Chapter I

REPRODUCTIVE PHYSIOLOGY OF FEMALE CRUSTACEANS:

A REVIEW
1.1. INTRODUCTION

Crustaceans are highly successful arthropods adapting to different kinds of aquatic environments and hold a position comparable to that held by insects on land. Unlike insects, most crustaceans continue to grow and moult even after attaining puberty. Crustaceans show slow breeding activity which in turn prompts them to accommodate somatic and ovarian growth either in some temporal succession as in higher Decapoda or in an overlapping fashion as in Amphipoda.

Even though bisexuality is the mode of reproduction among most crustaceans, other modes such as protandric hermaphroditism, gynandromorphism and parthenogenesis are also observed in several species. The isopod, *Excirolana chiltoni* is ovoviviparous with intra uterine development (Klapow, 1972). Sexual dimorphism is reported from the shrimp, *Upogebia stellata* (Eunice et al., 2001) and from fiddler crabs (Litulo, 2004a; Koch et al., 2005). On the other hand, in male spider crabs, *Libinia emerginata*, polymorphism is the rule (Hartnoll, 1985).

Previous investigations reveal that, in crustaceans, reproductive and survival strategies vary from species to species (Da Silva-Castiglioni and Negreiros-Fransozo, 2006; Da Silva-Castiglioni et al., 2007; Figueiredo et al., 2008; Costa and Soares- Gomes, 2009). Even among closely related groups, there is hardly any uniformity in duration of development or the stage at which the young ones are liberated. Further, the reproductive biology and breeding pattern of various
crustacean groups are reported to be quite different. For instance, many species breed continuously, while some are annual breeders. Some crustaceans require long duration to complete a reproductive cycle. Some species mate only at ecdysis, when their exoskeleton is soft; some others mate only during intermoult, while the exoskeleton is hard.

Reproduction and moult in crustaceans are programmed to a great extent in accordance with the cues received from the diverse environmental factors such as availability of food, water, temperature, salinity, lunar phases and tidal cycles (Da Silva-Castiglioni and Negreiros-Fransozo, 2006). In majority of crustaceans, seasonal shifts are known to influence programming of growth and reproduction. Environmental signals mediated through the central nervous system regulate physiological and morphological changes associated with growth and reproduction; as a result, these physiological events are restricted to specific period of the year (Adiyodi and Adiyodi, 1970).

Generally, species confined to the tropical regions and having access to perennial sources of water have prolonged breeding seasons that may extend for several months. Monsoon rains apparently affect the breeding pattern of estuarine decapods in Peninsular India (Pillay and Nair, 1971; Sudha, 1992) as changes in salinity affect the survival of their larva. The breeding season of the crabs (such as *Uca annulipes*, *Sesarma quadratum* and *Hyoplax gangetic*) inhabiting the coast of South Western India extends from August to the succeeding April (Pillay and Nair, 1973; Syama et al., 2010).

1.2. **Growth and reproduction in crustaceans-seasonal programming:**
Unlike insects, reproductive physiology of adult crustaceans is significantly influenced by moulting (Adiyodi and Adiyodi, 1970). Being the two high energy demanding processes, the relationship between moulting and reproduction has great significance in the physiology of organisms inasmuch as these physiological processes demand an effective and balanced mobilization of reserves from storage tissues. The balanced and integrated somatic and reproductive growth is accomplished mainly through different patterns of stimulatory and inhibitory hormonal interactions in various groups of crustaceans (Adiyodi, 1985; Charniaux-Cotton and Payen, 1988; Meusy and Payen, 1988; Laufer et al., 1993, 2002; Van Herp and Soyez, 1997 for review; Wilder et al., 2002; Diwan, 2005; Raviv et al., 2006). Hence, interaction between growth and reproduction is a topic gaining more attention among the investigators world over, both from the standpoint of academic interest and in view of its direct application in optimizing aquaculture. Many investigators (Anilkumar, 1980; Dugan and Hubbards, 1996, 2000; Sudha, 1992; Lardies and Castilla, 2001; Syama, 2009) studied the relationship between moult and reproductive cycles in crustaceans and revealed that there exists significant differences in the design of growth and reproduction of several crustacean groups. Moulting and ovarian growth appear as antagonistically programmed functions in several brachyuran crabs such as Carcinus maenas (Demeusy, 1965; Styrishave et al., 2004, 2008), Gecarcinus lateralis (Dorothy, 1966), Menippe mercenaria (Cheung, 1969;), Paratelphusa hydrodromous (Anilkumar, 1980), Macropipus pubes (Gonzales-Gurriaran, 1985); and S. quadratum (Syama, 2009). Metopograpsus messor, an intertidal crab, displays partial synergism between reproduction and moult. Like other brachyurans, the wild
population of *M. messor*, exhibits high degree of antagonism between premoult and reproduction; in nature, premoult females do not engage in reproduction until ecdysis (Sudha and Anilkumar, 1996). However, vitellogenic activity resumes soon after the completion of early postmoult recuperation and as the individuals attain intermoult, the ovaries are sufficiently matured to ensue spawning. From the available information, it is apparent that, there exists a co-ordination in the temporal arrangement of moult and reproduction in a given crustacean species, and seemingly these events are interdependent. *Euphausia superba*, however, displays an unique pattern of the programming of moult and reproduction; the occurrence of spawning in all stages of the moult cycle is a common phenomena in this Antartic krill (Stephen, 1989).

Synchronous pattern of vitellogenic and moulting cycle occurs in palaemonid shrimps such as *Macrobrachium nobilii* (Pandian and Balasundaram, 1982), *M. idella* (Vijaya, 1989) and *M. rosenbergii* (Okumura et al., 1992; Okumura and Aida, 2000), where vitellogenesis progresses paralleled with active proecdysial events. In these species, the intermoult period is comparatively too short with that of premoult. In *M. rosenbergii*, during the reproductive-moult cycle, the ovary develops synchronously along with the advancement of moulting stages (Okumura and Aida, 2001). In the Mediterranean deep water shrimp, *Aristeus antennatus*, reproductive growth is reported to be synchronous with moulting (Demestre, 1995). In adult *Daphnia magna*, ovarian activity is cyclic and closely entrained with moulting. Each reproductive cycle in this species starts with shedding of the old exoskeleton and the release of new eggs from the ovaries into the brood chamber (Creuzburg et al., 2007). Apart from natantian decapods and amphipods (Legrand and Juchault, 1972), anomuran crabs
(Gunamalai and Subramoniam, 2002) also exhibit overlapping reproductive and moulting activities, thereby allowing both new cuticle synthesis and ovarian maturation to occur synchronously.

