STUDIES ON THE BIOLOGY OF FRESHWATER CLADOCERA: CRUSTACEA

Thesis submitted to the University of Calicut

for the award of the Degree of

Doctor of Philosophy in Zoology

By

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CERTIFICATE

Certified that the thesis entitled "**Studies on the Biology of Freshwater Cladocera: Crustacea**" submitted by Mr. Britto Joseph. K is an original piece of work based on his studies under our supervision and guidance in the Research and Post-graduate Department of Zoology, St. Thomas' College, Thrissur.

It is also certified that no part of this thesis has been submitted to any other organization for the award of any Degree or Diploma.

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DECLARATION

I, Britto Joseph. K. do hereby declare that the present study entitled "**Studies on the Biology of Freshwater Cladocera: Crustacea**" has been conducted by myself at the Research and Post-graduate Department of Zoology, St. Thomas' College, Thrissur, under the guidance of Dr. C. K. Gopinathan Nayar and Dr. Joseph Louis Olakkengil as Co-guide. I further declare that the work has neither been published nor has it been submitted for the award of any degree, diploma, fellowship or any other similar title of recognition.

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ACKNOWLEDGEMENTS

I wish to express my gratitude and sincere thanks to my Research Supervisor Dr. C. K. Gopinathan Nayar, Professor & former Head of the Department of Zoology, Christ College, Irinjalakuda for his valuable guidance and sincere encouragement throughout the period of this work. I am deeply indebted to him for his constant guidance, without which this work would not have been completed.

I am also grateful to my Co-guide, Dr. Joseph Louis Olakkengil, Reader and Head of the Department of Zoology, St. Thomas' College, Thrissur for his valuable suggestions and constant encouragement. I express my gratitude to Prof. A. M. Francis, Principal and Rev. Dr. Andrews Thazhath, Manager, St. Thomas' College, Thrissur, for providing the necessary facilities for this study. The help rendered by Rev. Dr. Antony Porathur, former Principal; Prof. M.T Joy and Prof. K. T. George former Heads of the Department of Zoology are gratefully acknowledged.

I am grateful to Principal, Christ College, Irinjalakuda for his encouragement and for permitting me to avail the laboratory and library facilities. My sincere thanks are also due to Dr. John Thomas of Department of Zoology, Christ College, Irinjalakuda, for his encouragement and providing necessary facilities for taking the photographs.

I am deeply indebted to Dr. P.T.C. Ponnachan, of Zoology Department for his valuable suggestions which has been a source of inspiration for the completion of this thesis. I am very much obliged to Dr. K.K. Subhash Babu, K.L.R.I, Irinjalakuda, for his support, suggestions and assistance in preparing the illustrations.

I express my gratitude to Sri. K. K. Sankaran, Lecturer, Dept of Statistics and Dr. Francy K. Kakkassery, Reader in Zoology, St. Thomas College, Thrissur for their help. I wish to keep in mind my sincere thanks to my colleagues of Department of Zoology for their encouragement. I am also thankful to Sri. Jaimon Akkanath, Ernakulam, for his help in the compilation of the thesis.

I wish to record my sincere thanks to the Librarian and staff of C.M.F.R.I, Cochin; University of Kerala, Thiruvanandapuram; Kerala Agriculture University, Vellanikkara and K.F.R.I. Peechi for letting me to avail the library facilities. I am also grateful to Dr. M. B. Raghunathan, Zoological Survey of India, Chennai; Dr. S. S. S. Sarma and Dr. S. Nandini, University of Mexico; Dr. N. M. Korovchinsky, Moscow, USSR; Dr. Jochen Vandekerkhove, Laboratory of Aquatic Ecology, Belgium; Dr. J. Vijverberg, Limnological Research Institute, Netherlands and Dr. Jeppesen, National Environmental Research Institute, Denmark; for their valuable help and suggestions.

I am very much grateful to my loving parents, K. T. Joseph and Reetha Joseph for their encouragement. I am very much obliged to my brother Linson Joseph, for his assistance in field collections. Finally, I am very much thankful to my beloved wife, Beena for her assistance; my daughters Betzy Rita and Blessmy Rose; for their encouragement throughout the period of my research.

Above all, I am deeply indebted to the blessings of 'Almighty God' who enabled me to complete this research work and ascribes "Him" all honour and glory for ever.

Britto Joseph. K

ABSTRACT

The cladocerans, commonly known as "water fleas" constitute an important component among the microcrustacean assemblages of all aquatic habitats; they may be planktonic, phytophilic or benthic. Their body is divided into head, thorax, abdomen and postabdomen; the size often ranges from 0.2 to 3.0 mm. The group Cladocera is classified into 4 orders viz. Anomopoda, Ctenopoda, Onychopoda and Haplopoda under Class Branchiopoda of Superclass Crustacea. Cladocerans occupy an important position in the freshwater food web and are important as food of many aquatic organisms. Although, India is potentially rich in cladoceran fauna, information on biology of Indian Cladocera is meager. The early researchers of our country mainly concentrated on systematic studies.

The specimens for the present study were collected from the different freshwater habitats of Thrissur district, Kerala. In the present study 12 species belonging to 5 families viz. Family Sididae, Daphniidae, Moinidae, Macrothricidae and Chydoridae have been selected for biological studies. The life cycle studies were made by rearing them individually in the laboratory providing similar culture conditions.

The present study has given emphasis on biology of cladocerans with reference to their life cycle, pre-adult and adult instars, moulting, morphometric dimensions during growth, reproduction, ephippium production, embryonic stages and life span. These features are described and illustrated. The samples collected from the field were dominated by

parthenogenetic females while the ephippial females and males were scarcely represented. The males and ephippial females were developed under laboratory conditions.

Out of the 12 species studied, the biology of 9 species: *Diaphanosoma sarsi*, *Pseudosida bidentata*, *Latonopsis australis*, *Moina brachiata*, *Moinodaphnia macleayi*, *Ilyocryptus spinifer*, *Macrothrix triserialis*, *Alona pulchella* and *Oxyurella singalensis* is studied for the first time in our country.

The biology of *Ceriodaphnia cornuta*, *Scapholeberis kingi* and *Simocephalus serrulatus* has also been investigated to compare with earlier reports and the general trends in life cycle are discussed in detail. The studies made on the life cycle of males of 4 cladoceran species: *Pseudosida bidentata, Moinodaphnia macleayi, Macrothrix triserialis,* and *Oxyurella singalensis* is a new contribution to the cladoceran biology.

The rapid development, early maturity, constant number of pre-adult instars, longer primiparous instar duration, high fecundity, parthenogenetic reproduction, moulting, general pattern of embryonic development and laying of resting eggs enclosed in the ephippium are some of the important life history traits adopted by cladocerans. The important life history characters were studied and statistically analyzed. The pattern of ornamentation of ephippium is found to be diagnostic at species level.

This is a modest report on the biology of cladocerans from the freshwaters of Kerala. It is hoped that this thesis and interpretations made herein would pave way for a better understanding of the life of cladocerans and will be helpful for the future investigators in this field.

