CHAPTER III
THE POSTEMBRYONIC DEVELOPMENT OF
ALLODIAPTOMUS MIRABILIPES

III.a. Introduction

Copepods are ancient arthropods, abundant in tropical waters (Battish, 1992). Though abundant, their species diversity is low for various reasons. One of the major reasons is the uniform climatic conditions; another reason is the abundance of blue green algae, which is poor food for the zooplankton, as they are poorly utilized (Bernandi and Giussani, 1990). Predation is high in the tropics. Species that are small and highly agile succeed most in the tropics, because they can escape predation (Fernando, 1980). Copepods have a complicated lifecycle and some species are able to diapause. This has helped them colonise many habitats and have become one of the most successful metazoans on earth (Hardy, 1970).

Copepods reproduce sexually, and among crustaceans they exhibit the most complete example of metamorphosis. Their development is anamorphic, which refers to successive addition of somites and appendages at each moult.

Copepods are sexually dimorphic, with the males being smaller than females. Morphological differences also occur, in the antennules, P5 and urosome segmentation and structure (Keifer, 1932a). The strategies used by males to pursue females vary in different orders of copepods. In calanoids, the male on finding the female encircles her and catches her by the furca, using the geniculated
right antennules. Then the male encircles the female’s abdomen using the terminal claw of right P5. Using the left P5 it fastens the freshly expelled spermatophore on the genital segment near the genital aperture. Mating can last from minutes to hours, depending on species. Calanoid females carry one to several spermatheca as a result of several matings, however only one spermatheca discharges its contents. In cyclopoids and harpacticoids the female is held by both the geniculate antennules and P6 used to transfer the spermatophore (Sheriff and Altaff, 1993; Dussart and Defaye, 2001; Williamson and Reid, 2001). Cyclopoid and harpacticoid females can store eggs and produce several clutches of eggs in the absence of male, but calanoids must mate repeatedly to produce several clutches.

The development time from the extrusion of egg to its hatching in subitaneous eggs (nondiapausing) varies from one to five days and development from egg to adult takes about one to three weeks. Calanoids and harpacticoids are able to produce two types of eggs: subitaneous and resting (diapausing) based on environmental conditions. Resting eggs in some species can survive several years. In the case of subitaneous eggs, after fertilization the eggs are either shed into the water or retained in egg sacs. Eggs are usually spherical, protected by a chitinous envelope and contain vitelline reserves. The fertilized egg immediately enters into embryonic development that entails a series of divisions of primodial cells, taking place at intervals of 45 to 60 minutes. The earlier divisions are synchronous and it is the later divisions that bring about species differences. The cell division at the gastrula stage determines the larval shape and appendages’ structure. Many factors influence embryonic development, the two important ones being, temperature and food. After embryonic development is complete, the egg sac is
dropped by the female and the nauplii hatch together. The next stage in development is referred to as postembryonic development. The adult lifespan varies from one to several months. Development time and lifespan increase in lower temperatures (Herzig, 1983). On attaining maturity copepods begin to produce viable eggs within a few days, and under favourable conditions an adult female can produce several clutches of eggs in her lifetime.

The lifecycle of copepods consists of six naupliar stages (N1 to N6) followed by six copepodid stages (C1 to C6), of which the sixth is the adult stage (Williamson and Reid, 2001). The egg hatches into an unsegmented larva called orthonauplius, with three pairs of appendages (antennules, antenna and mandible), two caudal setae or spines and a somewhat rounded body. The following five unsegmented naupliar stages are referred to as metanauplii. During the development of the body in the naupliar stages, each moult results in a bigger, more elongate nauplius larva with new appendages added to the already existing ones and in the process making them more specialized. A pronounced metamorphosis occurs between the N6 and the first copepodid instar (C1). Copepodids are morphologically more similar to adults, with the body being divided into prosome and urosome bearing caudal rami. Development is determinate and the adults do not moult.

Reproductive biology of calanoids has been studied from the 19th century. A few of the pioneers in the field are Jurine (1820), Dietrich (1915) and Gurney (1931) (Devi, 1985). Copepod biology is less studied in comparison to taxonomic studies. In India though taxonomic studies have been done on copepods, not much
work has been carried out on their life cycle (Devi and Reddy, 1989a). Among the freshwater diaptomids of India, the two most common diaptomid genera are, *Heliodiaptomus* and *Allodiaptomus*. Of these, the former, *Allodiaptomus* occurs abundantly in Kerala and Karnataka states of South India and *Heliodiaptomus* more abundant in the other south Indian states. Of the species of *Allodiaptomus*, *A. mirabilipes* is endemic to the southern states of India, especially Karnataka and Kerala (Reddy, 1994). Studies on the postembryonic development of a few diaptomids have been done in India, but it is scanty when considering the number of species reported. (Reddiah, 1965; Devi and Reddy 1990a and b). Larval characters are important taxonomic tools in studying the phylogeny of ambiguous groups.

*Allodiaptomus mirabilipes* was originally described by Keifer (1936a) from a female specimen collected from an unidentified location in Karnataka State, South India. He later realised that he had erroneously described *Heliodiaptomus cinctus* as *Allodiaptomus mirabilipes* and accordingly corrected it in his publication of 1936b. Brehm in 1953 also gave a brief description of *A. mirabilipes*, but it was Abraham (1972) who re-described these two species and tabulated their differences. Reddy (1987) has added more detailed characters to *A. mirabilipes*, in his redescription of the genus *Allodiaptomus*. *A. mirabilipes* generally inhabits rice fields and ponds fed by surface run-off from nearby rice fields and irrigation ponds (Reddy, 1994).

*A. mirabilipes* was present in all the surface water samples from MRB and was the only calanoid representative of the study area. Nothing is known of its
postembryonic development or larval morphology; hence an attempt has been made to study its postembryonic development.