Surveying through the literature, it is evident that decapod crustaceans exhibit great diversity with respect to the number of broods released per year and fecundity. Most of the natantians are continuous breeders. For example, *Gonodactylus bredini* (Steven and Joseph, 1987) and caridean shrimp, *Palaemon macrodactylus* (Michio and Chida, 1988; Bauer, 1989) and the sand shrimp, *Crangon affinis* (Hong and Oh, 1989) release as many as five broods per year.

Occurrence of continuous breeding has also been reported in many of the brachyuran crabs especially among those inhabiting the tropical waters (Negreiros-Fransozo et al., 2002; Figueiredo et al., 2008; Da Silva-Castiglioni et al., 2007). Some of the multiparous crabs are known to release upto 12 broods annually (Kotb and Hartnoll, 2002; Costa and Soares-Gomes, 2009). Previous studies from our laboratory have shown that, *M. messor* and *S. quadratum*, members of the grapsid family, release more than 14 broods a year (Sudha and Anilkumar, 1996; Syama et al., 2010). Multiple spawning serves to increase the reproductive output of the species especially during favourable environmental conditions (Adiyodi, 1985 for review). Contrary to the practice of multiple broods, one brood per year appears to be the rule in some of the brachyurans such as the Calicut population of the field crab, *Paratelphusa hydrodromous* (Anilkumar and Adiyodi, 1980), *Gecarcinus steniops* (Santhamma, 1985) and *Cancer* sp. (Hines, 1991; Shields, 1991). Biennial spawning is known to occur
among macruran (Aiken and Waddy, 1980) and anomuran decapods (Jensen and David, 1989).

Reviewing the literature, it is apparent that, in most crustaceans, reproduction and growth are confined to different seasons. In the grapsid crab, *Plagusia dentipes*, ovigerous females occur in October-December (Tsuchida and Watanabe, 1997). In *M. messor* and *S. quadratum* (grapsid crabs), the reproductive and growth seasons are extended from August to the succeeding May, the peak season for reproduction being August-December; March-April is the season for peak somatic growth (Sudha and Anilkumar, 1996; Syama, 2009; Syama et al., 2010). Seasonal programming of growth and reproduction is also reported in other brachyuran crabs like, *P. hydrodromous* (Anilkumar and Adiyodi, 1980), and the shrimps, *Callianassa kraussi* (Forbes, 1977) and *Palaemon gravieri* (Kim and Hong, 2004) and *P. setifer* (Kim, 2008). Breeding period in *C. kraussi* appears to be in winter-spring (May-July/August); restricted breeding activity has been recorded also during summer (November-January) (Forbes, 1977). Year round breeding activity with peaks of spawning in summer is reported in the fiddler crabs, *U. annulipes* and *U. inversa* (Litulo, 2004b), in the hairy crab, *Pilumnus vespertilio* (Litulo, 2005) and in the red frog crab, *Ranina ranina* (Krajangdara and Watanabe, 2005). Continuous breeding activity with peak season during spring and autumn has been reported in *Uca rapax* (Costa and Soares-Gomes, 2009). However, no specific spawning season was reported in *Portunus sanguinolentus*, the first spawning takes place about 60 days after ecdysis followed by two more spawning at 30-40 days intervals during the same intermoult period (Ryan, 1967).
1.3. Oviposition:

Oviposition or spawning is a timely programmed and environmentally correlated process; it takes place when conditions are favorable for embryonic development and their subsequent hatching of larvae or the juveniles. Available literature showed that, oviposition is either seasonal or continuous. In *Libinia emerginata*, the first oviposition takes place in late May or early June followed by another spawning during early September (Hinsch, 1972). Occurrence of seasonal oviposition during March was reported in Calicut population of *P. hydrodromous* and liberation of the juveniles generally coincides with the onset of monsoon (Adiyodi, 1985 for review). In the shore crab, *Menippe mercenaria*, spawning period is extended from August to September (Cheung, 1969). Spawning period is June to August in *Rithropanopeus harrisii* (Bomirski and Klek, 1974) and January and May in the crab, *Ucides cordatus* (Mota-Alves, 1975). In the grapsid crabs, *M. messor* and *S. quadratum*, oviposition occurs throughout the period from August-May (Sudha and Anilkumar, 1996; Syama, 2009). Warm water and long photoperiod together induce oviposition in *Orconectes virilis*; a change in either of these factors could inhibit oviposition (Aiken, 1969). Synchronization of oviposition with specific phases has been reported in the stomatopod *Gonodactylus zacae* and *G. falcatus*. In both the species, oviposition occurs during neap tides or at the transition from spring to neap tides. In *G. graphurus*, oviposition occurs during spring tides (Reaka, 1976).

1.4. Moulting:
Moulting in Crustacea appears as a part of mechanism of growth. The crustacean body is ensheathed by a rigid cuticular exoskeleton and their periodic growth or moulting forms the most important metabolic event, which dominates the life cycle of crustaceans (Highnam and Hill, 1969). During moulting, the crustaceans show many cyclic changes in structure, physiology and biochemistry, leading to growth (Passano, 1960). The moult cycle is broadly divided into four stages; they are (1) proecdysis or premoult: preparatory to moulting; (2) ecdysis or moulting: which means shedding of old cuticle; (3) metecdysis or postmoult: stage immediately after ecdysis denoted by mineral deposition and protein synthesis and (4) anecdysis or intermoult when normal growth takes place (Carlisle, 1953; Kuballa and Elizur, 2008).

In *M. messor*, during preparation of ecdysis (D1), the new cuticle, begins to appear and it is more pronounced during D2; at stage D3, juvenile setae become clearly visible and the D4 stage, in this species is characterized by the partial extrusion of the juvenile setae from their grooves; complete extrusion of the juvenile setae occurs only during the process of exuviation (Sudha, 1992). Contrary to this pattern, in the Hawaiian spiny lobster, *Panulirus marginatus*, the juvenile setae appear as early as in D1 and complete extrusion of juvenile setae occurs in stage D4 (Mills and Lake, 1975; Lyle and Mac Donald, 1983).