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Symbols and Abbreviations

AFR	Age at First Reproduction
AID	Adult Instar Duration
CH	Carapace Height
E max	Maximum clutch size
FGT	First Generation Time
hrs	hours
Lmax	Maximum life span
(mm±SE)	Millimeter ± Standard Error
PID	Pre-adult Instar Duration
pH	Hydrogen ion concentration
r	Correlation Coefficient
REP	Rate of Egg Production
RH	Relative Height of neonate
RL	Relative Length of neonate
SaB	Size at Birth
SFR	Size at First Reproduction
TL	Total Length
viz.	Such as
Σlx	Mean life span
Σmx	Cumulative number of eggs produced

INTRODUCTION

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 1

INTRODUCTION

INTRODUCTION

Cladocerans which make up a considerable proportion of freshwater zooplankton constitute an important component among the microcrustacean assemblages of aquatic habitats, such as lakes, reservoirs, ponds, puddles, swamps, paddy fields, streams and rivers. They are known to be the inhabitants of both the permanent and temporary water bodies; they may be planktonic, phytophilic or benthic. A few species have been reported from some of the unique freshwater habitats like glaciated zones (Tappa, 1965), water trapped in mosses (Frey, 1980), acidic waters with pH 3.8 to 5.0 (Frey, 1982), underground water (Dumont, 1983; Dumont, 1987; Dumont and Brancelj, 1994; Dumont and Negrea, 1996), caves (Brancelj, 1990) and fast flowing rivers (Dole-Olivier *et al.* 2000).

Cladocerans exhibit wider diversity and are abundant in lentic environments than lotic environments. They are known to be more abundant in temporary water bodies than in permanent freshwater habitats (Hutchinson, 1967). Nevertheless, some species can tolerate considerable salinities (Potts and Durning, 1980; Negrea, 1983). The range of tolerance varies from nearer distilled water (Dodson, 1982) to the 3.2% salinity of oceans (Della Croce and Angelino, 1987). Members of only three genera viz. *Podon, Evadne* and *Penilia* are known to inhabit marine waters. The term "Cladocera" was derived from two Greek words, '*klados*' means branch and '*keras*' means horn, referring the two branched second antennae which are the chief locomotory structure of these animals. Most of the cladocerans move through the water with a series of hops and jumps and hence called "water fleas".

1.1 Systematic Account

The earlier classification (Calman, 1909) of branchiopod Crustacea into 4 orders viz. Anostraca, Notostraca, Conchostraca and Cladocera was a refinement of the scheme first put forward by Sars in 1867.

Fryer (1987) in his classical work, "A new classification of the Branchiopod Crustacea" considered Cladocera an artificial group comprising representatives of rather different phylogenetic origin. He classified them into 4 orders viz. Anomopoda, Ctenopoda, Onychopoda and Haplopoda. This classification has been widely accepted by taxonomists all over the world.

Fryer's (1987) classification is followed in the present study which is cited below.

Superclass Crustacea Lamarck, 1801

Class Branchiopoda Latreille, 1817

Order Anomopoda Sars, 1865

* Family Daphniidae Straus, 1820

* Family Moinidae Goulden, 1968

Family Bosminidae Baird, 1845

* Family Macrothricidae Norman and Brady, 1867

* Family Chydoridae Dybowski and Grochowski, 1894

Order Ctenopoda Sars, 1865

* Family Sididae Baird, 1850

Family Holopedidae Sars, 1865

Order Onychopoda Sars, 1865

Family Polyphemidae Baird, 1845

Family Podonidae Mordukhai- Boltovskoi, 1968

Family Cercopagidae Mordukhai-Boltovskoi, 1968

Order Haplopoda Sars, 1865

Family Leptodoridae Lilljeborg, 1861

*Species studied in the present investigation come under the family

The group 'Cladocera' thus includes 4 orders, 11 families and 80 genera comprising more than 450 species with only 2% marine representatives (Dodson and Frey, 1991; Amoros, 1996; Korovchinsky, 1996).

Martin and Cash-Clark (1995) using modern cladistic analysis studied the inter-relationships among the four orders of Cladocera and suggested that despite the large difference between the orders, they might still be monophyletic. Their theory envisages the derivation of Cladocera from a cyclestheriid-like ancestor (Olesen, 1998). Recent molecular evidence also suggests that the Cladocera is a monophyletic group (Crease and Taylor, 1998). In this system the Class Branchiopoda is divided into Subclass

Diplostraca retaining the Order status of Cladocera and further divided them into 4 suborders viz. Anomopoda, Ctenopoda, Onychopoda and Haplopoda. Subsequently, Negrea *et al.* (1999) proposed a scheme of classification of Branchiopoda giving due credit to Fryer (1987).

Cladocerans from the Indian subcontinent has been reviewed by Michael and Sharma (1988) and described 90 species (93 taxa) belonging to 37 genera. This includes 9 families namely Daphniidae Straus, 1820; Polyphemidae Baird, 1845; Sididae Baird, 1850; Leptodoridae Lilljeborg, 1861; Bosminidae Sars, 1865; Macrothricidae Norman and Brady, 1867; Moinidae Goulden, 1968; Chydoridae Stebbing, 1902 and Podonidae Mordukhai-Boltovskoi, 1968. The study was based on samples obtained from different localities in the Indian subcontinent.

In inland waters the Family Sididae was represented by 4 genera such as *Pseudosida, Sida, Latonopsis, Diaphanosoma* and Family Daphniidae by 5 genera namely *Ceriodaphnia, Daphnia, Daphniopsis, Scapholeberis* and *Simocephalus.* The Family Moinidae included 2 genera *Moina and Moinodaphnia* and Family Bosminidae included *Bosmina* and *Bosminopsis.* The Family Macrothricidae was represented by 4 genera such as *Ilyocryptus, Streblocerus, Macrothrix, Echinisca* and Family Chydoridae by 17 genera namely *Eurycercus, Pleuroxus, Alonella, Chydorus, Dunhevedia, Dadaya, Pseudochydorus, Alona, Acroperus, Camptocercus, Graptoleberis, Leydigia, Biapertura, Oxyurella, Kurzia, Euryalona* and *Indialona.* The Family Leptodoridae and Polyphemidae represented by a single Genus *Leptodora* and *Polyphemus* respectively. Subsequently, Sharma (1991) reviewed the cladoceran fauna of our country and listed 109 species.

The first study of cladocerans from Kerala is that of Michael and Hann (1979) who reported 2 species from Thiruvananthapuram. Michael and Sharma (1988) added 8 species from Thiruvananthapuram and 9 from Irinjalakuda. Raghunathan (1989a) studied the Cladocera from Wynad. Further, Babu and Nayar (2004) added 15 species from Periyar Lake. Recently, Babu and John (2007) added 1 more species from Muriyad, to the cladoceran fauna of Kerala thus raising the total number to 35.

