CHAPTER 6

Population characteristics and life history of *Eriopisa chilkensis* Chilton
(Gammaridae-Amphipoda)

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6.1. Introduction

6.1.1 Amphipods

Amphipods are extremely diverse, abundant and widespread crustaceans. The amphipods comprise more than 850 species in the whole southern ocean, 741 of which are benthic species (De Broyer et al., 2003). Most amphipods are free-living benthic gammaridians that can occur in such high densities that at times they dominate some communities (Conradi et. al., 1997; Vetter, 1998; Poore and Steinberg, 1999 and Cunha et al., 2000). Amphipods are often the main food for predatory fish and birds (Nair, 1971; Beare and Moore, 1997; Bocher et. al. 2001 and Dauby et. al., 2001) and there is considerable potential
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for metal accumulated by amphipods to be transferred along marine food chains (Wang, 2002). Internationally, amphipods are used widely for sediment testing (Mearns et. al., 1986; Schlekat et. al., 1992 and Fairey et al., 2001), because they are ecologically relevant, have a short life cycle and are suitable for laboratory experiments. They are also known as biomonitors for trace metals, some species are ecologically sensitive and therefore are potential bioindicators of disturbed communities (Conradi et al., 1997 and Warwick, 2001). Amphipods are selected as biomonitors of trace metal because they are net accumulators of particular metals, relatively sedentary, abundant, easy to identify and resistant to handling stress (Rainbow, 1993a and Rainbow, 1993b).

The benthic amphipods, especially Gammaridea, are an invaluable food source for many economically important fishes (Mason, 1974; Hobson and Chess 1976). Their limited mobility and their sensitivity to environmental changes suggest that their distribution and abundance can be used as an indicator of environmental quality (Albright 1982).

\textit{E. chilkensis}, the species selected for the present study is an estuarine gammarid amphipod which was first recorded from Chilka Lake by Chilton (1921) and Asari (1983) studied the biology of \textit{E. chilkensis} and reported that they are suspension feeders feeding on organic-rich detritus. Work done on the gammarid amphipods of Cochin backwaters by Nair et al. (1983) gave an account of the ecology and population dynamics of this group. \textit{E. chilkensis} occur in large numbers in the organically enriched sediments of the Cochin estuary and it can be regarded as a tolerant species of organic pollution. \textit{E. chilkensis} was encountered in varying densities in the benthic epifaunal communities in the mangrove swamps of Puduvypin (an area adjacent to Cochin backwater) in the present study. It also has an important trophic role in these areas in shredding leaf litter. In spite of its ecological importance, no detailed work on this species has been carried out in the Cochin estuary and adjoining areas. In this chapter, an attempt has been made to study the different
traits of its life cycle, such as age at maturity, life-span, number of broods per female, duration of embryonic development, life time fecundity (based on number of juveniles released), growth rate and biochemical components in the laboratory. Along with this, the population dynamics of this species in the Cochin estuary was also studied for comparative assessment. This investigation was designed to find whether this species could be cultured in large numbers economically to be used as a feed for fish culture owing to its nutritive value.

6.1.2 Food and feeding

Amphipods exhibit different types of feeding behaviour namely deposit feeding, suspension feeding, deposit feeding coupled with predation, opportunistic predation, micropredatory browsing, macropredation coupled with scavenging, opportunistic necrophagy and true necrophagy. They have a wide dietary spectrum.

6.1.3 Literature review

Our knowledge of the Amphipoda is based on the work of number of authors and dates back to 1816. Studies on the amphipods of the Indian and the neighbouring waters received the attention of zoologists when Giles (1885) published a paper on the occurrence of two species of amphipods from Bengal. Bernard (1935) had worked on the amphipods from the waters of Travancore, Cochin and Bengal coasts from the collections of Zoological Survey of India. Life history of amphipods are known to us from the accounts given by Sexton and Mathews (1913) and Sexton (1924). Amphipoda of the Madras coast was studied by Nagappan Nayar (1959). Shyamasundari (1973) studied the effect of salinity and temperature on the tube-building amphipod (Corophium triaenonyx Stebbing). Breeding biology of a brackish-water amphipod Melita zeylanica in India was studied by Krishnan and John (1974). Morino (1978) classified breeding activity and life history of amphipods into four categories and
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collapsed that low latitude species tend to breed throughout the year and have short life spans. Nair and Anger (1979 a,b) studied the life cycle of *Corophium insidiosum* and *Jassa falcata* using laboratory culture. Seasonal variation in population structure and biochemical composition of *Jassa falcate* was studied by Nair and Anger (1980). Nelson (1980) reviewed the reproductive pattern of 65 species of gammaridean amphipods. Laboratory studies on the reproduction and growth of the amphipod (*Gammarus pulex*) was carried out by Welton and Clarke (1980). Life histories of the amphipods *Lembos websteri* Bate and *Corophium bonnelli* Milne Edwards was studied by Moore (1981). Asari (1983) has studied the biology of *Eriopisa chilkensis* and *Idunella chilkensis* from mangroves of Pitchavaram, India. Work done on the gammarid amphipods of Cochin backwaters by K.K.C Nair et al. (1983) gives an account of the ecology and population dynamics of this group. Breeding periodicity and sex ratio of *Orchestia gammarellus* (Pallas) was studied by Moore and Francis. Sainte-Marie (1991) reviewed the reproductive bionomics of aquatic gammaridean amphipods. Steele and Steele (1991) pointed out that tropical species are characterized by small size, low fecundity, short brood intervals and multivoltine life cycle. Life-history traits of 214 amphipod species were reviewed by Sainte-Marie (1991) and stated that life-history patterns of gammarid amphipods are influenced by latitude, depth and salinity. Life history of the amphipod *Gammarus locusta* in the Sado estuary was studied by Costa and Costa (1999). Cunha et al., (2000) studied the life history and reproductive biology of *Corophium multisetaeum* and stated that low-latitude, warm-water amphipods show iteroparous, multivoltine life history patterns. Chandani and Alan (2004) studied the reproductive bionomics and life history traits of three gammaridean amphipods *Cymadusa filosa*, *Ampithoe laxipodus* and *Mallacoota schellenbergi* from the tropical Indian Ocean and reported multivoltinism and continuous reproduction.
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6.2 Life history and reproductive biology