Post-ecdysial changes show wide differences in different crustacean groups. In the crayfish, *Astacus leptodactylus*, the early postmoult (Stage A) is characterized by the presence of a fibrillar protoplasm in the setal lumen which is subsequently replaced by a homogeneous protoplasm during late postmoult (Stage B) (Van Herp and Bellon- Humbert, 1978). On the other hand, in the
spiny lobster, *Panulirus marginatus*, the post-ecdysial wide setal lumen is filled with a granular cytoplasm (Lyle and Mac Donald, 1983). In shrimps such as *Palaemon paucidens* (Kamiguchi, 1971) and *M. rosenbergii* (Peebles, 1977), the appearance of setal cones is used as the cue for identifying the postmoult events.

In brachyuran crabs, such as *P. hydrodromous* (Anilkumar and Adiyodi, 1985), *M. messor* (Sudha, 1992), and *S. quadratum* (Syama, 2009; Syama et al., 2010) intermoult appears as a lengthy period, contrary to that of *Ligia sp.* and *Leander sp.*, where intermoult is comparatively short (Freeman and Bartell, 1975).

In a deep water shrimp, *Aristeus antennatus* (Demestre, 1995) and *M. rosenbergii* (Okumura and Aida, 2000), the moulting activity is present throughout the year. But, in *P. hydrodromous* (Anilkumar, 1980), *M. messor* (Sudha and Anilkumar, 1996), and in crayfish, *Pacifastacus leniculatus* (Guan and Wiles, 1999), moulting is reported as an annual event. Puberty moult is reported in the portunid crab, *Charybdis bimaculata* (Doi et al., 2008).

### 1.5. Interaction between moulting and reproduction:

The relationship between moulting and reproduction is much more evident in females inasmuch as vitellogenesis and new cuticle formation could affect the physiology of the organisms by the competitive utilization of reserve materials from the storage organs. The interrelation between moulting and female reproduction has been demonstrated in few crustacean species. In crabs and lobsters, reproduction takes place during relatively long intermoult period (Adiyodi, 1985, 1988 for reviews). In lobsters, moult cycle is shown to override the reproductive activity by the suppression of vitellogenesis during premoult.
Normally vitellogenesis occurs in these decapods during the moult stages B, C and D0. If the moult cycle progresses into the early premoult stage (D1), the ongoing vitellogenesis is arrested, and the late premoult lobster with mature ovaries shows the tendency to reabsorb yolk rather than successful oviposition (Byard and Aiken, 1984). The spider crab, belonging to *Oxyrhynca* sp., enters vitellogenesis only after pubertal moult (Chaix and De Reggi, 1982). Temporarily separated moulting and female reproduction has also been reported in *P. hydrodromous* with annual breeding cycle and the non occurrence of moulting throughout the extended breeding season (Anilkumar, 1980).

Previous observations made in our laboratory showed that, *M. messor*, a highly fecund brachyuran crab exhibits two types of reproductive cycle in the annual cycle. During August-December, the population shows frequent reproductive cycle resulting in continuous spawning during the long intermoult period. However, during January-May season, reproductive cycle is followed by moult cycle. During this season, the ongoing vitellogenesis is arrested if the females enter premoult and soon after postmoult recuperation, the hitherto restrained ovary begins to accumulate yolk and spawning ensues during late postmoult or early intermoult. Like the spider crab, *Oxyrhyncha* sp. (Chaix and De Reggi, 1982), ovarian maturation of *M. messor* occurs concurrently with the embryonic development in the brood pouch (Sudha and Anilkumar, 1996). In an anomuran sand crab, *Emerita asiatica*, the reproductive cycle is invariably followed by moult cycle. Here, the brooding of eggs in the pleopods inhibits the onset of moult cycle. However, soon after hatching, the crabs enter the premoult stage followed by ec dysis. In the mean while, the ongoing vitellogenesis within the
ovary is extended throughout the premoult period and the next spawning occurs in postmoult stage after mating (Subramoniam, 2000 for review).

The synergistic programming of ovarian growth and moult cycles is also well demonstrated in freshwater prawns, *M. nipponense* (Okumura et al., 1992) and *M. rosenbergii* (Wilder et al., 1991; Okumura, 2004). Although the ovarian cycle begins during intermoult stage, vitellogenesis progresses as the premoult stages advance. Like *E. asiatica*, both of these shrimps start premoult stages, only after the release of larvae from the brood, to ensue the next spawning after ecdysis. Similar patterns of moulting and reproduction have been reported in penaeid shrimps, with a difference that the free spawning occurs during premoult stage followed by ecdysis. In *P. monodon*, active vitellogenesis precisely occurs during the lengthy premoult period (Crocos, 1991). The continuation of ovarian maturation during premoult stage is also evidenced in an anostracan, *Artemia* (Walgraeve et al., 1988) and the isopod *Armadillidium vulgare* (Suzuki et al., 1990). Both *M. nipponense* and *M. rosenbergii* also showed a non-reproductive moult cycle, when the ovary remains vitellogenically inactive throughout intermoult period resulting in consecutive moult cycles (Wilder et al., 1991; Okumura et al., 1992; Okumura, 2004).

1.6. The female reproductive system:

The crustacean ovaries are located either dorsally or dorsolaterally to the alimentary canal and morphologically it shows diversity in various crustacean groups. Generally, it appears as a paired structure longitudinally extending the entire length of the cephalothorax and the abdomen. In brachyurans, the ovaries are restricted to the cephalothorax and are interconnected at the centre and form
the shape of ‘H’ (Payen, 1973; Anilkumar, 1980; Sudha, 1992; Syama, 2009). In shrimps, the partly fused paired and bilaterally symmetrical ovaries are extended between the stomach and telson (King, 1948). In Emeritia asiatica (anomuran crab), the two ovarian limbs behind the interconnected bridge join at the mid region to form a ring, from which a median ring is extended to the abdomen (Subramoniam, 1981). In free living copepods, ovaries comprise a median lobe but in parasitic copepods, paired ovary shows bilateral symmetry (Ferguson, 1967). Ovarian symmetry is lost in hermit crab (Carayon, 1941). In the myodocopid and halocyprid ostracods, the paired sac-like ovary lies separately on both the sides of the gut tube in the posterior region of the trunk (Ikuta and Makioka, 1999, 2004).

Generally, oviducts are the lateral extensions of the ovary and appear as short tubules. The position of the opening of the oviduct may vary among different species. In many crustaceans, where internal fertilization is the rule, the saccular spermatheca with glandular epithelium is found proximal to each of the oviducts. In phyllopod anostracans, the two lateral oviducts open to a median ovisac where spermatozoa are received during copulation for fertilization (Linder, 1959; Munuswamy and Subramoniam, 1979). In ostracods and brachyurans, there is a pair of seminal receptacles each connected to the oviduct, but open to the exterior through separate opening (McGregor and Kesling, 1969; Anilkumar, 1980). In macrurans, the seminal receptacle opens out through the genital segment without any connection to the oviduct (Aiken and Waddy, 1980, 1990).