1. 2 Habit and Habitats

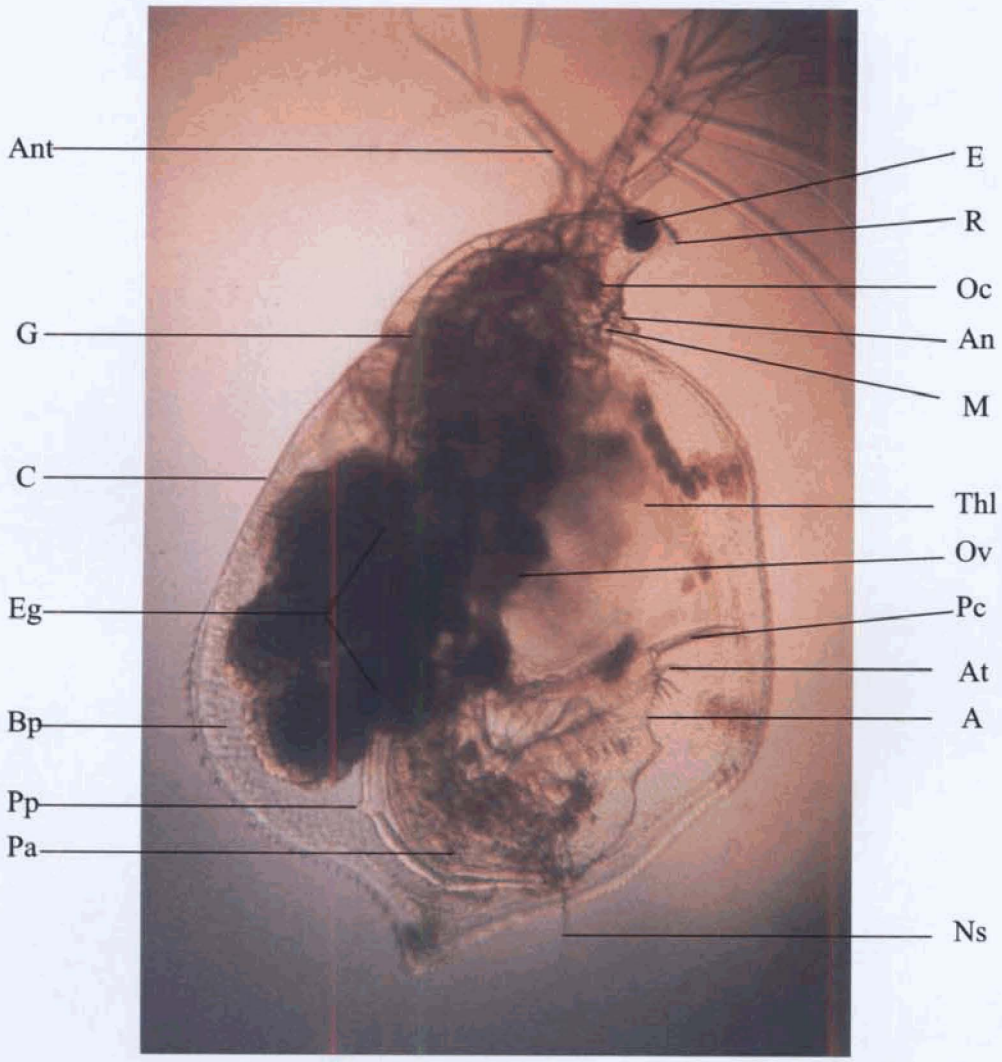
A good number of genera and species of Cladocera are encountered among the weeds in lakes, ponds, swamps and most of them are herbivores. Pennak (1978) has indicated that those found in ponds, ditches or in the weedy margins of larger water bodies are often coloured yellowish, brownish or reddish. Majority of species, are found to be living on submerged vegetation (epiphytic), organic sediments, decaying plant materials and swim only short distances. They are called "meiobenthos" by Frey (1988). Almost all members of the families Chydoridae and Macrothricidae and some members of the Sididae are benthic (Dodson and Frey, 1991). The bottom-dwelling forms may swim but more often scramble about on the bottom, pulling with their antennae and pushing with their postabdomen. However, a less number of species are truly planktonic, and appear transparent and colourless. The members belonging to Genus *Scapholeberis* are hyponeustonic forms often found associated with the surface films of water.

1. 3 General Morphology

Although no single genus or species can be considered typical of the group a generalized account of a cladoceran is given here based on Plate 1.

Plate 1

General organization of a cladoceran ***Simocephalus serrulatus*** (Koch)



Ant- Antenna
A- Anus
An - Antennule
At- Anal teeth
Bp- Brood pouch
C- Carapace
E- Eye
Eg- Eggs
G- Gut
M- Mouth
Ns- Natatorial setae
O- Ocellus
Ov- Ovary
Pa- Postabdomen
Pc- Postabdominal claw
Pp- Postabdominal process
R- Rostrum
Thl- Thoracic legs

The characters described include the body form and size, head morphology, nature of postabdomen; setation in antennae, antennule and shell. Vast majority of this group exhibit considerable variation in their general structural plan with an average body length often ranging from 0.2 to 3.0 mm. But the largest known species, *Leptodora kindti* attains a length upto 18.0 mm (Hutchinson, 1967).

Although, the cladoceran body is divided into distinct head, thorax, abdomen and postabdomen; the division between abdomen and postabdomen is not completely demarcated. In the great majority of the species the thoracic and abdominal regions are covered by a cuticular shell or carapace that has a general bivalved appearance but is actually a single folded piece that gapes ventrally. The carapace may vary in its shape, length, and the ventral surface architecture. In the lateral view the shell may be oval, circular, elongated or angular. There are often surface reticulations, striations, or other type of markings. Moreover the carapace margins in some forms are covered by hairs, spines or setae. The body lies free within the valves hanging from the dorsal carapace. Their internal anatomy is visible under a microscope, due to the translucent nature of the carapace.

The most conspicuous structure on the head is the large compound eye on each side, formed of a few lenses surrounding a mass of pigmented granules. A small ocellus is situated posterior to the compound eye. The compound eye together with the ocellus acts as the light sensitive organ. The presence of ocellus, its size, shape and position with reference to eye and rostrum are of taxonomical importance. Ocellus is clearly seen in family Daphniidae, Macrothricidae, and Chydoridae. But it is absent in Bosminidae and Moinidae except in *Moina oryzae* and Genus *Moinodaphnia.* The head is

often bent downwards with a ventero-posterior process called the rostrum. It is well marked in most of the members of Daphniidae and Chydoridae, while it is absent in some genera of Sididae. Chydorids have small structures on or near the mid-dorsal line known as 'head pores'. These are borne on the head shield, an unpaired plate covering the frontal and lateral surfaces of head. The number and arrangement of head pores and the shape of head shield are important taxonomic features of Chydoridae (Frey, 1959).

Mouth is located near the margin separating the carapace and head. The mouth parts include a median labrum, a pair of mandibles, a pair of maxillae and a median labium which are placed near the junction of head and body. The mandibles are stout structures meant for grinding food and provided with toothed or ridged surface. Maxillae are small pointed structures used for pushing food between mandibles. The labium bears a median keel in Macrothricidae and Chydoridae, which is considered an important feature for identification of different taxa (Birge, 1918).

The head of all cladocerans bear a pair of antennules and antennae. The antennules are located ventrally near the rostrum and bears sensory setae laterally or terminally. They are large and movable in Sididae while that of Daphniidae are rudimentary and immovable. The antennules are 'cigar-shaped' and longer than head in Moinidae. In Bosminidae, they are attached to the head and are parallel to each other and curve backwardly. Macrothricidae have long and club shaped movable antennules inserted in the anterior end at the ventral side of head. In Chydoridae, they are mobile and generally do not extend beyond the tip of the rostrum. The antennules of males are generally longer than females and are important in defining the species (Frey, 1987).

The main swimming organ of Cladocera constitutes a pair of large biramus antennae that originate from the posterior margin of the head. Each consists of a stout basal segment, a segmented dorsal ramus, and a segmented ventral ramus. The two rami bear variable number of plumose setae. The number of antennary setae may be expressed by a formula; for example in *Daphnia* it is (0-0-1-3) / (1-1-3), implies that the dorsal and ventral rami have four and three segments each and again starting from the base each of the segment bears 0, 0, 1, 3 and 1, 1, 3 setae. The number of segments in each ramus, arrangement and number of setae show variation with respect to species and hence antennary setation is also an important taxonomic feature. The antennae are activated by a set of powerful muscles which originate dorsally in the 'neck' region. A strengthening ridge or fornix is seen above the base of each antenna, which is well developed in Daphniidae.

