there is no decline in the protein level during stage D_1. All this might indicate that the premolt rise of hemolymph protein is primarily due to cuticular synthesis, although vitellogenin synthesis might also continue to a certain extent, as revealed by the existence of vitellogenin in the hemolymph.

In the decapod crustaceans, the integumentary tissues (epidermis and cuticle) underlying the exoskeleton synthesise cuticular proteins both during premolt and postmolt periods (Skinner *et al.,* 1992). Hence, as indicated in the present study, protein derivation

from the hemolymph during premolt may mean that these proteins are degraded into amino acids by specific proteolysis in the integumentary tissues in order to facilitate resynthesis of cuticular protein. This condition is in striking contrast to that of vitellogenesis, where a presynthesized hemolymph protein is sequestered into the ovary for deposition as lipovitellin (Adiyodi and Subramoniam, 1983).

Comparison of the molting and reproductive pattern of *Macrobrachium* and *Emerita* is instructive in this context. In *Macrobrachium*, two types of molting have been reported: (1) a reproductive molt cycle in which ovarian development and spawning occur alternately, and (2) a non-reproductive molt cycle, when there is no concurrent reproductive activities following the molting cycle (Okumura *et al.,* 1992). Furthermore, embryogenesis in the brood carried by the females and the vitellogenesis in its ovary overlap under optimal conditions (O' Donovan *et al.,* 1984). In such cases hatching is followed by molting and spawning. In *Emerita asiatica,* the repetitively breeding female population always exhibits concurrent molting and reproductive activities without any spatial separation. As shown in Fig. 7, three types of molting pattern could be distinguished in *Emerita asiatica*. In the immature females, body growth occurs through repeated molting, whereas when sexual maturity commences, there is intervention of the reproductive cycle with molting. Ovarian maturation commences in the females at 18-mm CL; however, ovarian development is slow, and another two molts occur before ovarian maturation is completed at 21-mm CL and spawning occurs after the female next molts to 22-mm CL. Then onwards (i.e., 23- to 33-mm CL), the female enters into repetitive reproductive and molting cycle in which there is total synergism in molting and reproductive events (see Fig. 7). Our unpublished observations of the molting of both the immature and mature females also show the continuous occurrence of molting throughout the year (1998–1999).

It may be summarised from the present study that the synchronized reproductive and molting events are accomplished through an efficient metabolic machinery supplying nutrient reserves both for molting and reproduction. Filter-feeding in *Emerita* species, coupled with the continuous availability of detrital food in the intertidal environment are favourable for setting up a nutritional status to ensure successful reproduction and molting throughout the year. The hormonal factors coordinating the molting and reproductive processes are without visible antagonism but are only secondary to the nutritional factors controlling these two energy-demanding processes.

ACKNOWLEDGEMENTS

Authors are thankful to the University Grants Commission, New Delhi, for the financial assistance through grants No. F.3-28/96(SR-II) to TS and F.No.3-16/94/SAP-II to the Department of Zoology. We are grateful to Prof. G. Anilkumar for his suggestions and useful discussion. Also thanks are due to Mr. R. Arun for his assistance in statistical analysis.

LITERATURE CITED

Adiyodi, R. G., and T. Subramoniam. 1983. Arthropoda-Crustacea. Pp. 443–495 *in* K. G. Adiyodi and R. G. Adiyodi, eds. Reproductive Biology of Invertebrates. Vol. 1. Oogenesis, Oviposition and Oosorption. John Wiley and Sons, New York.

Aiken, D. E. 1980. Molting and growth. Pp. 91–163 *in* J. S. Cobb and B. F. Phillips, eds. The Biology and Management of Lobsters. Vol. 1. Academic Press, New York.

Chang, E. S. 1992. Endocrinology. Pp. 53–91 *in* A. W. Fast and L. J. Lester, eds. Marine Shrimp Culture: Principles and Practices. Elsevier Science Publishers B.V., Amsterdam.

Chung, J. S., H. Dirckson, and S. G. Webster. 1999. A remarkable, precisely timed release of hyperglycemic hormone from endocrine cells in the gut is associated with ecdysis in the crab *Carcinus maenas*.—Proceedings of the National Academy of Sciences, USA 96: 13103–13107.

Crisp, D. J., E. M. Hill, and D. L. Holland. 1991. A review of the hatching process in barnacles. Pp. 57–68 *in* A. M.Wenner and A. M. Kuris, eds. Crustacean Egg Production. Crustacean Issues. Vol. 7. Balkema Press, Rotterdam.

Drach, P. 1939. Mue et cycle d'intermue chez les cructacés décapodes.—Annales de L'Institute Oceanographique, Monaco 19: 103–391.

Giese, A. C., and J. S. Pearse. 1974. Reproduction of marine invertebrates. Pp. 1–49 *in* A. C. Giese and J. S. Pearse, eds. Acoelomate and Pseudocoelomate Metazoans. Vol. 1. Academic Press, New York.

Goodbody, I. 1965. Continuous breeding in populations of tropical crustaceans, *Mysidium columbiae* (Zimmer) and *Emerita portoricensis* Schmitt.—Ecology 46: 195–197.