6.2.1 Materials and Methods

Amphipod Collection and maintenance

Live epibenthic samples were collected from Puduvypin, Cochin estuary (Figure 6.1) using van-Veen grab (0.048 m² mouth area) and brought to the laboratory along with the sediments. In the laboratory, the individuals of *E. chilkensis* were sorted out and kept in the tanks of the recirculating system designed and patented by Nair et al., (2006) where the environmental conditions were maintained as near to the in situ conditions for one month, to acclimatize the individuals for experimental studies. Mass culture of *E. chilkensis* was maintained in the tanks of the recirculating system (Plate 1). Later, pairs of this species (males and females) were isolated and transferred to separate finger bowls, each containing 125 ml of un aerated brackish water (Plate 2, 3). The temperature was maintained at 26 ± 1 °C, salinity 19 ± 1 and pH 7.4-8.6. Few strands of dried algal matter (*Chara* sp.) were added to each bowl as food for the animal. These bowls were labeled for later identification. Individuals were studied under 3 different categories: - juveniles, males and females.

Feeding rates

These were tested with different types of feeds like decaying mangrove leaves, natural detritus available at the sampling site, flesh of young mussel (*Villorita cyprinoids*), algae (*Chara* sp. Plate 4) and *Navicula* sp. scraped from the sides of the culture tanks. For further experiments *Chara* sp. was selected as feed based on its abundance and local availability, and considering the fact that the farmers in the field can culture these amphipods with ease.

Feeding experiments were conducted in 125 ml finger bowls with filtered brackish water. Algae *Chara* sp. (muskgrass) was soaked in water, dried using blotting paper and weighed before introducing into the culture bowls. The
quantity of feed used in each bowl ranged from 0.2 to 0.5 g w/wt (wet weight). Experiments were run using 10 amphipods each in 2 sets of culture bowls (adults and ovigerous females) and the experiment lasted for 24 hrs. Bowls containing algae and without experimental animals were used as controls. On termination of the experiment, the excess feed was netted (mesh size 10 µm) and re-weighed as before. Feeding rate was calculated as amount of feed consumed per day per individual.

Life span and mortality

Total life span was measured by rearing all the individuals released from a single brood in finger bowls until all of them died. Water was changed every day and fresh feed was added on every alternate day. The time of liberation of juveniles from the brood pouch until its death was recorded to measure the total life span. Experiments were run using five culture sets. Mortality in juveniles, females with brood and males after copulation were also assessed during this process.

Moulting and Growth rates

Individuals released from a single brood were reared together in a finger bowl and the moulting rate was assessed separately for males and females. Growth rates were assessed as the average length increment in body size. Live animals were removed from the culture bowls soon after their release from the brood pouch and measured for total length at regular intervals. An ocular micrometer was used for the body length measurements. Body size (length) was measured as distance from tip of the cephalon to the tip of the telson.

Sexual maturity and sex ratio

Newly hatched juveniles from a single brood was separated into finger bowls and reared. The reared animals when reached almost to maturity, a
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mature female was added to assess the time required for male maturity. The day at which the female was found to be ovigerous was recorded and this was the time for male maturity. Experiments were run using 5 sets of culture bowls to have a mosaic picture. Similarly a matured male was added to the female which have almost reached maturity. The appearance of the brood was taken as the time for female maturity. Number of males and females from various broods were counted to find out the sex ratio. Males were identified based on the structure of antennae and subsequently by the presence of an enlarged second gnathopod (Plate 5) and females based on the presence of brood / lamella (Plate 6).

Fecundity, embryonic development

After one month of acclimatization in the laboratory, pairs of amphipods (males and females) were removed from the main culture tank and placed in separate finger bowls. They were observed daily until the female was found to be ovigerous. The fecundity of E. chilkensis was estimated based on the total number of juveniles released by a female. The time for the embryonic development was recorded as the period from oviposition to the hatching of juveniles from the brood pouch.

Biochemical analysis

Live samples maintained during the culture were sorted out into three groups viz. juveniles, adults and ovigerous females for biochemical analysis. The specimens were rinsed with distilled water to remove salt, lyophilized and stored in a desiccator. Before analysis, the samples were thoroughly homogenized using a standard homogenizer. Protein was estimated according to the Faulin- Phenol method described by Lowry et al., (1951) using bovine albumin as standard. Total carbohydrate was estimated according to the colorimetric method using phenol and sulphuric acid, described by Dubois et
al., (1956) using glucose as standard. Lipid extraction was carried out according to Bligh and Dyer (1959) by direct elution with chloroform and methanol (1:2). The extracted lipid was dried at $80^\circ$ C (20 min.) and determined spectrophotometrically after carbonization at $18^\circ$ C in concentrated sulphuric acid according to Marsh and Weinstein (1966), tripalmitine solution were used as standard. Energy equivalents were calculated using the conversion factors given by Winberg (1971) and the values were corrected for biologically unavailable nitrogen oxidation energy according to Kersting (1972). Energy equivalents are expressed in Kcal g$^{-1}$.