The anterior part of head in front of the eye is called vertex. The members of family Moinidae has a distinct depression just above the eye called supraocular depression. This depression is caused by the attachment of bundle of muscles to the inner surface of the flexible exoskeleton. In some cladocerans there is a depression found at the junction of head and body called cervical depression.

The thorax holds 5 or 6 pairs of thoracic legs while the abdomen is suppressed and has no legs. In family Sididae all these legs are similar, but in other families the first two pairs are more or less prehensile and may aid in clinging to the substrate. The first and second pair of legs is also used for 'filter feeding' as filter screens to keep out large particles, while the other sets of legs create a continuous flow of water. This water current oxygenates the body surface as well as brings food materials towards the mouth.

Some smaller species, especially members of the Family Chydoridae use the abdomen as a sort of foot to kick along surfaces. The postabdomen (Plate 1) is the part of the body near the end of abdomen which is bent forward so that the dorsal side is downward and ends in two terminal claws. It is used for cleaning debris from the appendages as well as for locomotion. The postabdominal claws of some cladoceran species belonging to Daphniidae and Moinidae possess a comb like structure called pecten. The claws also may bear one or two basal spines; there may be a row of anal teeth and lateral setae which are either feathered or grouped in some species. The proximal part of the postabdomen has two long abdominal natatorial setae (Plate 1). Two to three outgrowths called 'postabdominal processes' are found anterior to the natatorial setae which serves to hold the eggs or embryos in the brood pouch (Plate 1). The structure of the antennae, antennules, carapace spines and postabdomen are important features in species identification.

1. 4 Reproduction and Life cycle

Absence of any larval stage during development is a unique feature of cladocerans except *Leptodora*, in which nauplii hatch from resting eggs (Warren, 1901). The reproduction can either be totally parthenogenetic (asexual) or may be intermixed with periods of gamogenetic (sexual) reproduction. Two female morphotypes are thus recognized in the populations which include the parthenogenetic individuals and ephippial forms. Although, parthenogenesis is the predominant mode of reproduction (Shan, 1969; Hebert, 1987), they resort to sexual reproduction occasionally or seasonally. The production of males and ephippial females indicate the onset of sexual reproduction. The shift from parthenogenetic to sexual reproduction ensures their survival under unfavourable environmental conditions.

1. 4. 1 Parthenogenetic Reproduction

Parthenogenesis in Cladocera was observed by several earlier workers (Von Siebold, 1856; Lubbock, 1857; Weismann, 1886). It is the method by which cladocerans reproduce for most of the year and hence the natural populations are dominated by parthenogenetic females.

Like any other crustaceans, cladocerans also grow by moulting; in which the chitinous exoskeleton forming the carapace is removed and renewed periodically and cast off as exuvium. After a limited number of pre-adult moults, the animal attains sexual maturity with the development of ovaries. Each female carries two ovaries which lie along the entire length of thorax lateral to the intestine. The bulk of the ovary contains growing oocyte clusters; the germarium occupies a small portion at the posterior end while the remaining large portion is reserved for growth and maturation (Lumer, 1937; Ojima, 1958).

The primiparous condition is attained when the first batch of eggs are formed in the brood pouch through parthenogenesis. The mature females release eggs into the 'brood pouch', which is a space dorsal to the body in between the valves of the carapace. Embryonic development takes place within the brood pouch and the young ones are released without passing through a larval stage. Normally one clutch of eggs is released into the brood pouch during each adult instar. These eggs develop into immature young ones, without fertilization, nourished initially by egg yolk and then by the secretion from the walls of the chamber. These neonates resemble the adult form but are smaller. Just before the next moulting, these juveniles are released to the exterior by the jerking movements of the female postabdomen

followed by the extrusion of another set of eggs into the brood pouch. These released juveniles then begin to feed and mature at a rapid pace to continue the parthengenetic cycle. Parthenogenesis allows for quick population growth under favourable conditions (Balcer *et al.* 1984).

1. 4. 2 Sexual Reproduction

The males are morphologically distinct from the females. The size of the body, nature of antennules and the presence of hooks in the thoracic legs make them distinct in populations. The testicles lie along both sides of the intestine and extend as far as the bend in the abdomen. In most newly hatched forms they appear as two small, solid cords consisting of relatively large, inactive spermatogonia (Taylor, 1914; Mori, 1933; Mortimer, 1936). As animals grow, the testicles enlarge and spermatogenesis begins, the maturing male germ cells are distributed along the entire length of the testicles (Zaffagnini, 1987).

Banta and Brown (1929) have suggested the effect of crowding in male production. Males appear in the population occasionally after a variable number of parthenogenetic generations (Hutchinson, 1967). Formation of male is induced by conditions like decrease in food concentration, over-crowding or due to variation in light intensity and temperature (Pennak, 1978; Dodson and Frey, 1991). Since the males appear only occasionally they are always found to be rare in natural populations and go unnoticed.

At the time of male appearance or a little later the females start producing specialized eggs. Appearance of males and mating with the females producing fertilized eggs (resting eggs) indicate the beginning of sexual cycle. The fertilized eggs are kept in the brood pouch of the female and are quite

different from the parthenogenetic eggs. The resting egg is enclosed in a thick chitinous protective case called 'ephippium' which is a semi-elliptical, opaque thickening of the carapace surrounding the brood chamber.

The fertilized eggs which are enclosed inside the ephippia are called the resting eggs which undergo several cell divisions (Banta and Wood, 1939; Ojima, 1958) and further enter into a period of dormancy called 'diapause'. Schultz (1977) has described the ultrastructure of ephippium of *Daphnia* and found that it has an external and internal wall. The diapausing embryos inside the ephippia require some stimuli such as water, long or increasing day lengths, proper temperature and high oxygen concentration for initiating the development (Schwartz and Hebert, 1987). These eggs are able to retain the structural integrity under extreme conditions, because they have honey comb like structure in the ephippium made up of crystalline calcium phosphate (Kawasaki *et al.* 2004a). When the ephippial female next moults, the ephippium is shed along with the enclosed egg. These females may again produce resting eggs or resume the production of parthenogenetic eggs to continue the life cycle.

1. 5 Food and Feeding

Cladocera form a key element in the functioning of the freshwater ecosystems of the world. Despite their smaller size, they are very important ecologically not only on account of their numbers, but also as filter feeders, they consume all particles within a size range. The most important component of their diet is made up of small algae in the range of 1-25μm (Lampert, 1987). They are grazers of the ecosystem, feeding on unicellular algae as well as on protozoans, bacteria, other micro-organisms and even organic detritus.

The food uptake and utilization of *Daphnia* have been studied by Naumann (1921). The edible food particles are ground up by tiny mandibles and pushed into mouth. The interpretations of gut analysis are difficult, since the fragile forms like flagellates or ciliates may be completely destroyed by the mandibles and appear in the gut as an unidentifiable mass. Hasler (1935) for the first time studied the digestive enzymes of *Daphnia magna* and reported the presence of protenases, peptidases, amylase and lipase in the gut. The particles very resistant to digestion may be accumulating in the hindgut when all other materials are digested (Porter, 1975; Nadin-Hurley and Duncan, 1976). The undigested residue is eliminated out through the anus situated usually on the dorsal border of the postabdomen.