### III.b. Literature Review

The pioneering work on calanoid development was done by Jurine in 1820. There have been many studies since then on the continental calanoids (Dussart and Defaye, 2001). Notable among these are the investigations carried out by Gurney (1931) who described the larvae of several continental copepods. Wilson (1932) described the fifth copepodid instar of *Diaptomus oregonensis* and Comita and Tommerdahl (1960) worked on the biology of *Diaptomus siciloides*. Comita and McNett (1976) studied the developmental biology of *Diaptomus oreconensis* and cited the work of Grandori in 1912 that provided the first somewhat complete study of the larval instars for a species, *Diaptomus vulgaris*. Cole (1953) carried out studies on copepod encystment. Published work on calanoid development up to about 1967 has been reviewed by Hutchinson (1967) in his, ‘Treatise on Limnology’.

There is however a paucity of literature on the developmental biology of freshwater calanoids in the Asian subcontinent. The calanoids of the family Diaptomidae abound in the inland waters of this region (Reddy, 1994). Published works are a plenty on their systematics, but their biology has not been well documented (Devi, 1985). Work on diaptomid copepods is very scanty. Reddiah (1965) gave the description of the last copepodid instar of *Tropodiaptomus lakhimpurensis*, *Arctodiaptomus shillongensis* and *Neodiaptomus kamakhiae*. Radhakrishna and Reddy (1977) described the developmental stages of
Heliodiaptomus viduus. Developmental stages of Pseudodiaptomus binghami, its growth and sex ratio was reported by Goswami (1978). Sehgal (1983) also described the developmental stages of Heliodiaptomus viduus and Neodiaptomus strigilipes. The descriptions by Sehgal are very brief and diagrams are a few to substantiate the description. Reddy and Devi (1985) described the complete postembryonic development of Megadiaptomus hebes. The complete postembryonic development of seven other Indian calanoids have also been studied they being; Heliodiaptomus contortus (Reddy and Devi, 1989), Allodiapptomus raoi (Devi and Reddy, 1989a), Phylodiapptomus greeni (Devi and Reddy, 1989b), Heliodiaptomus cinctus (Reddy and Devi, 1990a), Phylodiapptomus blanci (Reddy and Devi, 1990b), Neodiaptomus lindbergi (Devi and Reddy, 1990a) and Tropodiapptomus informis (Devi and Reddy, 1990b), with samples collected from Andhra Pradesh, India. McLaren et al., (1987) worked on the temperature dependant development in Pseudocalanus species. Dahms and Fernando (1993) described the naupliar development of Phylodiapptomus annae from Sri Lanka. Sheriff and Altaff (1993) studied the feeding in the planktonic copepod Heliodiaptomus viduus.

There are few studies on the effect of food and temperature on copepod lifecycle. Singh (1972) studied the diurnal variations in food selection and feeding properties of the freshwater calanoid Rhinediapptomus indicus Kiefer. Kamps (1978) studied the effect of temperature on the development time and brood size of Diaptomus pallidus. Jacobs and Bouwhuis (1979) worked out the lifecycle of Eudiaptomus vulgaris, and studied how temperature influences its developmental stages in the natural habitat. Elmore (1982) studied the influence of food
concentration and container volume on life history parameters of *Diaptomus dorsalis* from subtropical Florida. Herzig (1983) studied the ecological significance of the relationship between temperature and duration of embryonic development in planktonic freshwater copepods. Klein-Breteler and Gonzalez (1988) studied the influence of temperature and food concentration on body size, weight and lipid content of two calanoid copepod species. Vijverberg (1989) reviewed the culture techniques used for the growth, development and reproduction of copepods and cladocerans under laboratory and *in situ* conditions. Abdullahi (1992) studied the effect of diet on growth and development of three species of cyclopoid copepods. Nishikawa and Maly (1996) discussed the factors that influence the degree of sexual dimorphism within and among calanoid copepod species.

**III.c. Methodology**

Ovigerous females were sorted out from live plankton samples collected from SS – 5 (the garden pond in Nirmala College campus Kerala State, South India). Location: (9° 59’09.96” N and 76° 34’24.06”E). The samples were collected using plankton net of 68μm. mesh size. Each ovigerous female was introduced into an embryo cup containing 30 ml. pre-treated pond water (filtered, boiled and cooled). Gravid females were removed immediately after hatching of first nauplius (NI). No artificial food was given during naupliar stages. When copepodids emerged they were transferred to finger bowls containing 125 ml. pre-treated pond water and fed with centrifuged algal scum (Elmore, 1982). For every instar 20 specimens were collected, anesthetised in 2% ethyl alcohol and fixed in
5% buffered formalin. The specimens were stained in methylene blue, measured 10 specimens of each instar, and dissected them in glycerol medium using stereoscopic dissection microscope. The dissected parts and whole mounts of the larval stages were fixed on slides using bees wax. The fixed dissected parts and whole mounts were drawn to scale using camera lucida as described by Devi (1985), and Devi and Reddy (1985, 1989a and 1990a) and Vijverberg (1989).

III. d. Results and Discussion

The ovigerous females carried a single medial egg sac containing 12-18 opaque, spherical eggs. The eggs were without any surface ornamentation. The diameter of the eggs ranged from 0.07 to 0.09mm (Fig.19b). Adult females were often seen with one, rarely two spermatophores attached to their genital pore. The spermatophores (Fig. 19a) extended just beyond the caudal setae.

The post embryonic development included six naupliar stages and six copepodid stages, the sixth copepodid stage being the adult. Postembryonic development was completed in ambient temperatures of 28-34°C. Nauplius I (NI) larva was observed in less than 24 hours after the eggs were released. Almost all the eggs were released at the same time from the egg sac. The time required for development was about 8 hours for Nauplius I, 10 hours each for Nauplius II and III, 24 hours for Nauplius IV to VI and copepodid V. Copepodid stages I to IV took about 48 hours each. The adults were observed on the 14th day.