Kerr, M. S. 1969. The hemolymph proteins of the blue crab, *Callinectes sapidus*-II A lipoprotein serologically identical to oocyte lipovitellin.—Developmental Biology 20: 1–17.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent.—Journal of Biological Chemistry 193: 265–275.

Lyle, W. G., and C. D. MacDonald. 1983. Molt stage determination in the Hawaiian spiny lobster *Panulirus marginatus*.—Journal of Crustacean Biology 3: 208–216.

Nelson, K. 1991. Scheduling of reproduction in relation to molting and growth in malacostracan crustaceans. Pp. 77–113 *in* A. M. Wenner and A. M. Kuris, eds. Crustacean Egg Production. Crustacean Issues. Vol. 7. Balkema Press, Rotterdam.

O'Donovan, P., M. Abraham, and D. Cohen. 1984. The ovarian cycle during the intermolt in ovigerous *Macrobrachium rosenbergii*.—Aquaculture 36: 347–358.

Okumura, T., C. H. Han, Y. Suzuki, K. Aida, and I. Hanyu. 1992. Changes in hemolymph vitellogenin and ecdysteroid levels during the reproductive and non-reproductive molt cycles in the fresh water prawn *Macrobrachium nipponense*.— Zoological Science 9: 37–45.

———, and K. Aida. 2000. Hemolymph vitellogenin levels and ovarian development during the reproductive and non reproductive molt cycles in the giant freshwater *Macrobrachium rosenbergii*.—Fish. Sci. 66: 678–685.

Parvathy, K. 1967. Studies on blood in relation to molting in some arthropods.—Ph. D. Thesis submitted to the University of Madras, Chennai, India. [Unpublished.]

Phlippen, M. K., S. G. Webster, S. Chung, and H. Dircksen. 2000. Ecdysis of decapod crustaceans is associated with a dramatic release of crustacean cardioactive peptide into the hemolymph.—Journal of Experimental Biology 203: 521–536.

Rani, K., and T. Subramoniam. 1997. Vitellogenesis in the mud crab, *Scylla serrata*—an in vivo isotope study.—Journal of Crustacean Biology 17: 659–665.

Reaka, M. L. 1975. Molting in stomatopod crustaceans, 1. Stages of the molt cycle, setagenesis, and morphology.—Journal of Morphology 146: 55–80.

Skinner, D. M. 1985. Molting and regeneration. Pp. 43–146 *in* D. E. Bliss and L. H. Mantel, eds. The Biology of Crustacea. Vol. 9. Academic Press, New York.

———, S. S. Kumari, and J. J. O'Brien. 1992. Proteins of the crustacean exoskeleton.—American Zoologist 32: 470–484.

Soroka, Y., Y. Milner, and A. Sagi. 2000. The hepatopancreas as a site of yolk protein synthesis in the prawn *Macrobrachium rosenbergii*.—Invertebrate Reproduction and Development 37: 61–68.

Subramoniam, T. 1977. Continuous breeding in the tropical anomuran crab, *Emerita asiatica* (Milne Edwards) from Madras Coast. Pp. 166–174 *in* K. G. Adiyodi and R. G. Adiyodi, eds. Advances in Invertebrate Reproduction. Vol. I. Peralam-Kenoth, Karivellur, Kerala, India.

———. 1979. Some aspects of reproductive ecology of a mole crab, *Emerita asiatica* (Milne Edwards).—Journal of Experimental Marine Biology and Ecology 36: 259–268.

———. 1991. Yolk utilization and esterase activity in the mole crab, *Emerita asiatica* (Milne Edwards). Pp. 19–29 *in* A. M. Wenner and A. M. Kuris, eds. Crustacean Egg Production. Crustacean Issues. Vol. 7. Balkema Press, Rotterdam.

———, R. Tirumalai, V. Gunamalai, and K. H. Hoffmann. 1999. Embryonic ecdysteroids in a mole crab, *Emerita asiatica* (Milne Edwards).— Journal of Bioscience 24: 91–96.

———. 2000. Crustacean ecdysteroids in reproduction and embryogenesis.—Comparative Biochemistry and Physiology C 125: 135–156.

Tirumalai, R., and T. Subramoniam. 1992. Purification and characterisation of vitellogenin and lipovitellins of the sand crab *Emerita asiatica:* molecular aspects of crab yolk proteins.—Molecular Reproduction and Development 33:16–26.

Waddy, S. L., D. E. Aiken, and D. P. V. DeKleijn. 1995. Control of growth and reproduction. Pp. 217–266 *in* J. R. Factor, ed. Biology of the Lobster (*Homarus americanus*). Academic Press, Inc., New York.

Wolin, E. M., H. Laufer, and D. F. Albertini. 1973. Uptake of the yolk protein, lipovitellin, by developing crustacean oocytes.—Developmental Biology 35: 160–171.

Yang, W. J., T. Ohira, N. Tsutsui, T. Subramoniam, D. T. T. Huong, K. Aida, and M. N. Wilder. 2000. Determination of amino acid sequence and site of mRNA expression of four vitellins in the giant freshwater prawn *Macrobrachium rosenbergii*.—Journal of Experimental Zoology 287: 413–422

Zar, J. H. 1984. Biostatistical Analysis. Prentice Hall Inc., New Jersey.

RECEIVED: 11 December 2000.
ACCEPTED: 26 June 2001.