The cladocerans select their food based on taste and size. Porter (1977) has pointed out that cladocerans can discriminate their choice of food and can remove significant fractions of algae from a lake each day. The passive size selection has been determined at the upper end by the size of the gape between margins of carapace valves (Gliwicz and Siedlar, 1980) and at the lower end by setae spacing (DeMott, 1985). Persson (1985) pointed out that the food selection of cladocerans is generally based on a passive selection by size. However, DeMott (1986) suggested that their food selection is generally based on a combination of the taste and size of food item.

A vast majority of cladocerans obtain food by filtering water. The thoracic legs of *Daphnia,* together with the carapace, form a suction and pressure pump (Cannon, 1933). This was later confirmed by Strickler (1984). Lampert (1987) has investigated the filtering process and found that water enters the gap between carapace margins and then leaves the carapace just above the postabdomen. During this process the food particles are brought

continuously into the mouth through the current of water maintained by the movements of thoracic legs.

Scourfield and Harding (1941) reported cladocerans role in removal of some harmful bacteria from water. The members belonging to Family Chydoridae, typically feed by crawling along the surfaces or through the mud, where they scrape up or filter food (Fryer, 1968). A few genera such as *Polyphemus* and *Leptodora* are predatory feeding on small crustaceans and rotifers (Pennak, 1978). Recently, Shiny *et al.* (2005) also reported that the cladoceran *Daphnia magna* is highly efficient in the removal of suspended organic matter and bacteria from waste water.

1. 6 Importance of Cladocera

The central position of Cladocera in the food web has directed the interest of many investigators to its food uptake and utilization. Their grazing contributes to the transfer of algal primary production to higher trophic levels. In other words cladocerans can make organic material available to higher trophic levels, in a larger pellet form by themselves acting as 'live feed' and there by saving the foraging energy of their predators. Thus cladocerans have become important in the aquatic ecosystems as a suitable food item for all fish larvae and a wide variety of plankton-feeding fishes.

Cladocerans have been considered an important food item of plankton-feeding fishes by earlier investigators (Herrick, 1884; Forbes, 1888; Scourfield and Harding, 1941). The importance of Cladocera as live animal food in the nursery ponds was also noticed by Alikunhi (1952) and Alikunhi *et al.* (1955). They play a crucial role in the transfer of energy from producers to secondary and tertiary consumers within the aquatic food web

(Dodson and Frey, 1991; Dumont and Negrea, 2002). Thus cladocerans contribute as the resource base for the youngest ontogenetic stages of most fish species (Fernando, 2002).

During the last three decades aquaculture has received important application in the country as a means for augmenting fish production, an enterprise for improving rural economy and an operation for productive utilization of the derelict land and water spreads. Resources of land, water and cultivable species of fish and prawn are available in different states of India. So the prime requirement of aquaculture practice is the production of appropriate nutritionally-balanced and economically viable live feed which will ensure maximum growth and survival of the cultivable fish or prawn.

Murugan (1989) has reported high levels of protein, free amino acids, fats and carbohydrates in cladocerans like *Daphnia carinata, D. longispina, D. magna* and *D. pulex*, which are considered valuable live feeds. Live feed organisms are preferred by most of the cultured larvae as they are their principal food in the natural habitat (Pandian and Marian, 1991). Cladocerans have been selected as food sources in larviculture based on availability, nutritional quality, economic feasibility, easy digestibility and their high rate of reproduction (Watanabe and Kiron, 1994). Pandey and Yeragi (2000) have also pointed out the importance of cladocerans like *Moina* and *Daphnia* as live food organisms in freshwater larviculture and ornamental fish industry. Indulkar and Belsare (2003) pointed out that live *Moina* is superior to other foods for post-larvae of *Macrobranchium rosenbergii.*

The genetic characteristics of daphniids, especially *D. magna* have been studied by Ferrari and Hebert (1982). Cladocerans are used extensively

in genetic studies (Hebert, 1987; Hebert and Taylor, 1997; Crease and Taylor, 1998).

Cladocerans have also been identified as best indicator organisms for assessment of water pollution particularly nutrient linked eutrophication, resulting from pollution by untreated domestic sewage and toxicity associated with pesticides (Anderson, 1944; Makrushim, 1976; Gannon and Stemberger, 1978). Prasad (1980) showed that *Daphnia* and *Bosmina* are highly sensitive to kraft pulp mill effluents. Recently, Yogendra *et al.* (2005) suggested that *Daphnia* can serve as a good test model for biomonitoring of industrial effluents.

1. 7 Relevance of Present Investigation

The cladocerans occupy an intermediate position in the trophic levels of freshwater ecosystems as planktonic, epiphytic and benthic organisms. Their high abundance and role as transfer organisms from algae and dead organic matter to macro-invertebrates and fish often make them one of the most important organisms to influence the biological processes of freshwater ecosystems. Edmondson (1971) has pointed out that the laboratory studies of growth, instar duration, egg production and life span are valuable sources of information for a clearer understanding of secondary productivity.

Cladocerans have been used as experimental animals by several biologists in various studies pertaining to aquatic toxicology (Anderson, 1944; Makrushim, 1976; Gannon and Stemberger, 1978; Prasad, 1980 and Shiny *et al.* 2005). The small size, rapid parthenogenetic development, filter feeding habit, the capacity to survive and reproduce in different types of

freshwater habitats make Cladocera an ideal organism for culture purposes; hence can be used as a model organism in basic research.

Most of the earlier studies are confined to a few species of *Daphnia* especially of the temperate regions (Koivisto, 1995; Lampert and Sommer, 1997; Alekseev and Lampert, 2001). Therefore, daphniids serve as model organisms for understanding the life history. Information on the biology of other cladocerans from tropical areas may be useful for further works in other fields like ecology, behavior and genetics.

Cladocerans are used as live food in larviculture, mainly due to their superior nutritional value and easy availability (Murugan, 1989; Pandey and Yeragi, 2000). Natural diets mainly include different species of live cladocerans. Larval survival and growth rates in many species of fishes have been shown to be the highest when they were fed with live food organisms (Indulkar and Belsare, 2003).

Cladoceran biology has not been a subject of intense study from our country especially from Kerala. Therefore, the study on the biology of cladocerans simulating natural conditions of this region makes comparison among different species more reliable. Hence the present study has focused mainly on their morphology, reproduction, growth, life cycle, embryonic development and life span of species collected from the locality. Since differences in the life histories are likely to occur, it was felt that it would be of interest to study these features. The conclusions derived from the investigation will be useful for developing better culture methods.

The biology of twelve cladoceran species collected from different freshwater habitats of Thrissur district, Kerala, has been presently studied. The species included are:

1. *Pseudosida bidentata* var. *szalayi* (Daday, 1898)

2. *Latonopsis australis* Sars, 1888

3. *Diaphanosoma sarsi* Richard, 1895

4. *Ceriodaphnia cornuta* Sars, 1885

5. *Scapholeberis kingi* Sars, 1903b

6. *Simocephalus serrulatus* (Koch, 1841)

7. *Moina brachiata* (Jurine, 1820)

8. *Moinodaphnia macleayi* (King, 1853)

9. *Ilyocryptus spinifer* Herrick, 1882

10. *Macrothrix triserialis* (Brady, 1886)

11. *Alona pulchella* King, 1853 and

12. *Oxyurella singalensis* (Daday, 1898).