(In the figures 19 to 36 setules have been omitted to avoid confusion. All setae bear setules unless otherwise mentioned)
III.d.1. **Nauplius I** (Fig.19c): (N I)

Body length 0.110-0.113 mm; width 0.069-0.071 mm. Body oval in shape. The first three pairs of cephalic appendages (antennule, antenna and mandible) present. A pair of plumose caudal setae of equal length present at the rounded posterior end.

Antennule (Fig.19d): 2 segmented and uniramous. Both segments of almost equal length. The basal segment bears 3 setae. The distal segment bears 3 terminal setae, of which the central one is more slender than the two lateral ones. On the distal end of the dorsal side 2 small spinules present.

Antenna (Fig.19e): biramous. Coxa bears one seta and the basis bears 3, two of which are proximal and one distal. The exopodite is indistinctly 5 segmented with 1, 1, 1, 1 and 2 setae respectively. Of the two distalmost setae one is slender. The endopodite is unsegmented and reaches upto the proximal segment of exopodite. It bears 2 subterminal unequal setae and 2 terminal strong setae.

Mandible (Fig.19f): biramous. The coxa and basis almost fused, they can be distinguished by their lateral indentation. Coxa bears 1 seta and basis 2 setae; all three are strong, equal length and short. The exopodite is faintly 3 segmented with 2, 1, and 2 setae, of which one is slender. The endopodite is unsegmented, slightly rounded and shorter than exopodite, bearing 6 setae in three pairs.

III.d.2. **Nauplius II** (Fig.20a)

Body length: 0.134-0.14mm. width 0.70-0.72mm. The first three pairs of cephalic appendages more distinct. A pair of caudal plumose setae of equal length present at the now bilobed posterior end. The two lobes and setae are of equal length.
Antennule (Fig.20b): three segmented with 1, 2 and 3 setae. The seta on basal segment almost 2/3 length of second segment. The ventro-terminal seta of distal segment slender as long as segment and without setules, the other two longer than the segment.

Antenna (Fig. 20c): coxa with a strong lanceolate, masticatory (Devi and Reddy, 1989a) seta and 1 slender, smooth seta. As in the earlier stage the basis bears three setae, and the proximal two setae are strong. Exopodite faintly 5 segmented with 2, 1, 1, 1 and 2 unequal setae. Endopodite unsegmented with 2 inner and 3 terminal setae.

Mandible (Fig.20d): Coxa as in preceding stage. Basis with 2 unequal divergent setae. Exopodite slightly longer than endopodite and is 4 segmented, with 2, 1, 1 and 2 unequal setae. Endopodite unsegmented, bearing 8 setae in groups of 3, 2 and 3.

III.d.3. Nauplius III (Fig.21a)

Body length: 0.172mm. Maximum width 0.074-0.079 mm. Body oval in shape and posterior end of body with two transverse rows of small spinules laterally. The buds of the next cephalic appendage make its appearance. 2 pairs of unequal caudal plumose setae present at the bilobed posterior end.

Antennule (Fig.21b): basal two segments as in preceeding stage. The seta on basal segment slightly shorter in length than the succeeding segment. The distal seta of second segment longer than the distal segment. Terminal segment bears 6 setae, of which one is short, slender and bare. This seta is observed in all succeeding
naupliar stages. A sharp cuticular extension is also observed. The dorsal surface bears 3 spinules.

Antenna (Fig.21c): coxa with 2 strong, masticatory, divergent setae. The proximal setae on the basis unequal in length. Exopodite faintly 5 segmented with 3, 1, 1, 1 and 2 unequal setae. Endopodite unsegmented with 3 inner and 4 terminal setae.

Mandible (Fig.21d): coxa as in preceeding stage, basis with 3 setae, the distal one shorter and slender. Exopodite shorter than endopodite, with 2, 1, 1 and 2 setae. Endopodite unsegmented, bearing 9 setae.

Maxillule: simple lobe with feather like seta.

III.d.4. Nauplius IV (Fig.22a)

Body length 0.21-0.24mm. Maximum width 0.091-0.096mm. Body further elongated in shape. A transverse septum at the posterior end of the body. The row of transverse spinules more prominent. The posterior armature as in preceeding instar.

Antennule (Fig.22b): first two segments with one and two setae as in previous instar. Third segment with 9 setae. Spinule ornamentation as in earlier instar.

Antenna (Fig.22c): coxa bears 2 setae. Basis with 3 unequal setae proximally and 2 unequal setae distally. Exopodite 6 segmented with 10 setae in all. Endopodite as in earlier instar with 3 lateral and 4 distal setae.

Mandible (Fig.22d): further enlarged. Coxa converted into a masticatory process with about 4 teeth and 1 seta. Basis bears 4 setae. Exo and endopodite of almost
equal length. Exopodite 4 segmented with 2, 1, 1 and 2 setae. Endopodite unsegmented, with 8 setae in all.

Maxillule (Fig.22e): biramous. Exopodite shorter than endopodite. Exo and endopodite with 3 and 5 setae respectively. Outermost seta of exopodite strong and spinose.

III.d.5. Nauplius V (Fig.23a)

Body length 0.23-0.27 mm. Maximum width 0.130-0.135 mm. Posterior end of body further elongated, caudal lobes of equal size. Spinule armature present. Maxillary buds make their appearance. 3 pairs of unequal caudal plumose setae now present.

Antennule (Fig.23b): first two segments as in earlier instar. Third segment oar shaped with 11 setae. The cuticular structure observed in earlier stages present towards central distal end of third segment. Spinule pattern as in earlier stages.