1.7. Vitellogenesis:
Vitellogenesis, a process involving synthesis of yolk and its subsequent deposition in growing oocytes, is an important reproductive process in oviparous animals. Yolk is the nutritive material, often accumulated in substantial quantities in the ooplasm to meet the basic requirements of embryonic development. The composition of yolk could vary from species to species and sometimes even among individuals, depending on diet. Water forms a significant proportion of the yolk; and the solid part contains almost all the necessary organic and inorganic compounds that are needed to sustain life and ensure normal growth of the embryo. Proteins and lipids constitute the major part of the organic reserves of the yolk and represent 50-80% of dry matter in the mature ovary (Millamena and Pascual, 1990; Penaflorida and Millamena, 1990; Mohamed and Diwan, 1992; Syama, 2009).

Proteins provide the basic structural material needed for tissue build up and lipids serve as the energy source. The important process which takes place during crustacean oocyte development is the synthesis of large amounts of vitellin (Wallace et al., 1967; Lee and Walker, 1995). The chief fraction of the crustacean vitellin is a high-density lipoprotein/lipoglycoprotein frequently associated with carotenoid pigments and usually referred to as lipovitellin. The eggs of the brine shrimp, Artemia salina, contain orange red and those of Branchipus stagnalis contain blue canthaxanthin prosthetic groups attached to lipovitellin (Warner et al., 1972; Zagalsky and Gilchrist, 1976). The lipovitellin of blue crab egg yolk is an orange colored high density lipoprotein (Lee, 1991; Lee and Puppione, 1988).
ELISA and immunohistochemical studies conducted in *Callinectes sapidus* strongly suggest that developing oocytes within the ovaries are responsible for lipovitellin synthesis (Lee and Walker, 1995). The presence of yolk-nuclear complexes in small developing and late previtellogenic oocytes and its disappearance with the commencement of vitellogenesis has been reported in *C. sapidus* (Krol et al., 1992; Lee and Walker, 1995).

Polyacrylamide gel electrophoretic analysis of proteins present in the crustacean ovary shows different types of protein fractions. Lipovitellin of *Procambarus clarkii* shows 5 subunits (Lui and O’ Conner, 1976), *Pachygrapsus crassipes* have 3 subunits (Lui and O’ Conner, 1977), *Orchestia gammarella* has 2 fractions (more abundant lipovitellin I and less abundant lipovitellin II) (Junera et al., 1977) and *Branchipus stagnalis* contains 5 major subunits (Zagalsky and Gilchrist, 1976). In addition to lipovitellin, the yolk may contain both non-conjugated simple protein fractions and some glycoproteins.

The yolk lipid level of *M. rosenbergii* ranged from 18.2% to 55.8%. The ovary of *M. rosenbergii*, a significant increase in the content of lipid occurred from stages I to II; between stage II and IV, however, no significant differences were reported. On the other hand, from stages IV to V, a significant increase in ovarian lipid was observed (Cavalli et al., 2001). Total neutral lipid (NL) was more abundant in the ovary of *M. rosenbergii* than the phospholipids. Most of the NL consists of triglycerides (TG), which ranged from 43% (Stage I) to 64.8% (Stage V). Sterols are the second most abundant lipid class among NL in the ovary of *M. rosenbergii*, it increases from 2.0% to 6.3% between stage I and Stage V respectively. Levels of FAA and diacylglycerol fluctuated throughout
maturation. Total phospholipid (PL) levels in the ovary of *M. rosenbergii* at Stage V were significantly greater than that of all other stages. Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) are quantitatively the major components of the PL fractions, collectively representing from 69.3% (Stage I) to 91.4% (Stage IV) of all PL in the ovary. Levels of these phospholipids are significantly higher at Stage V of ovarian maturation in *M. rosenbergii*. The level of PI (Phosphatidyl inositol) also increased significantly during maturation (Cavalli et al., 2001).

The predominant fatty acids in the ovary of *M. rosenbergii* includes palmitic acid (16:0), oleic acid (18:1 n-9) and linoleic acids (18:2 n-6), whereas myristic acid (14:0), stearic acid (18:0), eico-sapentaenoic acid (20: 5n-3; EPA), arachidonic acid (20:4 n-6; ARA) and docosahexaenoic acid (22: 6n-3; DHA) are present at intermediate levels. The levels of saturated and mono-unsaturated fatty acids increased significantly in the ovary of *M. rosenbergii* as gonadal development progressed (Cavalli et al., 2001). Among poly unsaturated fatty acids (PUFA), the levels of 18:2 n-6 and 18:3 n-3 significantly increased from Stage I to III, while ARA (arachidonic acid) levels decreased significantly during the same period. A significant increase in the levels of docosahexaenoic acid (DHA) and total n-3 HUFA (highly unsaturated fatty acids) was observed between Stage I to Stage III (Cavalli et al., 2001).

Ovaries, hepatopancreas, haemocytes, fat body and adipose tissues have been reported to date as sites of vitellogenin (Vg) synthesis in crustaceans (Eastman-Reks and Fingerman, 1985; Yano and Chinzei, 1987; Quackenbush, 1989a; Rankin et al., 1989; Suzuki et al., 1989; Browdy et al., 1990; Fainzilber et al.,
1892; Shafir et al., 1992; Chen and Chen, 1994; Han et al., 1994; Vafopoulou and Steel, 1995). The sites of yolk biosynthesis vary according to the species examined. Some crustaceans (e.g., Penaeus japonicas) rely solely on the ovary for the entire quantity of yolk materials (Yano and Chinzei, 1987). Others may use the fat body as a primary source of yolk (Amphipods, Isopods), as is the case with most insects (Charniaux-Cotton, 1978; Gohar et al., 1984; Souty and Picaud, 1984; Bowner, 1986). The hepatopancreas and ovary are common sites for yolk biosynthesis in several penaeid shrimps (Penaeus vannamei, Penaeus semisulcatus, Penaeus monodon, Parapenaeus longirostris) (Quackenbush, 1989a, b; Browdy et al., 1990; Quintio et al., 1990; Tom et al., 1992; Chen and Chen, 1993, 1994; Tseng et al., 2001; Marsden et al., 2007). In crabs and shrimps, there is evidence that ovary can synthesis lipovitellins (Adiyodi and Subramoniam, 1983; Quackenbush, 1989 a, b; Lee and Walker, 1995).