It is hoped that, this attempt will be helpful in making some generalizations and to draw certain conclusions which will be useful for future investigators. The present investigation entitled ***"Studies on the biology of freshwater Cladocera: Crustacea"*** has been undertaken in order to fill the existing lacunae of our knowledge on cladoceran biology.

REVIEW OF LITERATURE

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A General review of Cladocera

The early description of the cladocerans was associated with the invention of microscope. The Dutch microscopist Swammerdam (1669) described these creatures as 'water fleas' in his book "Insects" on creatures with branching arms. Subsequently Schaeffer (1775) gave a better description for the first time. These authors used different names and did not separate the cladocerans taxonomically from other microcrustaceans.

A scientific study of cladocerans was made by O.F. Müller (1785) who included them under Entomostraca and gave scientific binomial names. It was Milne-Edwards (1840) who divided Branchiopoda into two independent orders Phillopoda and Cladocera. The Order Cladocera included the genera *Macrothrix* Baird 1843, *Diaphanosoma* Fischer 1850, *Moina* Baird 1850, *Ceriodaphnia* Dana 1853, *Simocephalus* and *Scapholeberis* Schoedler 1858, *Ilyocryptus* Sars 1862, *Pseudosida* Herrick 1884, *Latonopsis* Sars 1888 and *Oxyurella* Dybowski and Grochowski 1894.

Monographs by Fischer (1850), Lilljeborg (1853) and Leydig (1860) represented descriptions of many European species. Sars (1862) showed that Cladocera constituted the largest group of freshwater crustaceans in number of species, and a most diversified group in structure and habits. He later classified them into two divisions called Calyptomera and Gymnomera (Sars, 1865) and further grouped them into four well marked 'tribes' namely

Anomopoda, Ctenopoda, Onychopoda and Haplopoda which comprised eight families. Subsequently Kurz (1875), Claus (1876) and Schoedler (1877) published their works on the European species.

In the latter half of nineteenth century several workers started active cladoceran studies outside Europe as well, particularly in North America (Birge, 1879; Herrick, 1879, 1884) in Australia (King, 1853; Sars, 1885; 1888) and in tropical Asia (Richard, 1894; Daday, 1898). Sampling populations and studies of environmental features became a regular part of limnological investigations during this period (Birge, 1895).

Calman (1909) grouped the branchiopod crustaceans into four orders Anostraca, Notostraca, Conchostraca and Cladocera as a significant step in their earlier classification. By the beginning of 20th century the interest on general cladoceran morphology had declined and researchers paid more attention to study aspects pertaining to the ecology of *Daphnia*.

The central position of *Daphnia* in the food web of the freshwaters has directed the interest of numerous investigators to its food uptake and utilization. Naumann (1921) already recognized that daphniids provided an important link between plankton and fish production. There were several studies on seasonal changes, population dynamics, and cyclomorphosis during this period. Many contradictory observations were made and various explanations were offered for these seasonal changes by Wesenberg-Lund (1926) and Berg (1931). Subsequently the work on filter feeding mechanisms of *Daphnia* by Cannon (1933); growth and reproduction by Wood and Banta (1936), Anderson *et al.* (1937) and Ingle *et al.* (1937) were also published. Techniques for the maintenance of cladoceran culture were also devised by

Lutz *et al.* (1937) and Banta (1937). Later these techniques helped the study of population growth and dynamics by Brooks (1946) and Edmondson (1955).

In the second half of 20th century a number of workers were actively engaged in the studies of these crustaceans from different parts of the world. A review on egg development was done by Green (1956). Revisionary studies have already been undertaken in a number of genera and species complexes around the world during this period. Contributions on the 'Systematics and Evolution of Moinidae' Goulden (1968); 'Evolution and Adaptive radiation in the Chydoridae' Fryer (1968); 'World Chydoridae' Smirnov (1974); 'Evolution and Adaptive radiation in Macrothricidae' Fryer (1974); 'Revision of Australian Cladocera' Smirnov and Timms (1975) were important in this regard.

Arnold (1971) evaluated the effect of different species of algae on the assimilation, survival and reproduction in *Daphnia pulex*. Vijverberg (1976) studied the effect of food quality and quantity on the growth, birth rate and longevity of *Daphnia hyalina*. The general life history characteristics of cladocerans were reviewed by Lynch (1980). The influence of biotic factors on cladoceran morphology was presented by Kruger and Dodson (1981).

Subsequently the 'Revision of Scapholeberinae' was done by Dumont and Pensaert (1983) while Idris (1983) published the 'Freshwater Cladocera of Malaysia'. Consequently there was an increase in the cladoceran researchers especially in the field of systematics. Fryer (1987) in his publication, 'A New Classification of the branchiopod Crustacea' considered Cladocera as an artificial group comprising representatives of rather different

phylogenetic origin and classified them into four orders such as Anomopoda, Ctenopoda, Onychopoda and Haplopoda. A significant contribution to cladoceran taxonomy was made by Frey (1987) and his followers. They used a population approach especially the study of large groups of specimens, their morphological variability, ontogenetic changes, experimental crosses and electron-microscopic examinations.

Vijverberg (1989) made an excellent review of culture techniques of cladocerans under laboratory and *in situ* conditions. Subsequently, demographic and population growth approaches were published by Gliwicz (1990) and Stearns (1992). Chemical communication in predator-prey relationships received much attention in aquatic ecology (Larsson and Dodson, 1993). Several studies have demonstrated that the presence of info-chemicals released by predators into water especially the 'fish kairomones' may lead to behavioural as well as morphological changes (Machacek, 1993). This ultimately leads to changes in their life history characteristics (Weider and Pijanowska, 1993). Contributions of Ringleberg (1993), Boersma and Vijverberg (1994) and Spaak and Hoekstra (1995) were also of major importance in ecology.

Aquatic toxicology, has received increasing attention over the last few decades as problems of water pollution are faced in both industrialized and developing countries. *Daphnia magna* has been used for ecotoxicological studies all over the world (Koivisto, 1995). Alberdi *et al.* (1996) assessed the potential of *Daphnia* species as biological indicators for pesticides. Barry (1996) evaluated the effects of endosulphan on the growth, reproduction and population dynamics of *Daphnia carinata* when fed at low and high food levels.

Besides the traditional domains, the new trends emerged in cladoceran studies was the widespread application of molecular techniques in taxonomy and ecological studies. Molecular evidences suggested Cladocera as a monophyletic group (Crease and Taylor, 1998). A reclassification of Anomopod families was made by Dumont and Silva Briano (1998). In this system Suborder 'Radopoda' was divided into seven anomopod families that gave a new status for some previous subfamilies: the Superfamily Eurycercoidea included three families (Eurycercidae, Sayciidae and Chydoridae) and Superfamily Macrothricoidea included four families (Ophryoxidae, Acantholeberidae, Macrothricidae, and Neothricidae).

Later Negrea *et al.* (1999) proposed a scheme of classification of Branchiopoda in which the Class Branchiopoda was divided into five superorders and eleven orders. Cladocera has been reinstated as Superorder Cladocera and included 3 orders viz. Ctenopoda, Anomopoda and Onychopoda. The Order Haplopoda was placed under a new Superorder Leptodorida.