Antenna (Fig.23c): further increase in size. Coxa and basis as in earlier instar, with 3 and 5 setae. Endopodite bears 4 inner lateral and 5 terminal setae. Exopodite 6 segmented with, 5, 1, 1, 1, 1 and 2 setae.

Mandible (Fig.23d): further enlarged; mostly similar to N IV. Exopodite slightly shorter than endopodite. Exopodite as in earlier instar, 4 segmented, segments bearing 2, 1, 1 and 2 setae. Endopodite bears 10 setae in all.
Maxillule (Fig.23e): biramous. Exopodite shorter than endopodite, bearing 4 setae. The second proximal seta of exopodite spinose. Endopodite bears 5 setae. The coxal and basal laciniae observed, with 2 and 1 setae respectively.

Maxilla (Fig.23f): a simple lobe with 2 unequal setae.

III.d. 6. **Nauplius VI** (Fig.24a)

Body length: 0.30-0.34 mm. Maximum width 0.113-0.114 mm. Body highly elongated. All the cephalic appendages have made their appearance. The buds of the first two swimming legs also present. Posterior part of body 2 segmented. The armature of posterior end of NVI as in earlier instar.

Antennule (Fig.24b): first two segments as in preceeding stage. Third segment with 13 setae and its distal end slightly tapering.

Antenna (Fig.24c): coxa and basis as in preceeding stage with 3 and 5 setae. Exopodite 6 segmented, with 12 setae and much longer than endopodite. Endopodite unsegmented and with 10 setae.

Mandible (Fig.24d): endopodite unsegmented and bears 11 setae. Exopodite 4 segmented as in earlier instar with 2,1,1 and 2 setae from proximal to distal segments.

Maxillule (Fig.24e): Praecoxa with 3 setae. Coxal and basal laciniae with 2 setae each. Exopodite much shorter than endopodite, setation as in N V. Endopodite with 5 setae.

Maxilla (Fig.24f): seven median lobes and 13 setae.
Maxilliped (Fig. 24g): a small elongated lobe with 2 unequal setae at tip.

First swimming leg (Fig. 24h): biramous. An indistinct division between coxa and basis observed. Exo and endopodites single segmented with 4 and 3 setae.

Second swimming leg (Fig. 24i): as in first swimming leg, but exo and endopodites with 3 and 2 setae.

Nauplius VI metamorphosed into copepodid I. Copepodids have elongate and cylindrical bodies, similar to that of the adult of the species, but smaller in size. The body divided into two regions, prosome and urosome. The articulation is between the fifth segment of the prosome and first segment of urosome in all copepodids and the adult. The time span and body length of each instar of the nauplius is given in table 10. The morphometry of the copepodid instars and the adults are given in table 11.

Table 10. Duration, length and anamorphic addition of appendages in Naupliar instars

<table>
<thead>
<tr>
<th>Instar (n=10)</th>
<th>N I</th>
<th>N II</th>
<th>N III</th>
<th>N IV</th>
<th>N V</th>
<th>N VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (Hours)</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>0.110</td>
<td>0.134</td>
<td>0.172</td>
<td>0.230</td>
<td>0.265</td>
<td>0.320</td>
</tr>
<tr>
<td>Cephalic appendages</td>
<td>Antennule Antenna Mandible</td>
<td>Antennule Antenna Mandible</td>
<td>Antennule Antenna Mandible Maxillule</td>
<td>Antennule Antenna Mandible Maxillule Maxilla</td>
<td>Antennule Antenna Mandible Maxillule Maxilla</td>
<td></td>
</tr>
<tr>
<td>Thoracic appendages</td>
<td>Maxilliped</td>
<td>1st and 2nd swimming legs</td>
<td></td>
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Table 11. Comparative morphometry of Copepodid instars

<table>
<thead>
<tr>
<th>Instar (n=10)</th>
<th>C I</th>
<th>C II</th>
<th>C III</th>
<th>C IV</th>
<th>C V</th>
<th>C VI</th>
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<tr>
<td>total length (mm)</td>
<td>0.45</td>
<td>0.52</td>
<td>0.63</td>
<td>F - 0.87</td>
<td>F - 0.98</td>
<td>F - 1.23</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M - 0.75</td>
<td>M - 0.85</td>
<td>M - 1.19</td>
</tr>
<tr>
<td>Ratio Cephalon/Metasome</td>
<td>1.9</td>
<td>1.6</td>
<td>1.3</td>
<td>F - 1.14</td>
<td>F - 1.04</td>
<td>F - 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M - 1.16</td>
<td>M - 1.06</td>
<td>M - 0.75</td>
</tr>
<tr>
<td>Ratio Prosome/Urosome</td>
<td>3.09</td>
<td>2.73</td>
<td>2.54</td>
<td>F - 2.68</td>
<td>F - 2.54</td>
<td>F - 2.53</td>
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<td></td>
<td></td>
<td></td>
<td>M - 2.54</td>
<td>M - 2.43</td>
<td>M - 2.20</td>
</tr>
<tr>
<td>Ratio Caudal Ramus L/W</td>
<td>1.6</td>
<td>1.64</td>
<td>1.54</td>
<td>F - 1.66</td>
<td>F - 1.78</td>
<td>F - 1.96</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>M - 1.75</td>
<td>M - 2.00</td>
<td>M - 2.50</td>
</tr>
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III.d.7. Copepodid I (Figs.25 and 26)

Prosome 5 segmented and urosome unsegmented (Fig.25a and 26d). Maximum body width near posterior end of cephalothorax. Cephalothorax bears five cephalic appendages. They are similar in structure to the adult appendages, but differences are observed in segmentation and setation. Four pairs of swimming legs present. The caudal rami do not bear any hairs/setules/spines, and are bare with 5 + 1 unequal setae. This stage lasted for about 48 hours.