6.2.2 Results

Food and feeding

Experiments with different feeds showed that the *E. chilkensis* fed on a variety of feeds like decaying mangrove leaves, natural detritus available at the sampling site, flesh of young mussel (*Villorita cyprinoides*), algae (*Chara* sp.) and *Navicula* sp. scraped from the sides of the culture tanks. Juveniles were found to survive only on the diatom (*Navicula* sp.) scraped from the sides of culture tanks and natural detritus. Based on the observation made during the study, *E. chilkensis* can be categorized as an omnivorous feeder showing a preference for natural detritus and decaying mangrove litter collected from the sampling site. At times they showed cannibalistic behaviour by feeding on their own juveniles and would also feed on moulted exoskeleton.

Feeding rates of adults on dried algal matter (*Chara* sp.) were calculated, after subjecting them to 24 hr starvation. Average feeding rate was 615 $\mu$g day$^{-1}$ individual$^{-1}$. Adults showed an average feeding rate of 1095 $\mu$g day$^{-1}$ individual$^{-1}$ whereas females with broods showed lower rate of 205 $\mu$g day$^{-1}$ individual$^{-1}$.
Life span and mortality

Females lived on average 1.4 times longer than males (average for females: 189 days and for males 132 days) Table 6.1. Mortality was high towards the latter half of the life span. Mortality in juveniles was relatively less, ranging from 8-33%. Mortality in females was also observed during the reproductive phase (usually towards the end of embryonic development - 7th to 9th day). Mortality in males was related to copulatory behaviour, was evident as it was found that 10-20% of males died after copulation (Table 6.2).

Moulting and Growth rate

Moulting rate was found to be higher in females, as the female always moulted after the release of juveniles from the brood pouch. Total number of moults in females ranged between 6-9 as against 5-7 in males (Table 6.1). A higher size increment per moult was observed in males as compared to females as the males attained larger size in a shorter duration.

Newly-born juveniles were in the size range of 1.63-1.92 mm and the number of antennal segments were 6. Initially growth of the body was exponential for around 20 days after hatching with a growth rate of 0.12 mm day⁻¹. Individuals from the same brood exhibited different growth rates, even the number of antennal segments varied from one to another, as some 15-days old individuals had 9 segments for females while males had 11 segments in their antennae. Size of individual or number of antennal segments cannot be often taken as an exact measure of their age. Growth rate was generally higher in early developmental stages. The maximum length observed during the laboratory experiment was 15.2 mm in males and 14 mm in females, whereas in the natural population males attained a size up to 17.76 mm and females attained a size up to 16.42 mm.
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Attainment of sexual maturity and sex ratio

Females of *E. chilkensis* attained sexual maturity within 30-49 days (39.25 ± 5.99) at sizes ranging from 5.48 to 5.76 mm. Males attained sexual maturity much earlier than females, within an average of 26.5 ± 5.55 days with a size range of 5.08 to 5.81 mm. According to this observation females attained a size of 5 mm within 39 days whereas males attained the same size within 27 days. This clearly indicates that the length increment per moult was higher in males compared with females and males grew faster than females. The sex ratio remained more or less the same at the time of maturity (Table 6.2). The number of males decreased as they died at an early age, and as a consequence, the number of males was relatively lower than females towards the end of the total life span. This might be a reason for relatively lower number of males than females in the natural benthic amphipod populations (Nair and Anger 1979; Kemp et al. 1985; Moore and Francis 1986). Age of the female at the time of brooding significantly affected the time required for the attainment of maturity in the next generation. Increase in the age of the female delayed the attainment of maturity of the offspring (Figure 6.2).

Mating behaviour

Sexually mature males were observed to grasp mature females and mount on them. Precopulatory riding behaviour, a condition known as amplexus was not observed in this case. A mature female amphipod is available for fertilization only for a short time immediately after moultion when the cuticle is sufficiently flexible to allow release of the eggs into the brood pouch through the genital pores. The copulatory process continued several times even after the release of eggs, may be to ensure complete fertilization. It was found that females kept separately in culture bowls had no eggs in their brood pouch, but with the introduction of the male it was found that they become gravid.
Therefore it can be inferred that egg bearing and release of eggs into the brood pouch takes place only in the presence of a male.

**Brood**

Amphipods, are peracarid crustaceans and thus are characterized by the presence of a brood pouch, or marsupium, in females during the breeding period. The brood pouches are formed by four pairs of brood plates (lamellae), projecting medially and the plates are fringed with long and thin hairs on their margin (Plate 7). These interlace to form a continuous brood pouch which is efficient in retaining the brood, whilst allowing the free exchange of water. The eggs are laid into this pouch, where they are fertilized owing to repeated copulation by the male. The developing embryos within the brood pouch are continuously exposed to the external medium. These brood plates or lamellae were found to be lost soon after the release of the juveniles.

**Embryonic development and hatching**

The brood pouch, which was initially yellowish in colour, turns to white as the embryo develops. The time for the embryonic development was recorded as the period from oviposition to hatching of juveniles from the brood pouch. Time between oviposition and hatching of juveniles (incubation period) varied from 8 to 14 days. There was no significant relation between the status of the brood (early or late) and incubation period. Mature eggs hatch directly into juveniles that resemble adults. Different stages of embryonic development were identified which were similar to those observed for other amphipods (Dick et al. 2002). 1\(^{st}\) and 2\(^{nd}\) day: -formation of morulla and blastula. 4\(^{th}\) day: -eggs were black, homogeneous ovals with a clear zone beneath the egg membrane (chorion). 5\(^{th}\) day: - movement of the early embryo towards the periphery of the membrane. 8\(^{th}\) day: - curled very early embryos, eyespots and appendages were not yet visible and the embryo was tightly expanded within the egg membrane.
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10th day: Eggs were larger and more elongated at the head region of the embryo and appendages were well developed (Plate 8). The juveniles were released one at a time by the female and the whole process took hours, depending upon the number of juveniles.