Influence of environmental factors to cladoceran resting eggs was studied by Rojas *et al.* (2001). Crispim and Watanabe (2001) analysed the resting egg banks. Ovie and Egbore (2002) evaluated the effect of different algal densities of *Scenedesmus acuminatus* on the population growth of *Moina micrura* Kurz; and found an inhibition of population growth at higher algal densities. The percentage of egg-bearing females and the number of eggs per egg-bearing females followed a similar pattern.

Mooij *et al.* (2003) evaluated the influence of temperature and food on the population dynamics and demographic characteristics of temperate Genus

Daphnia. Tatarazako *et al.* (2003) studied the mechanisms associated with the switch from parthenogenetic to gamogenetic reproduction and suggested the role of juvenile hormone in the chemical signaling responsible for inducing the production of male offspring.

The Sixth International Symposium on Cladocera at Poland in August 2002, threw some light on the present trends in cladoceran research. The important papers presented for the symposium included that of Arbaciauskas (2004), who demonstrated that although ex-ephippial daphniids and those of parthenogenetic origin differ in life histories, there is no ex-diapause effect on fitness of successive parthenogenetic generations. Mikulski *et al.* (2004) showed that short term exposure of various instars of *Daphnia* to simulated predation threat induces different defensive responses. Alekseev and Lampert (2004) demonstrated that photoperiod and maternal effects are important factors influencing life history and population dynamics in *Daphnia.* Slusarezyk and Rygielska (2004) looking for potential sources of environmental clues inducing the formation of resting eggs in *Daphnia*, concluded that kairomone extracted from fish faeces was the most active agent for induction. Vandekerkhove *et al.* (2004) demonstrated that isolation of resting eggs from sediments enhances overall hatching success, reduces hatching time, inter and intraspecific variability. Nandini *et al.* (2004) using standard life table approach quantified the life history parameters of *Moina macrocopa* subjected to different environmental stress.

Kawasaki *et al.* (2004a) studied the chemical composition of *Daphnia* resting eggs. The study demonstrated that the resting eggs have shells that are made up of crystalline calcium phosphate. This property of the resting eggs may ensure *Daphnia* survival in harsh environments. Kawasaki *et al.* (2004b)

studied the chemical composition, microanatomy and physical properties of the resting eggs using X-ray analytical microscope. The analysis demonstrated that phosphorus, sulphur, potassium and calcium are present in the resting eggs. Sarma *et al.* (2004) have made an elaborate review of recent works on different life history variables of cladoceran taxa in tropical and temperate freshwater bodies. Their study concluded that tropical and temperate species differ in several life history characteristics and environmental factors contribute to these differences.

Effect of temperature and photoperiod in hatching of ephippium was recently studied by Vandekerkhove *et al.* (2005a). Decaestecker *et al.* (2005) investigated the effect of eight endoparasites in natural *Daphnia magna* populations. Chadwick and Little (2005) studied the impact of microsporidian parasite in the life history of *Daphnia magna* and showed that the parasite causes shift in life history strategy towards early reproduction.

Review of Indian Cladocera

Although systematic studies on Cladocera was initiated in different parts of the world from 17th century, the earliest records of studies on Indian Cladocera dates back to the report of a new species of genus *Daphnia* from Nagpur by Baird (1860). Further studies in this direction were made during the first half of the 20th century by Gurney (1906, 1907). This was followed by the isolated reports from different parts of the country which included that of Daday (1911) from Tibet and Arora (1931) from Punjab. Subsequently Sewell (1935) and Brehm (1936, 1950) reported a few more species. Biswas (1964, 1966 and 1971) and Nayar (1971) published a series of reports from Rajasthan.

One of the important contributions from South India during this period was that of Michael (1973) who made studies on Cladocera from Madurai. Further, Patil (1976) reported Cladocera from Shillong and Nasar (1977) from Bihar. Quadri and Yousuf (1978) reported for the first time from Kashmir followed by Sharma (1978) from Calcutta and nearby areas. Michael and Hann (1979) also made some contributions to the studies on Chydoridae. A good number of studies on freshwater cladocerans of Tamil Nadu were made by Raghunathan (1986), Venkataraman and Krishnaswamy (1984a, 1984b, 1985, 1986) and Hudec (1987). Another important contribution during this period was that of Rane (1985a, 1985b) from Madhya Pradesh. All studies up to this period have been neatly compiled by Michael and Sharma (1988) in their monograph on 'Indian Cladocera' wherein he has listed 90 species from Indian subcontinent, of which 19 species are from Kerala.

Later important contributions in this aspect were by Raghunathan (1989a, 1989b); Sharma (1991), Rane (1992), Battish (1992), Venkataraman (1992, 1993, 1994, 1995) and Murugan *et al.* (1998). Venkataraman (1999) made an extensive study and added 34 species to the cladoceran fauna of Tamil Nadu. Subsequently Raghunathan and Sureshkumar (2002) published a check list of the cladoceran fauna of Tamil Nadu. Further, Durga Prasad and Padmavathy (2003a, 2003b) made cladoceran studies in Lake Kolleru of Andhra Pradesh. Chandrasekhar (2004) studied cladocerans from Adra Lake in West Bengal. Babu and Nayar (2004) studied the Cladocera of Periyar Lake, and added 15 species to the fauna of Kerala. Recently, Raghunathan (2006) indicated that the total number of cladoceran species of Tamil Nadu is 77, of which Chydoridae represented the most dominant group.

Review of Biological studies

Studies on the biology of Cladocera were initiated in India by Michael (1962). All the subsequent studies were done in the last four decades. A detailed review of important contributions on these aspects from Indian subcontinent is given below.

Michael (1962) made studies on the seasonal variations of *Ceriodaphnia cornuta* Sars collected from a natural pond at Barrackpore, West Bengal. He reported that *C. cornuta* passes through 1-2 pre-adult instars followed by 9 adult instars producing a total number of 42.0 eggs in a life span of 12.0 days.

The other subsequent contributions during this period was that of Parabrhmam *et al.* (1967), who made studies on the occurrence, growth and feeding habits of *Moina dubia* Gurney and Richard in sewage stabilization ponds. The culturing methods and its phytoplankton relationships were discussed in detail. Their study indicated that 28°- 30°C and pH 7.5 to 8.2 are favourable for the growth of *Moina dubia.*

Navaneethakrishnan and Michael (1971), while studying the egg production and growth in *Daphnia carinata* King under the laboratory conditions observed that the number of eggs produced gradually increased from the first to the last instar. They reported that *D. carinata* passes through 5 pre-adult and 8 adult instars at a temperature range of 29-31°C in a life span of 24.0 days, producing 42.4 eggs.

Murugan and Sivaramakrishnan (1973) studied the biology of *Simocephalus acutirostratus* King under laboratory conditions. They studied

the life span, instar duration, egg production, growth and stages in embryonic development. They reported that *S. acutirostratus* passes through 4 pre-adult instars and 18 adult instars at a temperature range of 28 -30°C in a life span of 44.0 days. A total number of 248.0 eggs were produced with a bimodal pattern of egg production.

Murugan (1975a) conducted studies on egg production, development and growth in *Moina micrura* Kurz from Madurai and showed the presence of 2 pre-adult and 11 adult instars producing about 61.2 eggs in a life span of 13.0 days at 28 -30°C. One of the interesting observations in that study was the uniform instar duration (24 hrs) of both the pre-adult and adult instars. There was only a single peak of egg production at 4th adult instar followed by a very gradual decline until the last instar.