Antennule (Fig.25b): 11 segmented, it reaches up to the midlength of the caudal ramus, both antennules similar in structure.

Antenna (Fig.25c): the coxa and basis are fused, bearing one and two setae. Exopodite weakly divided into six segments, with 1, 3, 1, 1, 1, and 5 setae. The
endopodite two segmented. The basal segment with 2 setae and terminal segment with 4 + 6 setae and do not bear any spinules.

Mandible (Fig. 25d): biramous in nature. The gnathosomal structure blade-like and chitinized. It bears 7 fine teeth and a small seta. The basis bears 3 setae. The exopodite four segmented with 2, 1, 1 and 2 setae. The endopodite is indistinctly divided into two segments. The basal segment bears 3 setae and terminal segment with 5 unequal setae.

Maxillule (Fig. 25e): The praecoxa bears 7 spiniform and 3 normal setae with setules. The coxalexite with 4 long and thick setae. Coxal and basal laciniae with 3 and 2 setae. Basal posterior lacinia bears 1 seta, and is small in size. Basis bears two setae. The exopodite bears 4 setae at its distal end and the endopodite bears 5 setae at its tip.

Maxilla (Fig. 25f): It bears 6 endites with 3, 3, 3, 3, 3, and 1 setae. The fifth endite is the largest. The endopodite is unsegmented bearing 4 setae.

Maxilliped (Fig. 25g): uniramous. The coxa and basis are distinct. The coxa bears three lobes along inner margin with 1, 2 and 2 setae from proximal to distal region. Of the three lobes the third is the largest and is spinose distally. The basis is slightly dialated distally and bears 2 setae and its inner margin bears short hair like structures proximal to the setae. Endopodite is two segmented. The basal segment is large and bears 1 seta distally and the distal segment much smaller with 4 setae distally.

Swimming legs (Fig. 26a - c): Four pairs of swimming legs present.
First swimming legs (Fig.26a): are paired and biramous. The coxa and basis do not bear any setae or spinous processes. Though exopodite and endopodite are present they are unsegmented. The exopodite is slightly longer than endopodite. The exopodite bears a terminal spine and 2 terminal and 2 inner setae. The endopodite 2/3 length of exopodite and bears 1 outer, 3 terminal and 2 inner setae.

Second swimming legs (Fig.26b): are similar to the first pair, but slightly smaller in size.

Third swimming leg (Fig.26c): is a bilobed rudiment, outer lobe with 2 setae and inner lobe with 2 setae.

Fourth swimming legs are small, bare, lobed rudiments.

III.d.8. **Copepodid II** (Figs.27 and 28)

Larger than copepodid I. The prosome is five segmented urospine (Figs.27a and 28e) is two segmented. Caudal rami similar to earlier stage both in structure and setation. This stage was observed for about 48 hours.

Antennule: (Fig. 27b) reaches uptomidlength of the caudal ramus and is 18 segmented. Segments 3 to 6 are ill defined. A1 of both sides identical. Aesthetasc are present on segments 1, 2, 3, 7, 9 and 10. 16th and 17th segments bear two setae each.

Antenna (Fig.27c): Terminal exopodite segment with 11 setae and first endopodite segment with spinules, otherwise as in copepodid I.
Mandible (Fig.27d): The cutting edge now bears 8 fine teeth and one seta. Exopodite as in previous stage. Basis and first endopodite segment with 3 setae each. Second endopodal segment with 7 unequal setae.

Maxillule (Fig.27e): the praecoxa bears 11 setae. The coxal exite with 6 setae. Coxal and basal laciniae with 3 setae each. Basal posterior lacinia as in copepodid I. Basis bears two setae. The exopodite bears 4 setae at its distal end and the endopodite bears 5 setae as in earlier stage.

Maxilla (Fig.27f): it bears 6 endites with 5, 3, 3, 3, 4, and 1 setae. The fifth endite is the largest. The endopodite unsegmented bearing 5 setae.

Maxilliped (Fig.27g): the coxa bears four lobes along inner margin with 1, 2, 2 and 3 setae from proximal to distal region. Basis as in previous stage. Endopodite is three segmented, with 3, 1 and 4 setae.

Swimming legs (Fig.28a-d): Four biramous swimming legs present.

First swimming leg (fig.28a): the coxa bears one inner seta and basis is bare. Exopodite two segmented, basal segment with an outer spine. Distal segment with one outer spine 2 terminal and 3 inner setae. The endopodite unsegmented as in C1 with 7 setae. The exopodite is slightly longer than endopodite.

Second swimming leg (Fig.28b): coxa, basis and exopodite as in first leg. Endopodite two segmented; basal segment with one inner seta, distal segment with 2 outer, 2 terminal and 3 inner setae.
Third swimming leg (Fig.28c): coxa and basis unarmed. Both exo and endopodite unsegmented. Exopodite with 2 outer, 2 terminal and 2 inner setae and 2 outer spines. The endopodite with 2 outer, 2 terminal and 3 inner setae.

Fourth swimming leg (Fig.28d): seen as bilobed rudiments, outer lobe with 3 unequal setae, inner lobe with two setae.

III.d.9. Copepodid III (Figs. 29 and 30)

Larger than the earlier two copepodid stages, otherwise similar in shape. The prosome is 5 segmented and urosome three segmented (Figs.29a and 30f). First two urosomites of almost equal length and third urosomite segment longer than the first two urosomites combined. Caudal ramus as in previous stage. This stage too lasted for about 48 hours.

Antennule (Fig. 29b): extending beyond caudal ramus; 23 segmented. Right and left antennules not identical, segment 18 of right antennule bears a spine in addition to seta. Some specimens were observed to have 24 segments.

Antenna (fig.29c): similar to earlier stage, but terminal endopodite segment with 12 setae.

Mandible (Fig.29d): as in CIII

Maxilla (Fig.29e): as in CIII.