**Fecundity**

The fecundity of *E. chilkensis* was estimated as the mean number of juveniles released by a female in each replicate. Newborn juveniles were removed soon after their release from the brood pouch to avoid cannibalism. The number of eggs produced from a single brood increased with the age of the female. The number of broods produced during the life span of *E. chilkensis* ranged from 4 to 7 (Figure 6.3). It was observed that a female could produce at least four broods consecutively. Females are able to carry eggs at a size of 5–6 mm. During the study, only one set was observed with a maximum of 7 broods. Total number of juveniles produced in a brood ranged from 4 to 29 and the total number of juveniles from a single female varied from 35 to 139. Significant positive correlation was obtained between age of the female and number of juveniles from a single brood (Table 6.3). In amphipod population, two kinds of variation of fecundity are usually recognized: one related to size (size-specific fecundity) and the other to individual differences among females with the same size (scatter) (Cunha et. al. 2000).

**Biochemical composition**

Protein, carbohydrate, lipid and energy equivalents for all the three categories studied are given in table 6.4. Carbohydrate content was higher in all the three categories studied compared to protein content, the highest being in ovigerous females (87.95 mg. g⁻¹) and the lowest in juveniles (51.04 mg. g⁻¹). Protein showed a decreasing trend during individual growth- higher in juveniles (27.12 mg. g⁻¹) and lower in adults (20.74 mg. g⁻¹). Lipid content was higher in
ovigerous females (89.75 mg.g\(^{-1}\)) compared to adults and juveniles. Protein content presently recorded was less compared to observations on other amphipods (Nair and Anger, 1980). It has been observed by Nair and Anger (1980) that variation in carbohydrate did not follow any regular pattern with the availability of food.

6.2.3 Discussion

*E. Chilkensis* can be cultured on different types of feeds like dead and decaying animals, algal matter and detritus. They also exhibited cannibalistic behaviour in the laboratory, by feeding on their own young ones. They even ingested moulted exoskeleton, which indicates that this species requires a high nitrogen food source at times. A similar observation was made by Agnew and Moore, (1986) while studying the feeding ecology of two littoral amphipods. According to our observation, average ingestion rate of adults feeding on dried algal matter was found to be lower in females with brood compared to other adults. Body distension caused by cramed stomach or swollen ovaries might constrict the gut and impair feeding mechanically (Thruston, 1979).

Females lived longer than males and mortality was high towards the last weeks of the life span. Nair and Anger (1979a) found that female *Jassa falcate* (Leach) lived up to 3.6% longer, but grew slower, than males in the laboratory. Mortality was low in juveniles, but increased with age. Death of males after copulation was observed, which might be due to energy used up during the behavioural activities associated with breeding. Mortality occurred even during brooding in females, due to the energy used up during incubation, and was usually towards the end of the developmental stages of the embryo (7th to 9th day). The energy allocated for reproduction is divided between the reproductive output (brood size, egg volume) and behavioural activities associated with breeding (mating amplexus, brooding of embryos and juveniles). The above
activities can be substantial in amphipods and may vary with environmental conditions (Clarke, 1987).

Moulting rate was higher in females with less increment per moult compared to males. Males attained sexual maturity earlier than females. Nair and Anger (1979) reported that males and females of *Corophium insidiosum* moulted approximately at the same time until they reached sexual maturity after which the females moulted more frequently at regular intervals. Higher length increment per moult in males was also observed in this species. In *Ampithoe valida* females reached sexual maturity before males but males lived slightly longer than females (Pardal et al., 2001). Cunha et al., 2000(a) also reported that length increment per moult was higher in males of *Corophium multisetosum*.

There was a shift from an equal number of males and females at the time of attainment of sexual maturity to a female domination towards the end of the life cycle. Sexual differences in growth rate, longevity and rate of maturation may affect the apparent sex ratio (Moore, 1981). According to Moore (1981) sex ratios in populations can be either 1:1, skewed towards females or males, or may show seasonal variations. Female biased sex ratio towards the end of life cycle indicates an increased female longevity. When conditions are stable, any population would maintain an unbiased (1:1) sex ratio (Wildish, 1977).

Incubation period varied from 8 to 14 days and after hatching, the juveniles stay in the brood pouch for few hours before being released and were not allowed to re-enter the brood pouch. Nair and Anger (1979a) also reported similar type of brood protection in *Corophium insidiosum*.

The number of offsprings produced from a single brood varied with the age of the female. Significant positive correlation was found between the number of juveniles released and the age of the female. The number of eggs carried by a female amphipod generally increased at each successive moult (Fincham, 1974). Brood size in gammarideans is often found as being
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proportional to body length of females (e.g. Beare and Moore 1996; Costa and Costa, 1999; Persson, 1999; Cunha et al, 2000a). Age of the mother at the time of breeding influenced life cycle characteristics of its offspring namely attainment of sexual maturity in the next generation. In E. chilkensis, individuals from early brood attained sexual maturity earlier than those from late brood, but in Corophium insidiosum (Nair and Anger, 1979, a) individuals from late broods attained sexual maturity earlier. In E. chilkensis, there was no significant relation between age of the mother and duration of incubation. Again, this observation contradicted the observation in C. insidiosum wherein; early brood females incubated their offsprings for longer duration. Such contradictory observations may be due to phylogenetic and physiological constraints (Sainte-Marie, 1991).