1.8. Physiology of hepatopancreas in relation to reproduction:

The hepatopancreas is an important site of intermediary metabolism in crustaceans and an important storage depot, like the vertebrate liver and the insect adipose tissue. The hepatopancreatic resources are suggested to mobilize to meet the needs in reproduction (Adiyodi, 1969; Anilkumar, 1980; Sudha, 1992; Vazquez-Boucard et al., 2002; Hasek and Felder, 2005). The role of hepatopancreas in supplying nutrients to the developing ovary has been reported in some prawn species (Penafloirda and Millamena, 1990; Millamena and Pascual, 1990; Tseng et al., 2001; Buckup et al., 2008).

The lipid, the major source of energy is reported as the chief organic component of the hepatopancreas; its metabolism in the hepatopancreas is subject to
greatest modification during vitellogenesis, embryogenesis, and larval
development. Lipids play an essential role as an energy reserve
(triacylglycerols), and as the basic component of membranes (phospholipids),
and for hormone synthesis and forms the most important part of vitellin
(Kanazawa and Teshima, 1971; Galois, 1983).

The growing oocytes are suggested to utilize the hepatopancreatic lipid reserve
in brachyuran crabs such as Portunus pelagicus (Pillay and Nair, 1973), P.
hydrodromous (Anilkumar and Adiyodi, 1978; Anilkumar, 1980), M. messsor
(Sudha, 1992), and U. tangeri (Mourente et al., 1994) and the shrimps such as P.
japonicus (Teshima and Kanazawa, 1983), P. indicus (Galois, 1984),
Metapenaeus affinis, Parapenaeus styliera, Cardina weberri (Nagabhushanam
et al., 1985), P. setiferus (Teshima et al., 1988; Castelle and Lawrence, 1989), P.
monodon (Millamena and Pascual, 1990) and Fenneropenaeus indicus
(Vazquez-Boucard et al., 2004). Recent reports show a decrease in the
hepatopancreatic lipid content of Penaeus monodon with an associated increase
in the lipid profile of spontaneously maturing ovaries (Marsden et al., 2007;
Marsden, 2008). The amount of lipid mobilized in P. monodon is reported to be
in excess of immediate ovary uptake in contrast to the findings for P. indicus
where lipid mobilized from the hepatopancreas, is insufficient to account for
increase in the ovary lipid content (Galois, 1984; Vazquez-Boucard et al., 2002).

In M. rosenbergii, the hepatopancreas is the main lipid storage and processing
organ (D’ Abramo and Sheen, 1993). However, its reserves contribute only
partially to vitellogenesis (Cavalli et al., 1999). Significant decrease in some
neutral lipid classes, particularly triacylglycerol, free fatty acids and
diacylglycerols, occurs in the hepatopancreas of *M. rosenbergi* at the final stages of maturation (Cavalli et al., 2001). However, towards the end of the maturation period, the level of lipid in the hepatopancreas of *P. monodon* and *M. rosenbergii* females are reported to be relatively high and subsequently it shows significant decline following spawning suggesting a possible storage of energy at the end of ovarian maturation, necessary to cover the metabolic demands for spawning (Millamena and Pascual, 1990; Cavalli et al., 2001). The high levels of neutral lipid mainly triglycerides in both ovary and hepatopancreas, have been reported in shrimps such as *P. japonicus* (Teshima et al., 1989), *P. monodon* (Millamena and Pascual, 1990) and *M. rosenbergii* (Cavalli et al., 2001) and in brachyuran crab, *U. tangeri* (Mourente et al., 1994) throughout the maturation period of ovary. The predominant phospholipids such as PE (phosphatidylethanolamine) and PC (phosphatidylcholine) appear in the ovary and hepatopancreas of *U. tangeri* and *M. rosenbergii* during the vitellogenesis (Mourente et al., 1994; Cavalli et al., 2001).

In many crustaceans, significant variations are reported in the pattern of hepatopancreatic soluble protein in relation to vitellogenesis (Ajmal Khan et al., 1977; Anilkumar, 1980; Sudha, 1992; Vazquez-Boucard et al., 2004; Marsden et al., 2007). Evidences are also available on the role of crustacean hepatopancreas as the site of vitellogenin synthesis (Lee and Chang, 1999; Soroka et al., 2000; Yang et al., 2000). In the shrimp, *Penaeus vannamei*, apart from the ovary, the hepatopancreas was found to be the common site for vitellogenin synthesis (Yano and Chinzei, 1987). Presence of an immunologically identical protein to vitellogenin has been reported in the hepatopancreas of the reproductively active females of *L. emarginata* (Wolin et al., 1973; Paulus and Laufer, 1987). In *C.*
maenas, Laufer et al., (1986, 1987) could identify specific cells for vitellogenin synthesis and reported that, maximum vitellogenin synthesis occurs in the intermediate stages of vitellogenesis; synthesis was declined towards the final phase of ovarian growth. The hepatopancreas of vitellogenic females is reported to be involved to some extent, in vitellogenesis in P. vannamei and P. semisulcatus (Quackenbush, 1989a,b; Shafir et al., 1992). Vitellogenin synthesis in hepatopancreas is also reported in Scylla serrata (Rani and Subramoniam, 1997). In M. rosenbergii, Chen and co-workers (1999) demonstrated the role of hepatopancreas in vitellogenin synthesis through gene expression studies. Studies in the Chinese mitten-handed crab, Eriocheir sinensis strongly suggest the involvement of hepatopancreas for the synthesis of vitellogenin (Li et al., 2006). Some species are capable of synthesizing vitellogenin in ovaries also (Lui and O'Connor, 1976; Browdy et al., 1990; Fainzilber et al., 1992; Lee and Watson, 1995). Several crustaceans can produce vitellogenin in both ovary and hepatopancreas (Paulus and Laufer, 1982; Shafir et al., 1992; Sagi et al., 1995; Tsutsui et al., 2000). Ovary and hepatopancreas in Potamon potamios are regarded as the sites for vitellogenin synthesis (Pateraki and Stratakis, 2000). Investigations on the isopods (Porcellio dilatatus and Oniscus asellus) and the amphipods (O. gammarellus) revealed that the fat body synthesizes vitellogenin (Picaud and Souty, 1980; Vafopoulou and Steel, 1995).