Maxillule (Fig.29f) – Praecoxa with 12 setae; coxalexite bears 8 setae. Other details of appendage as in CIII.
Maxilliped (Fig.29g): coxa with 4 endites bearing 1, 2, 3, and 4 setae. Basis with 3 setae. Endopodite 4 segmented with 3, 1, 2 and 4 setae respectively.

Swimming legs (Fig.30a–e): 5 pairs, the fifth being rudimentary.

First swimming leg (Fig.30a): coxa and basis similar to CII. Exo and endopodites 2 segmented. First exopodite segment bears 1 outer spine and 1 inner seta. Distal segment has one outer spine; 2 terminal and 3 inner setae. Endopodite basal segment with 1 inner seta, distal segment bears 7 setae.

Second swimming leg (Fig.30b): coxa and basis as in leg 1. Exo and endopodite 2 segmented. Exopodite-basal segment with 1 outer spine, 1 inner seta; distal segment with 2 outer spines; 2 terminal and 4 inner setae. Endopodite as leg 1, but distal segment with 7 setae.

Third swimming leg (Fig.30c): similar to leg 2.

Fourth swimming leg (Fig.30d): coxa unarmed and basis with an outer seta. Exo and endopodites unsegmented. Exopodite with 2 outer spines, 2 terminal and 2 inner setae. Endopodite with 1 outer, 2 terminal and 3 inner setae.

Fifth leg (Fig.30e): rudimentary, lobed and bare.

III.d. 10. **Copepodid IV** (Figs.31 to 33)

Sexual differentiation observed from this instar.

Female (Fig.31a): the prosome six segmented; the last two pedigers not distinctly demarcated and bears dorsal row of spinules. Urosome three segmented (Fig.32f);
first two segments of almost equal length and the last urosomite almost 2.25 times longer than the two preceding segments. The caudal rami as in earlier stages.

Antennule (Fig. 31b and c): It reaches beyond caudal ramus and is 25 segmented. Both antennules identical.

Antenna (Fig. 31d): same as earlier stage, except exopodite 7 segmented. The endopodite is two segmented and terminal segment with 13 setae.

Mandible (Fig. 31e): As in preceding stage. Second endopodite segment now armed with 8 setae.

Maxillule (Fig. 31f): The praecoxa bears 14 setae and the coxal exite 9. Coxal and basal laciniae bear 4 setae each. Basis bears 3 setae. The exo and endopodite bear 4 and 6 setae respectively.

Maxilla (Fig. 31g): as in preceding stage.

Maxilliped (Fig. 31h): As in preceding stage. Endopodite 5 segmented, with 4, 2, 1, 2 and 4 setae.

Swimming legs (Fig. 32 a-e): Five pairs, the fifth showing sexual dimorphism.

First swimming legs (fig. 32a): as in previous stage.

Second swimming legs (Fig. 32b): also as in previous stage, but second endopodite segment with 7 setae.

Third swimming leg (fig. 32c): coxa and basis as in previous stage. First exopodite with 1 outer spine and 1 inner seta, second with 2 outer spines, 2 terminal and 4
inner setae and hair on proximal margin. First endopodite segment unchanged; second with 3 outer, 2 terminal and 3 inner setae in addition to hair on proximal margin.

Fourth swimming leg (Fig. 32d): coxa with 1 inner seta. Basis unarmed. Exo and endopodites two segmented. Basal exopodite segment with 1 outer spine and 1 inner seta, distal segment with 2 outer spines, 2 terminal and 4 inner setae.

Fifth swimming leg (Fig. 32e): coxa unarmed. Basis with one seta. Exo and endopodites twice as long as broad. Exopodite with two unequal terminal spines, outer spine being shorter. Endopodite almost 2/3 longer than exopodite with rounded spinous tip.

**Male** - the prosome is six segmented; spinules on last two segments as in female. Urosome three segmented (33c). The caudal rami as in earlier stages.

Antennule (33a): extending beyond caudal rami. Left antennule 25 segmented and similar to that of female. Right antennule 22 segmented. Segments 17, 18 and 19 elongated.

All other appendages except fifth swimming leg as in female.

Fifth swimming leg (Fig. 33b): coxa unarmed. Basis without seta. Exopodite of right leg 1.3 times longer than left leg and with 1 outer strong apical spine, almost twice as long as its counterpart on left leg. It also bears an inner slender subapical spine which is almost equal to its counterpart. Endopodite of both legs 2/3 as long as exopodite and has a spinose apex.
III.d.11. **Copepodid V** (Figs.34 to 36)

Female (Fig.34a): the prosome is six segmented; the last two pedigers not distinctly demarcated and bears dorso-lateral row of spinules. Metasomal wings on either side of last prosomal segment. Each wing bears a small spine. Urosome with three segments (Fig. 35f). Genital somite and anal somite of urosome almost of same length. The genital somite bears a hyaline spine, one on either side of segment somewhat medially. The middle urosomite is almost half the length of the genital and anal urosomite. The caudal rami as in preceeding stage.

Antennule (Fig.34b): It reaches beyond caudal setae and is 25 segmented, both antennules identical.

Antenna (Fig.34c): same as earlier stage, except terminal enopodite segment with 15 setae.

Mandible (Fig.34d): As in preceeding stage, but first endopodite segment with 4 setae.

Maxillule (Fig.34e), Maxilla (Fig.34f) and Maxilliped (Fig.34g) as in preceeding stage.

Swimming legs (35a-e): First four almost similar. The fifth showing sexual dimorphism.

First swimming legs (Fig.35a): Coxa, basis and endopodite as in preceeding stage. Exopodite 3 segmented. Basal segment with 1 outer spine and 1 inner seta, second
segment with 1 outer spine and 1 inner seta, distal segment with 1 outer spine and 5 setae.