Males and females of E. chilkensis attain different sizes at the same age, the males being larger. Based to our observations made, on attainment of sexual maturity, we found that females of E. chilkensis needs an average of 39 days to attain a size of 5mm whereas males attain a similar size within an average of 27 days. According to Sainte-Marie (1991), about 97% of gammarids show larger males compared to females. Growth rates were higher in the early phases, where it is commonly accepted that individuals grow exponentially (Welton and Clark, 1980). The maximum body size attained by individuals under laboratory conditions was smaller (15.2mm) compared to organisms in the field (17.76mm). Growth variations could also be associated with poor food quality. In addition to the quality of the food supplied, the small body size of individuals under laboratory conditions might be due to a decrease in the scope for growth in response to stress. For example, Chen et al. (1990) recorded lower growth rates of penaeid shrimps in culture due to deterioration of water quality caused by ammonia. Even in the present case, it may be possible that water quality suffered problems caused by ammonia since the excess food supplied was removed only once every week. Maximal body length attained in culture
experiments was about 30% smaller than observed in the field for both sexes and the difference in maximal number of eggs is up to 60% in Jassa falcata (Nair and Anger 1980).

The average protein content was less compared to other amphipods, whereas the carbohydrate content compare favorably with other species of amphipods (Nair and Anger, 1980). This might be due to the difference in the type of feed used during the experimental set up. Lipid content was comparatively higher in ovigerous females than adults and juveniles.

6.3 Population Dynamics

Population dynamics of E. chilkensis was studied from the monthly samples collected over a period of one year from mangrove rich area of Puduvypin. The mangrove ecosystem is mostly self-balanced and is mainly detritus based unlike the coastal system that is mainly based on multi variables. Since these detritus rich mangrove areas are used by fishes, crabs and oysters for their reproduction or growth, such swamps are considered of great economic importance for capture as well as captive fisheries. Mangroves of Puduvypin are dense and dominated by Avicennia officianalis, Exoecaria agallocha, Rhizophora mucronata, R. apiculata, Acanthens ilicifolius and Brugueira sp.

6.3.1 Materials and Methods

Amphipod Collection

Population density and dynamics of E. chilkensis was studied from the monthly samples collected over a period of one year (October 2003-September 2004) during the low tide from Puduvypin (9° 59' N and 76° 13' E), adjacent to mangrove vegetation (Figure 6.1). The selected area had a depth of 3-4 m and was influenced by tides. Population density of E. chilkensis was also estimated at the polluted regions of Cochin Estuary (9° 59’ N 76° 16’ E). The mud
samples were collected using van Veen grab (mouth area 0.048 m²), sieved using 500-μm screen and preserved in 4% formaldehyde coloured with Rose Bengal. From these samples *E. chilkensis* was separated, identified and counted in the laboratory as juveniles (< 5 mm) and adults (> 5 mm). Sediment grain size was analysed by pipette method (Krumbein and Pettijohn, 1966) and organic carbon was estimated by wet oxidation method (El. Wakeel and Riley, 1957). Organic matter was calculated as 1.724 times the carbon (Trask, 1955).

### 6.3.2 Results

#### Hydrography

Temperature, salinity, pH, dissolved oxygen, nitrate, nitrite, silicate, phosphate and ammonia, measured during different months are given in Figure 6.4 (a, b and c) and Figure 6.7. All the environmental parameters measured were subjected to considerable variations throughout the period of observation. Water temperature ranged from 27.5-34.0°C. High temperature was observed during the pre-monsoon period (February-May) and ranged from 27.5-34.0°C. In monsoon (June-September) the values were comparatively lower and ranged from 29.0-32.0°C and during post-monsoon (October-January) the temperature varied from 29.0-33.0°C. The salinity values ranged from 5.11 (June 2004) to 33.61 psu (February 2004). Comparatively low salinity was recorded during the period May-September and the value fluctuated between 5.11 and 13.23 psu. From December to April high salinity was recorded and it ranged from 21.37-33.61 psu, highest was recorded during February. In the post monsoon months October and November moderate salinity was recorded 18.71 and 16.68 psu respectively. pH ranged from 7.42 to 8.21 and D.O ranged from 2.43 ml/l (May) to 6.34 ml/l (March). Seasonal comparison showed that monsoon average in D.O was higher. Nitrate values ranged from 2.37μM (December) to 60.91μM (June) and nitrite ranged from 0.16-1.80μM, highest value recorded during July. Phosphate ranged from 3.33 to 19.75μM high value was recorded
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during July. Seasonal comparison showed that monsoon average was higher for nitrite, nitrate and phosphate values. Silicate ranged from 5.15 to 109.40 μM; high value was recorded during June 2004. Ammonia ranged from 4.90 to 35.32 μM and high value was recorded during March.

Sediment texture (Figure 6.5) was characterized by a high sand and clay fraction (mean: 43% and 38% respectively). The organic matter (Figure 6.6) in the sediment was generally high ranging from 16.6 (October 2003) to 72.74 mg g⁻¹ (February 2004).

Annual Cycle

The distribution and population dynamics of *E. chilkensis* are concordant with previous records of the amphipods in Cochin estuary (Nair et al. 1983). *E. chilkensis* was encountered in varying densities in the epifaunal community in the mangrove rich regions of Puduvypin (21-1583 ind. m⁻²). Changes observed in the proportion of adults and juveniles over a period of 12 months are shown as densities per m² (Table 6.5 and figure 6.8). These data were compared with the total gammarid population, contributed by *Eriopisa chilkensis*, *Corophium triaenonyx*, *Melita zeylanica*, *Quadrivisio bengalensis*, *Grandidierella gilesi* and *G. bonneri*. High densities of *E. chilkensis* have been attributed to its short life-span (4-8 months) and continuous possible gonad maturation. It attained large standing stock in these areas due to high fecundity and survival. *E. chilkensis* population underwent considerable fluctuation during the annual cycle. Laboratory culture of *E. chilkensis* showed that it breeds throughout the year, but in the field they were absent during February. The maximum population density of 1583 m⁻² was observed during November. A similar peak in density (20,416 ind. m⁻²) of total gammarid amphipods was also observed during the same month. Population density of gravid females ranged from 21 to 250 ind. m⁻² and high abundance was recorded during the month of November. Population density of juveniles ranged from 42-333 ind. m⁻² and high
abundance was recorded during December. High abundance of gravid females were followed by the peak in juvenile abundance in the very next month showing high recruitment. Juveniles were also not recorded during February and March. High population density coincided with a salinity of 16.68 p.s.u (November 03) and moderate organic enrichment, 35.34 mg g\(^{-1}\) (November 03).