The apparent mobilizations of the hepatopancreatic carbohydrates in relation to vitellogenesis are suggested in brachyuran crabs (Adiyodi and Adiyodi, 1972; Anilkumar, 1980; Nagabhushanam et al., 1985; Sudha, 1992). A possible conversion of hepatopancreatic lipid to glycogen through the enzyme glycogen synthetase has been suggested (Passano, 1960; Hohnke, 1971).
The exact involvement of hepatopancreatic free amino acids (FAA) in vitellogenesis is still enigmatic in crustaceans. In brachyuran crabs such as *P. hydrodromous*, *C. weberi* and *M. messor*, the intense depletion of hepatopancreatic aminoacids during the final stages of vitellogenesis seem to indicate that they are significantly utilized for yolk formation (Anilkumar, 1980; Nagabhushanam et al, 1985; Sudha, 1992). In *C. maenas*, the hepatopancreatic aminoacid concentration is shown to vary in accordance with the different phases of vitellogenesis (Pochon-Masson et al., 1982).

1.9. Physiology of hepatopancreas in relation to moult:

Reports are also available on the contribution of hepatopancreatic reserves for somatic growth. Significant histological and histochemical changes in the hepatopancreas in relation to moult cycle have been described in the shrimp *Palaemonatus argentinus* (Sousa and Petriella, 2001).

Variation in lipid concentration was seen in the hepatopancreas of *P. japonicus* (Tetsuo et al., 1977) in relation to moult cycle. It is reported that, the lipid concentration reached a maximum at Stage D0 and this pre-ecdysial increase of hepatopancreatic lipids was reported to be derived from both polar and neutral lipids. In *M. messor*, the total lipid is found to be maximum at stage D1 and declined significantly by moulting (Sudha, 1992), suggesting their utilization as a source of energy during the proecdysial period. Moult-related fluctuation in the hepatopancreatic triacylglycerol and free fatty acid has been demonstrated in *Pleoticus muelles* (Jeckel et al., 1990). Triglycerides appear as an important lipid reserve in the hepatopancreas in female crayfish, *Parastacus defossus* (Buckup et al., 2008). A cyclic fluctuation in the hepatopancreatic cholesterol in
relation to moult cycle of the prawn, *Penaeus japonicus* has been reported (Guary and Kanazawa, 1973). According to Kanazawa and Teshima (1971), cholesterol is a structural component of the cell membranes and a precursor of sex hormones involved in reproductive control in crustaceans.

Moult-related utilization of hepatopancreatic protein has been found in brachyuran decapods such as *Menippe rumphi* and *M. messor* (Babu, 1984; Sudha, 1992). However, in the prawn *Metapenaeus monoceros*, the hepatopancreatic profiles of protein, glycoprotein and lipoprotein did not show any significant fluctuation in relation to moult cycle (Bojan, 1987).

Evidences, scarcely though, are available on the carbohydrate metabolism in relation to moult cycle. Increased glycolytic fluctuation in relation to late premoult has been demonstrated in *Orconectes virilis* (McWhinnie and Chua, 1964). In *Palaemon serratus*, high level of hepatopancreatic oligosaccharides such as β-glucosidase and β-galactosidase are shown to be coupled with the premoult (Chuang and Yang, 1991).

### 1.10. Control mechanisms of growth and reproduction:

Crustacean physiological processes such as reproduction and moult are primarily controlled by the central nervous system which receives the environmental cues and induces appropriate responses. Intense research has been carried out during the past years in order to understand the control mechanism of the crustacean growth and reproduction (Subramoniam, 2010; Nagaraju, 2011, for reviews). The regulatory hormones include a group of active
stimulatory and inhibitory principles, through the coordinated activity of which, the up and down regulation of growth and reproduction is accomplished.

**a) Neurohormones:**

Crustacean reproduction is regulated by a complex chain of neurohormones present in the thoracic ganglion, brain and the X-organ sinus gland complex. The brain and thoracic ganglion have been confirmed as source of gonadal stimulatory hormone in crustaceans (Otsu, 1963; Hinsch and Bennet, 1979; Fingerman, 1997; Nagaraju, 2011, for review).

The neurohormones produced from the neuroendocrine cells of the X-organ sinus gland complex, situated in the crustacean eyestalks inhibit gonadal growth in both males and females (Adiyodi and Adiyodi, 1970; Aiken et al., 1979). Studies on the adult *Potamon koolense* suggest that, neurohormone synthesized from the type-3 neurosecretory cells of the medulla terminalis ganglion of the eyestalk, inhibits ovarian growth during the period of reproductive quiescence (Joshi and Khanna, 1987). However, in *P. japonicus*, it is the medulla externa of the eyestalk that acts as the site of synthesis of the ovarian inhibitory factors (Kaworu, 1988). Eyestalk ablation in female crustaceans caused accelerated oocyte proliferation (Snyder and Dhainaut, 1977; Syama, 2009) and ovarian maturation (Leny and Khoo, 1984; Persis and Sarojini, 1985; Anilkumar and Adiyodi, 1985; Okumura and Aida, 2001; Sudha and Anilkumar, 2007; Marsden, 2008). Unilateral eyestalk ablation has been employed to induce ovarian maturation and spawning and also shorten the moult interval in shrimps (Zaib Un Nisa, 2001; Lin et al., 2001; Venkitraman et al., 2004; Hesni et al., 2008; Marsden, 2008). Sinus gland (SG) extracts have also been shown to
regulate the synthesis of non-vitellin proteins in pre-vitellogenic prawn ovaries (Avarre et al., 2001; Tsutsu et al., 2005). The involvement of sinus gland hormones in the regulation of ovarian and hepatopancreatic reserves at very early stages of ovarian development in *P. monodon* has been suggested (Van Herp and Soyez, 1997; Marsden et al., 2007).

**b) Ecdysteroids:**

Ecdysteroids, a group of polyhydroxylated ketosteroids, synthesized from the ecdysial glands (Y-organ) appear as the chief hormonal factors primarily responsible for controlling growth in arthropods (Chang et al., 1976; Buckmann, 1989; Snyder and Chang, 1991; Hopkins, 1992). The ecdysone synthesized from the Y-organ is released directly into the haemolymph and subsequently it is converted into its active form, 20-hydroxyecdysone by the peripheral tissues in order to promote the physiological changes associated with moulting (Adiyodi and Adiyodi, 1970; Chang et al., 1976; Van Herp and Soyez, 1997, for reviews). In crustaceans, several ecdysteroids such as 25-deoxyecdysone, ponasterone A, and 20-hydroxyecdysone circulate in the haemolymph (Hopkins, 1983; Lachaise and Lafont, 1984; Snyder and Chang, 1991; Hopkins, 1992). Changes in these ecdysteroid titers and ratios during the molt cycle are temporally correlated with major physiological events involved in molting and regeneration of lost limbs (Chang, 1989, for review; Hopkins, 1992).