Second and third swimming legs (Fig.35b and c): coxa and basis as in preceding stage. Exopodite and endopodite three segmented. First and second exopodite segment with 1 outer spine and 1 inner seta, third with 1 outer spine, 2 terminal and 4 inner setae in leg 2; 3 terminal and 3 inner setae in leg 3. First endopodite segment with 1 inner seta, second with 2 and third with 2 outer, 3 terminal and 2 inner setae.

Fourth swimming leg (Fig. 35d): as in legs 2 and 3, except basis with a delicate seta at outer distal end.

Fifth swimming leg (Fig.35e): coxa and basis as in preceding stage. First exopodite segment 2.28 times longer than second one; second segment bears 2 small hyaline spinules disto-laterally and 2 unequal apical hyaline spines; outer stumpy and inner pointed. Endopodite extending upto the end of the first exopodite segment and armed with spinous tip.

**Male:** the prosome is six segmented; spinules on last two segments as in female prosome. Urosome three segmented (Fig.36c). The caudal rami as in earlier stages.

Antennule (Fig.36a): extending beyond caudal rami.

All other appendages except fifth swimming leg as in female.
Fifth swimming leg (Fig. 36b): coxa and basis as in preceeding stage. Exopodite 2 segmented and right exopodite 1.3 times longer than the left. It bears 1 outer strong apical spine, almost twice as long as its counterpart on left leg. It also bears an inner slender subapical spine larger than its counterpart. Endopodite of right leg reaches beyond first exopodite segment and is twice as long as that on the left. Left endopodite is 0.7 times shorter than first exopodite segment. Both endopodites have a spinose apex.

III.d.12. Adult female (Figs. 1 to 3 in Chapter II)

Prosome six segmented. Metasomal wings on either side of last prosomal segment. The left wing longer, extends upto the spine on the genital segment; is ovate bearing 2 hyaline spines. The right metasomal wing shorter, broader and bears 1 terminal and 1 subterminal spine. Urosome 3 segmented. The basal segment being the genital somite, bearing a stout triangular shaped, postero-laterally directed spine on left side and a pointed spine at right angles to segment on right side. The segment is dialated proximally. The metasomal wings and genital somite armature are characteristic of *A. mirabilipes*. The caudal rami are similar to the earlier instars, but its outer margins are hairy.

Antennule – extends a little after caudal setae. 25 segmented. Both alike.

Antenna – terminal endopodite with 16 setae.

Mandible, Maxillule, Maxilla and Maxilliped as in preceeding stage; except now the endites of coxa of maxillipeds bear hairs.
First swimming leg – similar to Copepodid V. Coxa bears hairs at outer margin; it also bears hairs on exo and endopodite.

Second, Third, Fourth legs similar to previous instar, except addition of hairs on inner and outer margins of exo and endopodites. Fourth leg bears a delicate seta on basis.

Fifth swimming leg – characteristic of species as described in Chapter II.

**Adult male**

Prosome 6 segmented. Spinules on dorso–lateral demarcation between last 2 prosomal segments. Asymmetrical metasomal wings are present, left slightly bigger than right, each with 2 delicate spines. Urosome 5 segmented. Caudal ramus similar to female.

Antennule – the right antennule 22 segmented and modified for grasping. Modifications observed from 8th–19th segment. The penultimate segment bears a smooth spinous process extending upto ¾ of next segment. Left antennule as in female.

All the other appendages except leg 5 as in female.

Fifth leg – characteristic of the species. Basis bears a hyaline membrane on its inner edge. The second exopodite bears two lateral spines of which the distal one is prominent. A thumb like extension on inner side of this segment and a terminal conspicuous spine and a subterminal small pointed spine.
The postembryonic development of *A. mirabilipes* is similar to those recorded in other calanoid species (Devi, 1985). The adult population that emerged in the laboratory showed a female biased trend. This result is in accordance with the population structure of this species in the parental stock. The ovigerous females for postembryonic studies were collected from SS-5. In this pond the female population at all times dominated the male population.

The development was anamorphic. About 15% mortality was noted at the Nauplius III stage and about 10% mortality during metamorphosis from C II to C III. Each succeeding naupliar stage was bigger than the former instar and new appendages were either added or segmentation and setation of already developed appendages increased. The length width ratio of naupliar stages increased as growth progressed, and an elongation of the naupliar stages was observed with each passing instar (Fig.37). The elongation of nauplii with each moult was observed in calanoids studied earlier (Reddy and Devi, 1989a, 1990a). In the later stages of naupliar development an asymmetry was noted in the caudal lobes and its armature, this phenomenon was reported in many earlier studies on calanoid nauplii (Alvarez and Kewalramani, 1969; Devi and Reddy, 1989a and b; Dahms and Fernando 1993).
The copepodid stages resembled the adults and from table 11 and fig.38 it can be observed that as development progressed morphometric changes occurred. The cephalon/metasome ratios progressively decreased in succeeding copepodid instars, as the size and number of metasomal segments increased during development. The prosome/urosome ratios decreased as development proceeded up to the copepodid III stage, it continued to decrease in the male copepodids of C IV, C V and C VI. In the females it increased during the C IV stage, and then gradually decreased in the next two stages. The caudal ramus too showed an increase in its proportion as growth progressed.
Sexual dimorphism was apparent from copepodid IV stage. In copepodid III, though a differentiation was observed between the right and left antennules of specimens, it could not be definitely attributed to a particular sex. Sexual differences have been reported from CIII in copepods, and it is reported that in some species it is obvious even from copepodid I (Dussart and Defaye, 2001). In the present study it was observed from CIV stage. The same was reported in the eight calanoid species studied earlier by Devi and Reddy (1985, 1989a and b, 1990a and b). The sexual differences noted in CIV, were in the morphology of the male right A1 and the thoracic leg, P5. It is these two appendages that the adult male uses to hold the female during copulation. The CIV males were smaller in size than the females and the ratios of their prosome/urosome differed from the female. In CV the sexual dimorphism became even more evident in these characteristics. In addition, differentiation is also noted in the segmentation and thus length of the urosome is different in the two sexes. While the female urosome is three segmented, in the male it is four segmented. CV females resemble the adult females in most characteristics. The differences that are striking, are the length ratios of the urosomal segments and the shape and armature of genital somite. The CV urosome has the basal and distal segments of almost same size, while in the adult the genital somite is much longer than the succeeding two segments.