*E. chilkensis* is found to tolerate a wide range of salinity (5.11-35.35 p.s.u) and temperature (27.5-34 °C). Gravid females were observed in high numbers during November and December (250 m\(^{-2}\); 229 m\(^{-2}\) respectively) when the salinity was around 16.68 and 28.85 p.s.u respectively indicating their preference for medium saline conditions during the breeding period, and were absent during February, March and June (Table 6.5, Figure 6.8). Highest number of juveniles (333 m\(^{-2}\)) was also recorded during the same period (November-December). It may be also possible that ovigerous females are under recorded due to egg spillages during fixation. Females with broods accounted for 13% of the total *E. chilkensis* population. A maximum of 46 eggs/brood was recorded during the field study. Juveniles were absent during February and March but were present during the low saline period (May-October), indicating their ability to tolerate low to moderate (5.11-18.71 psu) salinity conditions. Generally, there was lower number of males than females throughout the study period. Total number of males recorded during the study ranged from 21-458 ind. m\(^{-2}\).

*E. chilkensis* could tolerate wide salinity fluctuations whereas other gammarid amphipods were absent during this (March-May) period. (Figure 6.7, 6.9 and Table 6.5). In the laboratory *E. chilkensis* within the finger bowls were subjected to natural evaporation and experiments confirmed they could survive a change in salinity from 18.39 (initial) to 35.35 p.s.u (final) over four days and with no mortality. This implies that *E. chilkensis* could tolerate short duration salinity fluctuations in the field whereas the other gammarid amphipods moved out to locations of preferred salinity. The percentage contribution of *E.
Chapter 6 Population characteristics and life history of E.chilkensis

*E. chilkensis* to the total gammarid amphipods on an average was 45.91% (Table 6.5). Population dynamics in the organic-rich environment of Cochin estuary, adjacent to the sewage discharge site followed a similar trend with the high abundance in November (42-667 ind. m$^{-2}$).

### 6.3.3 Discussion

The distribution of *E. chilkensis* is concordant with previous records of the amphipods in Cochin backwaters (Nair et al., 1983). It was encountered in varying density in the epifaunal community of this estuary. *E. chilkensis* generally tolerates a wide range of salinity (5.22-32.64 p.s.u) and temperature (27.5-34°C), but preferred a range between 16 and 28 psu. The presence of ovigerous females throughout the year appears to be a widespread feature of amphipods (Mark and Alan, 1989). Asari (1983) also reported that *E. chilkensis* were found in bottom samples throughout the year. *E. chilkensis* could survive in water with D.O levels ranging from 2.43 to 6.34 ml l$^{-1}$ and ammonia ranging from 4.9 to 35.32 μM l$^{-1}$. Maximum density of *E. chilkensis* including the gravid females and juveniles were obtained during November and December, when the salinity values ranged between 16 and 28 psu, organic matter ranged between 35-37mg g$^{-1}$ and the sediment was a perfect mixture of sand and clay in almost equal proportion. According to Asari (1983) *E. chilkensis* was absent in sandy areas. Experimental studies carried out with *Corophium multisetosum* showed that temperature, salinity and sediment type affect survival, sexual maturation and number of offspring (Cunha et al. 2000b). Relative importance of physico-chemical parameters revealed that salinity was the prominent factor controlling the abundance of gammarid amphipods followed by temperature and dissolved oxygen (Nair et al., 1983).

*E. chilkensis* could tolerate wide range (euryhaline) and wide fluctuation in salinity when compared with other amphipods. Salinity is often considered less important for reproductive activities, but some studies have suggested that
Chapter 6 Population characteristics and life history of *E.chilkensis*

Salinity influences the brood sizes. Vlasblom and Bolier (1971) found that although eggs of *Echinogammarus marinus* could develop in salinities ranging between 4 and 7 psu, the number of juveniles produced was less. Cunha et al., (2000a) also reported the significance of salinity on the brood size in wild populations of *Corophium multisetosum*. When salinities do not vary significantly, other factors may determine amphipod distribution such as algal cover, shelter or interspecific relationships (Nicolaidou and Karakiri, 1989). Tanks used for mass culture contained *Corophium triaenonyx* and *E.chilkensis* and it was observed that in the presence of *Corophium triaenonyx*, the number of *E. chilkensis* decreased drastically, which may be due to competition for food and space. According to the observations made during the present study, *E. chilkensis* seek shelter under the algal covers, leafs and detritus which were added into the culture tanks. Our observations indicate that the population of *E. chilkensis* produces several generations per year.