Although there are ample evidences to demonstrate the role of ecdysteroid in insect reproduction (Carney and Bender, 2000; Koslova and Thummel, 2000; Riddiford et al., 2001), this aspect has not been successfully attempted in crustaceans (Subramoniam, 2000; Okumura, 2004; Diwan, 2005; Hopkins et al.,
2009, for reviews). The effect of exogenously administered ecdysteroids on moulting and reproduction in *Emerita asiatica* has been investigated (Gunamalai et al., 2004). Current studies, aimed at determination of circulating levels of ecdysteroids during moulting and reproduction, are expected to shed more light on the endocrine regulation of these processes in crustaceans. The interrelationship between hemolymph and ovarian ecdysteroids has been studied by direct radioimmunoassay during different moult and reproductive stages (Chaix and De Reggi, 1982; Rotllant and Takac, 1999; Subramoniam, 2000; Gunamalai et al., 2004; Sudha and Anilkumar, 2007). In the freshwater prawn, *Macrobrachium nipponense*, Okumura et al., (1992) demonstrated a close positive correlation between the haemolymph ecdysteroid titre and the ovarian maturation during the reproductive-moult cycle. A different situation exists in the brachyuran crabs where the ovarian maturation is completed in the intermoult period when the ecdysteroid titre is kept at a very low level. In the Oxyrhynchian species, where the moulting is terminated by the pubertal moult, the Y-organ gets atrophied (Chaix and De Reggi, 1982). In shore crab, *C. maenas*, the vitellogenesis is shown to occur during the intermoult period, when the ecdysteroid level is low (Soumoff and Skinner, 1983; Styrishave et al., 2008). Further more, a study on the penaeid shrimp, *Penaeus vannamei*, administration of ecdysteroids did not induce any ovarian maturation, as it specifically lacks any positive effect on vitellogenein synthesis (Chan, 1995). The ecdysteroid assay performed on a brachyuran crab, *M. messor* reveals the presence of relatively low ecdysteroid levels during intermoult when active vitellogenesis occurs (Sudha and Anilkumar, 2007). Nevertheless, that this low ecdysteroid titre is a requisite for successful female reproduction, is another
question that deserves to be addressed. Bilateral eyestalk ablation in females of *M. messor* resulted the precocious vitellogenesis and premoult, along with the elevated ecdysteroid levels, clearly demonstrate that vitellogenesis can occur under a high ecdysteroid titre in brachyuran crabs, much higher than the premoult levels. 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 (Okumura et al., 1992; Wilder et al., 1994; Wilder and Aida, 1995; Sudha and Anilkumar, 1996, 2007; Subramoniam, 2000 for review).

c) Ovarian ecdysteroids:

Although the ambiguity exists in respect of the role of ecdysteroids in vitellogenesis, several crustacean species accumulate large quantities of ecdysteroids within the ovary (Subramoniam, 2000 for review). The composition of ovarian ecdysteroids is however, variable among different species. In the crab, *Emerita asiatica*, the purified yolk protein (lipovitellin) contains both free and conjugated ecdysteroids (Subramoniam et al., 1999; Subramoniam, 2000 for review). In the spider crab, *Acanthonyx limulatus*, the relative concentration of free ecdysteroids, as well as the conjugated ecdysteroids show a significant change during ovarian maturation, suggesting the metabolic conversion of free ecdysteroids into conjugated forms and vice-versa (Chaix and De Reggi, 1982).

In those crustaceans, where there is perfect synchronization between moulting and reproduction, accumulation of ovarian ecdysteroids takes place during premoult. In *M. rosenbergii*, the ovarian ecdysteroids show sharp increase as the
premoult stage advances towards D3 stage and maintain the titre up till spawning (Wilder et al., 1991; Young et al., 1993). Since the ovarian accumulation of ecdysteroids occurs under high titre of haemolymph ecdysteroids, it is presumed that, the hemolymph ecdysteroids are transported to ovary along with the yolk precursor materials (Wilder et al., 1991; Young et al., 1993; Subramoniam, 1999). Supporting evidences are also provided by the work in terrestrial isopods Armadillidium vulgare (Suzuki et al., 1996) and Oniscus ascellus (Vafopoulou and Steel, 1995) where ovarian conjugated ecdysteroids show substantial increase during premoult, when the haemolymph ecdysteroid level is also high.

d) Methyl farnesoate:

Methyl farnesoate (MF) is an unepoxidated sesquiterpene structurally related to the juvenile hormone JH III of insects, synthesized by the crustacean mandibular organ (MO) (Laufer et al., 1987). Over the past years, both direct and indirect substantiation have been emerged for the role for MF as a hormone in crustaceans. MF is synthesized de novo by a ductless gland and is secreted into the haemolymph at nanomolar levels. The enzyme that metabolizes MF are found in the hepatopancreas and other peripheral tissues such as the gonad (Gunawardene et al., 2002). After release by the MOs, the MF is transported through the haemolymph to the target tissues by a protective lipoprotein called MF binding proteins which has been identified and characterized from crustacean haemolymph (Prestwich et al., 1990, 1996; Li and Borst, 1991; Takac et al., 1993; King et al., 1995; Tamone et al., 1997). The MF is thought to be involved in the regulation of moulting and reproduction in crustaceans.
i) The role of MF in reproduction:

Available reports show that MF may have a stimulatory effect on reproduction in both the sexes (Borst et al., 1987; Linder and Tsukimura, 1999; Rodriguez et al., 2002; Nagaraju, 2007, 2011 for reviews). MF synthesis has been correlated with ovarian maturation in *L. emarginata*, *C. maenas* and *U. pugilator* (Borst et al., 1987; Laufer et al., 1987). By using an anti-vitellogenin immunoserum, Laufer and Borst (1988), demonstrated the synthesis of vitellogenin after MF administration. Injection of MF increased the vitellogenin titre in haemolymph of eyestalk ablated *L. emarginata* (Borst et al., 1987; Laufer et al., 1987, 1999; Jo et al., 1999), *M. rosenbergii* (Wilder et al., 1995), *P. indicus* (Nagaraju et al., 2002), *M. malcolmsonii* (Nagaraju, 2003) and *O. senex senex* (Reddy and Ramamurthi, 1998; Nagaraju, 2003; Reddy et al., 2004). MF stimulates ovarian protein kinase C in the crayfish *Cherax quadricarinatus* (Soroka et al., 2000).