The long antennules of copepodids II to Copepodid V extend beyond the caudal rami; presence of spinous process on outer distal end of exopodite segments of swimming legs 1 – 4 in all the copepodid stages and presence of 7 setae on swimming legs 2 – 4 of copepodid III and all succeeding instars are
unique to *A. mirabilipes*. The last two mentioned characteristics are unique to the species belonging to subgenus *Allodiaptomus*.

The species is identified by the structure of male P5 prosomal wings of last thoracic appendage and spinule armature of female thoracic wings; morphology and armature of genital urosomite. All these characters become apparent only in the last moult.

On comparison of the postembryonic development of *A. mirabilipes* with *A. raoi* (Devi and Reddy, 1989a) it is observed that the naupliar stages are very similar, but a few differences noted in morphometry. In the copepodid stages the above mentioned distinguishing characteristic features appear. Differences in the comparative ratios in morphometry are also evident in the copepodid stages. The morphometry of the fifth leg of both sexes of copepodid V are characteristic, but segmentation and armature are similar. The species specific characteristics of male and female P 5 of the two species appear only in the adult stage. In both species sexual differentiation occurred from the copepodid stage IV. The instars of *A. mirabilipes* are slightly larger than *A. raoi*, and the development was completed in 14 days, whereas in *A. raoi* it took 15 days. According to Herzig (1983) temperature plays an important role in the duration of embryonic development. Lower temperatures increase the duration in larval development, while in higher temperatures development is completed in a shorter period. Klein-Breteler and Gonzalez (1988) have also reported the positive influence of food and temperature on developmental rates. Here, however both species developed in the same ambient temperature. So, the difference in duration of larval development must be
a genetic one. According to Devi (1985), the larger calanoids completed their postembryonic development in shorter durations of time. The small size difference may be the reason for the difference in development time.

III.e. Conclusion

The stages in postembryonic development of *A. mirabilipes* are similar to that observed in all calanoids.

Development was anamorphic and postembryonic development was completed in 14 days. The population that evolved was female biased. Mortality was low. 15% mortality between NII and NIII and 10% when CI moulted to CII.

Generic characteristics make their appearance from the latter naupliar stages and species specific characteristics appear in the copepodid stages; especially from copepodid III onward.

Sex differentiation was observed from copepodid IV onwards. The differences were observed mainly in; A1, P5, urosome, prosome /urosome ratio and length of the two sexes.

Species specific characteristics were observed only in the adults.
Fig. 19. *Allodiaptomus mirabilipes* Nauplius I: a - spermatheca, b - egg, c - NI, d - A1, e - A2, f - Md
Fig. 20. *Allodiaptomus mirabilipes* NI: a - NII, b - A1, c - A2, d - Md
Fig. 21. *Ailociaptomus mirabilipes* Nauplius III: a - N III, b - Al, c - A2, d - Md.
Fig. 22. *Allodiaptomus mirabilipes* Nauplius IV: a - N IV, b - A1, c - A2, d - Md, e - Mxl.
Fig. 23. *Allodiaptomus mirabilipes* Nauplius V: a - N V, b - A1, c - A2, d - Md, e - Mx1, f - Mx.
Fig. 24. *Allodiaptomus mirabilipes* Nauplius VI: a - N VI, b - A1, c - A2, d - Md, e - Mxl, f - Mx, g - Mxp, h & i - P1 and P2
Fig. 25. *Altodiaptomus mirabilipes* copepodid I: a - C I, b - A1,
c - A2, d - Md, e - Mxl, f - Mx, g - Mxp.
Fig. 26. Allodiaptomus mirabilipes copepodid I: a - P1, b - P2, c - P3, d - urosome
Fig. 27. *Allodiaptomus mirabilipes* copepodid II: a - C II, b - A1, c - A2, d - Md, e - Mxl, f - Mx, g - Mxp.
Fig. 28. Afiodiaptonus mirabilipes copepodid II: a - P1, b - P2, c - P3, d - P4, e - urosome.
Fig 29. Allodiaptomus mirabilipes copepodid III: a - C III, b - A1
    c - A2, d - Md, e - MdI, f - Mx, g - Mxp.
Fig. 30. *Allodiaptomus mirabilipes* copepodid III: a - P1, b - P2, c - P3, d - P4, e - P5, f - urosome.
Fig. 31. Allodiplomus mirabilipes copepodid IV female: a - C IV, b - A1 1 to 20, c - A1 21 to 25, d - A2, e - Md, f - Mxl, g - Mx, h - Mvp.
Fig. 32. *Ailodiaptomus mirabilipes* copepod IV female: a - P1, b - P2, c - P3, d - P4
e - P5, f - uroscope
Fig. 33. *Allodiaptomus mirabilipes* copepod IV male: a - A1, b - A2, c - urosome
Fig. 34. Allocodiaptomus mirabilipes copepodid V female: a - C V female, b - A1, c - A2, d - Md, e - Mx, f - Mxp.
Fig. 35. *Allodiamopectus mirabilipes* copepodid V female: a - P1, b - P2, c - P3, d - P4, e - P5, f - urosome.
Fig. 36. *Allodiaptomus mirabilipes* copepodid V male: a - A1, b - P5, c - urosome.