Laboratory culture of *E. chilkensis* showed that it breeds throughout the year, but in the field during February they were completely absent. During this month high salinity (33.61p.s.u) and organic matter (72.74 mg g\(^{-1}\)) was observed. Salinity was found to be the prominent factor controlling the abundance of the gammarid amphipods, followed by temperature and dissolved oxygen (Nair et al., 1983). Our observation cannot be related with the high salinity (33.61psu), because experiments have shown that they could tolerate salinity up to 35.35 psu. Hence, such an observation may be attributed mostly to the high organic matter present during that month. This is in accordance with the classic model of Pearson and Rosenberg (1978), under which, increase in organic matter above a critical level causes hypoxia, smothering and change in physiochemical properties of the sediment and further leads to decreasing faunal density. The total benthic macrofauna was also less during this month. Nair et al., 1983 also reported high organic carbon and nitrogen content in sediments during the period of occurrence of gammarid populations. Remani et
al. (1980) observed that high organic carbon and C/N ratios in sediments seem to have no influence on the gammarid populations. A distinct density peak in *E. chilkensis* and total gammarid amphipods was observed in November. During this month the salinity was 16 p.s.u and organic matter was 35.34 mg.g\(^{-1}\). Steele and Steele (1975) observed a correlation between the abundance of organic matter in the sediment and the seasonal release of young in Gammarids of the North- Western Atlantic. The authors suggested that the release of young must coincide with the optimum conditions for their survival.

In Cochin estuary, *E. chilkensis* reproduce throughout the year showing continuous recruitment. Since *E. chilkensis* females produce broods consecutively (iteroparity) under laboratory conditions, based on the present study (duration of embryonic development, maturation and life span), one should expect six or seven generations annually, thereby exhibiting a multivoltine life cycle. Morino (1978) in his review stated that tropical species such as *M. zeylanica* in India breeds throughout the year. Steele and Steele (1991) pointed out that in tropics where there is a continuous supply of food, young ones could be produced continuously. Fenwick (1984) emphasized the importance of phylogenetic constrains and concluded that there is no simple optimum life history, but instead, a combination of different life-history traits may be equally successful in a given environment. This opinion was confirmed by Sainte- Marie (1991), who suggested that phylogenetic and physiological constraints should be best considered for the interpretation of gammaridean life history patterns.

Analysis of the gut contents of *Daysciaena albida* collected from the Cochin backwaters showed that amphipods and isopods formed the major part of their diet (Kurup and Samuel, 1988). Gammarids like *Anisogammarus pugettensis*, *Gammarus lacustrus* and *Gammarus tigrinis* have already been examined for their potential in fish culture. *Anisogammarus pugettensis* was proposed as an alternative to brine shrimp as food for young salmon (Chang
Chapter 6 Population characteristics and life history of *E. chilkensis*

and Parsons, 1975). *Gammarus lacustrus* in Hudson Bay meets dietary requirements for rainbow trout (Mathias et al., 1982). *Gammarus tigrinis*, a shoreline amphipod has been introduced into brackish streams as food for fishes (Reish and Barnard, 1979). Similarly *E. chilkensis* can be used as an alternative fish feed, as this gammaridean can tolerate wide range of temperature and salinity and consumes a wide variety of plant and animal material, in addition to scavenging dead and decaying organisms. They being resistant to handling stress can be easily mass cultured in the laboratory and used as natural feed.

*E. chilkensis* has been encountered in two different environments, moderately polluted regions (Harbour) and organic rich environments (Puduvypin). This shows that this organism can tolerate a wide range of environment in the estuary. In the Cochin backwaters, moderate enrichment of Zn, Cd, Pb, Fe and Cu have been reported and metal enrichment is high especially in case of Zn (x 25 – fold) and Cd (x 10 – fold) (Balachandran et al. 2005 and according to their study, the pollution in this region places the estuary as one among the impacted estuaries in the world. Sediment heavy metal contamination is a cause for concern as these metals undergo bioaccumulation and adversely affect the benthic organisms. Gammarid amphipods are used as biomonitors not only because they are net accumulators of trace metals, but they are relatively pollution tolerant, sedentary, abundant and easy to identify (Rainbow 1998). Here there is considerable scope for in-situ experimental studies and laboratory studies. Any impact on the reproductive and fecundity of these invertebrates may have significant ramifications on estuarine food chains and secondary production. The present study gives a basic knowledge about the life history and ecological aspects for evaluating the potential role of this amphipod as a test species in estuarine areas and to further develop assays for pollution monitoring at sub-lethal levels.
Fig. 6.1. Study region (Cochin backwaters) showing the sampling sites
Figure 6.2 *E. chilkensis* - Attainment of sexual maturity in the F1 (First) generation

Figure 6.3 *E. chilkensis* fecundity pattern mean ± S.D
Figure 6.4 Environmental conditions at Puduvypin
Figure 6.5 Variations in sediment texture at Puduvypin

Figure 6.6 Variations in organic matter in sediment at Puduvypin
Figure 6.7 Salinity variations, showing the most favourable and adverse range in salinity from March-May at Puduvypin

Figure 6.8 *E. chilkensis* total density, gravid females and juveniles
Figure 6.9 Percentage contributions of *E.chilkensis* towards other gammarid amphipods
Table 6.1 Life cycle criteria of *Eriopisa chilkensis*