In *Nephrops norvegicus*, the females showed oscillations in MF synthesis that seem to parallel the vitellogenic cycle (Rotllant et al., 2001). It was also reported that the exposure of daphnid oocytes to MF during late ovarian development causes the oocytes to develop into males, whereas only females are produced from the control (Olmstead and Leblanc, 2002; Mu and Leblanc, 2004). These results suggest that MF may act as a sex determinant.

It was shown that physiological doses of MF could stimulate an increase in oocyte diameter when ovaries were cultured *in vitro* (Laufer, 1992; Rodriguez et al., 2002). This result indicates that MF acts directly on ovarian tissues due to the presence of an MF receptor (MF binding proteins) (Takac et al., 1993).
Several contrasting results on the non-involvement of MF in ovarian maturation have also been reported. For example, no significant effects were detected in lobster, *H. americanus*, and *M. rosenbergii* when MF was injected into senescent females (Tsukimura et al., 1993; Wilder et al., 1994). Incubation of active ovarian tissue of tadpole shrimp, *Triops longicaudatus* with MF shows no effect on the further progress of vitellogenesis (Riley and Tsukimura, 1998; Tsukimura et al., 2006). Linder and Tsukimura (1999) reported that MF significantly reduced the number of developing oocytes when administered continuously to juvenile *T. longicaudatus*.

ii) The role of MF on moulting:

In *M. rosenbergii*, MF levels in the haemolymph increase during the premoult stage and decline in the postmoult stage (Wilder et al., 1995; Ahl and Laufer, 1996; Laufer et al., 2005). Administration of MF also caused moult acceleration in the crayfish *C. quadricarinatus* (Abdu et al., 2001), *P. clarckii* (Laufer et al., 2005) and the crab *O. senex senex* (Nagaraju, 2003; Reddy et al., 2004).

1.11. Ecdysteroid receptors:

Most of the critical events occurring in arthropod life are controlled by ecdysteroids and its action has been clearly demonstrated in insects in relation to moultng, metamorphosis (Thummel, 1995; Kozlova and Thummel, 2000), embryogenesis (Kozlova and Thummel, 2003) and reproduction (Raikhel et al., 2002; Swevers and Iatrou, 2003, 2009; Oscar et al., 2005). It has been shown that ecdysteroid receptor gene is required for normal oogenesis in *Drosophila*.
The functional receptor for 20-hydroxyecdysone is a heterodimer of two proteins belonging to the nuclear receptor gene family, the ecdysone receptor (EcR) and the retinoid X receptor (RXR) - a homologue of ultraspiracle (USP) (Yao et al., 1992). The heterodimer EcR-RXR/USP binds to sequence-specific DNA elements in target genes and directly induces the expression of a number of primary response genes which in turn amplify the effect of 20-hydroxyecdysone by triggering the expression of a large battery of secondary-response genes (Thummel, 1995; Riddiford et al., 2001 for reviews). RXR appears to be ubiquitous among animal kingdom. In vertebrates, RXR is known to bind with retinoids, and has been implicated in a variety of physiological functions including growth, reproduction and development (Mangelsdorf and Evans, 1995; Lu et al., 1997; Kostrouch et al., 1998; Wiens et al., 2003).

Among crustaceans, studies on the hormone receptor gene has so far been pursued in only very few species such as *U. pugilator* and *U. annulipes* (brachyuran crabs) and *Daphnia* (brachiopod). In *U. pugilator*, the genes (*Up EcR* and *Up RXR*) encoding the homolog of the functional insect ecdysteroid receptor have been isolated and by using the probes derived from these genes, putative ecdysteroid target tissues including the regenerating limbs, the ovary and the developing embryo have been identified (Durica et al, 2002). Anilkumar et al., 2005 sequenced the DNA binding domain (DBD) of the ecdysteroid receptor gene (*EcR*) from the *Uca annulipes*. The alignment, performed using clustal 1.8, have revealed the extreme sequence conservation with the coding
region and extensive similarity with in the intron region of *Uca pugilator*. Further, in *U. pugilator*, the expression of *EcR* and *RxR* genes related to circulating levels of ecdysteroid has been studied at the RNA level using Northern blot and Ribonuclease Protection Assays (RPA) and at the protein level using immunocytochemistry in both non-regenerating and regenerating tissues (Durica et al., 2002).

**1.12. Concluding remarks and future prospects:**

As growth and reproduction are mutually interacting physiological phenomena in decapod crustaceans and as these functions are diversely programmed even in closely allied groups, a precise understanding of the annual physiological cycle is a prerequisite for pursuing any work on endocrinology of the species in question. Being a highly diverse group, with varied reproductive patterns, decapod crustaceans exhibit no uniformity with respect to the synthesis and incorporation of yolk. Although the utilization of hepatopancreatic reserves for somatic and reproductive growths has been suggested in penaeid shrimps, the role of hepatopancreas in brachyuran growth and reproduction is only inadequately researched.

The up and down regulation of growth and reproduction in crustaceans is accomplished by the interaction between stimulatory and inhibitory principles. Ecdysteroids are known to promote growth in insects and crustaceans alike. Eyestalks are known to be the sites of production and storage of inhibitory hormones. However, the removal of the eyestalks has produced varying results, apparently based on the phylogeny and the physiological state of the species. Moreover, our knowledge is quite meagre on the hormonal control of
physiology of hepatopancreas in relation to growth and reproduction in crustaceans. Despite the fact that the role of ecdysteroids in insect reproduction has been well documented, the question of possible ecdysteroid influence on crustacean vitellogenesis has not yet been resolved. This deficiency is primarily driven by the significant diversity existing among various crustacean groups in programming growth and reproduction. Moreover, the antagonistic programming of growth and reproduction in female brachyurans arises the question, whether a high ecdysteroid titre could impede the reproduction in this group.

Keeping all these facts in view, we have undertaken the present study on the physiology of storage tissues in relation to growth and reproduction in the candidate brachyuran crab, *Uca triangularis*. The study also addresses the question of hormonal control of mobilization of reserves in response to somatic and reproductive needs.