<table>
<thead>
<tr>
<th>Life cycle criteria</th>
<th>M/F</th>
<th>Range</th>
<th>Mean (±S.D)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life span (days)</td>
<td>M</td>
<td>124 -175</td>
<td>132</td>
<td>22.88</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>170 - 220</td>
<td>189</td>
<td>20.84</td>
</tr>
<tr>
<td>Number of moult(s)</td>
<td>M</td>
<td>5 - 7</td>
<td>6.38</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>6 - 9</td>
<td>7.63</td>
<td>0.92</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>M</td>
<td>15.2</td>
<td>13.4</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8.56 - 14</td>
<td>10.38</td>
<td>1.79</td>
</tr>
<tr>
<td>Growth rate from 0 – 20 days (mm per day)</td>
<td>M/F</td>
<td>-</td>
<td>0.12</td>
<td>-</td>
</tr>
<tr>
<td>Broods per female</td>
<td>F</td>
<td>4 - 7</td>
<td>5.14</td>
<td>1.07</td>
</tr>
<tr>
<td>No. of juveniles per brood</td>
<td>F</td>
<td>4 - 29</td>
<td>13.72</td>
<td>7.39</td>
</tr>
<tr>
<td>Juveniles per female (sum)</td>
<td>F</td>
<td>35 - 139</td>
<td>72.16</td>
<td>39.74</td>
</tr>
<tr>
<td>Incubation time (days)</td>
<td>F</td>
<td>8 - 14</td>
<td>12</td>
<td>2.45</td>
</tr>
<tr>
<td>Sexual maturity of female (days)</td>
<td>F</td>
<td>30 - 49</td>
<td>39.25</td>
<td>5.99</td>
</tr>
<tr>
<td>Size (mm)</td>
<td></td>
<td>5.48 -</td>
<td>5.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Sexual maturity of male (days)</td>
<td>M</td>
<td>20 - 38</td>
<td>26.5</td>
<td>5.55</td>
</tr>
<tr>
<td>Size (mm)</td>
<td></td>
<td>5.08 -</td>
<td>5.05</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Table 6.2 *E. chilkensis* - mortality and sex ratio (‘*’ indicates total density in the finger bowls)

<table>
<thead>
<tr>
<th>Culture set</th>
<th>Juveniles</th>
<th>Mortality of Female with brood</th>
<th>Male after copulation</th>
<th>Sex ratio at maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (8*)</td>
<td>1 (12%)</td>
<td>1 (50%)</td>
<td>1 (20%)</td>
<td>M 5  F 2</td>
</tr>
<tr>
<td>B (9*)</td>
<td>1 (11%)</td>
<td>1 (25%)</td>
<td>0</td>
<td>M 4  F 4</td>
</tr>
<tr>
<td>C (25*)</td>
<td>9 (33%)</td>
<td>0</td>
<td>1 (12%)</td>
<td>M 8  F 8</td>
</tr>
<tr>
<td>D (24*)</td>
<td>2 (8%)</td>
<td>0</td>
<td>1 (9%)</td>
<td>M 11 F 11</td>
</tr>
<tr>
<td>E (22*)</td>
<td>2 (9%)</td>
<td>1 (12%)</td>
<td>2 (16%)</td>
<td>M 12 F 8</td>
</tr>
</tbody>
</table>

Table 6.3 Correlation between age of the females and fecundity

<table>
<thead>
<tr>
<th>Culture set</th>
<th>n</th>
<th>r</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>0.885272*</td>
<td>P &gt; 0.01</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>0.738187*</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>0.492465</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>0.860559*</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>0.809762*</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 6.4 *E. chilkensis* major biochemical composition and energy contents in adults, juveniles and ovigerous females.

<table>
<thead>
<tr>
<th></th>
<th>Juveniles</th>
<th>Adults</th>
<th>Ovigerous females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (mg. g⁻¹)</td>
<td>51.04</td>
<td>71.57</td>
<td>87.95</td>
</tr>
<tr>
<td>Protein (mg. g⁻¹)</td>
<td>27.12</td>
<td>20.74</td>
<td>21.38</td>
</tr>
<tr>
<td>Lipid (mg. g⁻¹)</td>
<td>63.05</td>
<td>70.25</td>
<td>89.75</td>
</tr>
<tr>
<td>Energy equivalent (KCal. g⁻¹)</td>
<td>0.93</td>
<td>1.03</td>
<td>1.31</td>
</tr>
</tbody>
</table>
Table 6.5 Population densities of total gammarid amphipods and *E. chilkensis* in (No.m\(^{-2}\)) as observed from the field data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Total number of (No.m(^{-2}))</th>
<th>Gammarid amphipods</th>
<th><em>E. chilkensis</em> (No.m(^{-2}))</th>
<th>Percentage contribution of <em>E.chilkensis</em> among other gammarids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Gravid females</td>
</tr>
<tr>
<td>Oct-03</td>
<td>3250</td>
<td>250</td>
<td>63</td>
<td>83</td>
</tr>
<tr>
<td>Nov-03</td>
<td>20416</td>
<td>1583</td>
<td>458</td>
<td>250</td>
</tr>
<tr>
<td>Dec-03</td>
<td>1646</td>
<td>1063</td>
<td>313</td>
<td>229</td>
</tr>
<tr>
<td>Jan-04</td>
<td>3605</td>
<td>667</td>
<td>229</td>
<td>63</td>
</tr>
<tr>
<td>Feb-04</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar-04</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Apr-04</td>
<td>271</td>
<td>271</td>
<td>83</td>
<td>21</td>
</tr>
<tr>
<td>May-04</td>
<td>271</td>
<td>271</td>
<td>83</td>
<td>42</td>
</tr>
<tr>
<td>Jun-04</td>
<td>605</td>
<td>292</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>Jul-04</td>
<td>459</td>
<td>271</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>Aug-04</td>
<td>1666</td>
<td>458</td>
<td>104</td>
<td>125</td>
</tr>
<tr>
<td>Sep-04</td>
<td>3459</td>
<td>563</td>
<td>167</td>
<td>104</td>
</tr>
</tbody>
</table>
Plate 6.1 Tanks of the recirculating system used for mass culture of Eriopisa chilenensis

Plate 6.2 Finger bowls used to maintain isolated culture

Plate 6.3 Enlarged view of the finger bowl with E.chilenensis
Plate 6.4 Chara sp. (used as feed)
Plate 6.6 Female with brood

Plate 6.7 Enlarged view of the brood plates /lamellae
Plate 6.8 Different stages during the embryonic development of *E. chilkensis*