Murugan (1975b) studied the biology of *Ceriodaphnia cornuta* Sars from Tamil Nadu. The study revealed that the species had 2 pre-adult instars followed by 18 adult instars. In a mean life span of 21.21 days it produced 123.60 eggs at 28-30°C.

Murugan and Sivaramakrishnan (1976) conducted studies on longevity, instar duration, growth, reproduction and embryonic development of *Scapholeberis kingi* (Sars) from Tamil Nadu. They found that *S. kingi* produced 239.4 eggs during a life span of 20.56 days at 28-30°C and in the meanwhile the animal passed through 2 pre-adult instars followed by 17 adult instars. There was a gradual increase in the number of eggs from the first adult instar till the 6th and showed uniform high rate of egg production in the succeeding eight instars with minor fluctuations. There was a sharp decline after 14th adult instar and absence of eggs towards the last instar.

Murugan (1977) studied the effects of different artificial media on the development of parthenogenetic egg of *Simocephalus acutirostratus* Sars collected from a seasonal pond at Madurai. The hatchability of the different stages of the embryo was tested in an artificial medium which contained varied proportions of sodium chloride, potassium chloride and calcium chloride. The hatchablity was found to be 100% in 0.001 to 0.1 M of sodium chloride. Also 100% hatchablity was noted from 0.001 to 0.05 M of calcium chloride. High percentage of hatchablity was recorded when isotonic solutions sodium and calcium were mixed. The results were discussed and compared with an allied temperate species *Simocephalus vetulus* (O.F.Müller).

Murugan and Venkataraman (1977) studied the *in vitro* development of the parthenogenetic egg of *Daphnia carinata* King and described eight stages during the process. They observed that the basic pattern of development was similar to that of other daphniids. But duration of the total period of development and the individual stages in *D. carinata* differed from those of other tropical and temperate forms. The total duration of embryonic development was found to be 28-30 hrs.

O' Brien and Vinyard (1978) studied the polymorphism and predation of *D. carinata* in South Indian ponds. They found that the crested forms of *D. carinata* evaded the waterbug *Anisops* more easily than normal forms. In experiments with *D. carinata* they also found a higher feeding rate and population growth rate in uncrested forms.

Venkataraman and Job (1980) made studies on the effect of temperature on the growth, development and egg production in *D. carinata.*

Sharma *et al.* (1981) conducted the laboratory studies on the life cycle of a male cladoceran *Daphnia lumholtzi* Sars from a lake at Shillong for the first time in India. The study indicated that the average life span of male *D. lumholtzi* was 36.33 days. *D. lumholtzi* passed through 13 instars at 12-18°C with a gradual increase in the duration of the different instars throughout the life span.

Venkataraman (1981) studied the seasonal variation in egg production of *Daphnia carinata* King with reference to physicochemical as well as biological factors in a temporary pond at Madurai. Based on laboratory observation on the life history of *D. carinata* he concluded that the animal had undergone 3 pre-adult instars followed by 15 adult instars in a life span of 26 days and produced a total number of 57.8 eggs at 29±1°C.

Murugan and Job (1982) studied the life cycle of *L. acanthocercoides* based on collections obtained from a pond at Madurai College campus, Tamil Nadu. They observed *L. acanthocercoides* had undergone 3 pre-adult instars and 13 adult instars in a life span of 23 days at temperature of 28-30°C under laboratory conditions. The pre-adult instar duration was found to be uniform with 24 hrs while the adult instar duration varied from 19-60 hrs. One of the notable observations in their study was that *L. acanthocercoides* produced a constant number of 2 eggs per brood in all adult instars unlike many other cladocerans.

Kanaujia (1982) studied the life cycle of *Ceriodaphnia cornuta* Sars collected from a pond in Cuttack under laboratory conditions at two different temperature ranges 16-25°C and 28-31°C. Based on the observations he

concluded that the instar duration, number of instars, egg production and longevity of *C. cornuta* showed a direct relationship with temperature.

Khan (1983) studied the effect of food on growth, life span and reproduction of *Ceriodaphnia cornuta*. It was noted that moulting, instar duration, growth and reproduction rates were lowered and life span was shortened in diluted culture medium. The growth and reproduction rates were high in the medium containing moderate quantity of food.

Kanaujia (1984) studied the life history, ephippia development and cyclomorphosis of *Daphnia lumholtzi* Sars under laboratory and field conditions. He observed that *D. lumholtzi* had undergone 3 to 4 pre-adult instars followed by 20 adult instars and produced 301.0 eggs during a life span of 54 days at a temperature range of 18-26°C. Moreover, he reported that seasonal water temperature exerted an important influence in the life cycle of *D. lumholtzi.*

Sharma *et al,* (1984) studied the in *vitro* development of *Daphnia lumholtzi.* The embryonic duration was 72 hrs at 10°C and 60 hrs at 20°C. When the temperature increased further to 28°C, there was a reduction in embryonic duration to 40 hrs.

Sharma and Dattagupta (1984) studied the process of cyclomorphosis leading to morphological changes in *Daphnia lumholtzi* under the influence of environmental factors. They observed that head and tail lengths of *D. lumholtzi* showed variations with the rise and fall of water temperature.

Venkataraman and Krishnaswamy (1985) during the laboratory studies on growth and reproduction of *Diaphanosoma senegal* Gauthier collected

from a seasonal pond at Madurai showed that this species had a life span of 18.7 days. Based on the observations he concluded that *D. senegal* has passed through 3 pre-adult and 16 adult instars at 28°-30°C. His study showed that *D. senegal* attained the maximum body size at the end of its life cycle and the growth increment was more during pre-adult instars.

Jana and Pal (1985a) made studies on *Moina micrura* in different culture media. Jana and Pal (1985b) also studied the relative growth and egg production of *Daphnia carinata* in different culture media.

Further, Sharma and Pant (1985) studied the oxygen consumption in *Simocephalus vetulus* in relation to size, density and temperature.

Venkataraman and Krishnaswamy (1986) conducted experimental studies with the notonectid water bug *Anisops bouvieri* as a predator to study the benefits of helmet development in cyclomorphic form of *Daphnia cephalata*. The observations were compared with another non-helmeted form of *Daphnia similis*. It was observed that the development of crest is a predation avoidance mechanism.

Manimegalai *et al.* (1986) further studied the helmet development in *D. cephalata* in relation to predation under laboratory conditions.

Kanaujia (1987) studied the biology and ephippia development in *Simocephalus vetulus* (O. F. Müller) collected from a fish pond at Cuttack, Orissa. The study revealed that this species had undergone 3-4 pre-adult instars followed by 20 adult instars during the life span of 41 days and produced a total number of 496.7 eggs at 21-31°C.

Kanaujia (1988a) made some observations on the life cycle of *C. cornuta* under laboratory conditions, for which collections were made from a pond at Cuttack, Orissa. In that study he observed 2 pre-adult and 25 adult instars for this species which produced 155 eggs during a life span of 31 days at 28-31°C. Moreover, the influence of different culture medium on the life cycle was also studied and discussed.

Kanaujia (1988 b) studied the annual life cycle of *Simocephalus vetulus* (O. F. Müller) under laboratory conditions. From the study he concluded that temperature and food influenced the life cycle of *S. vetulus*. Body size increased with suitable food in the medium and there was reduction in the duration of instar and total life span.

Murugan and Moorthy (1988) studied the longevity, instar duration, growth and fecundity of *Daphnia cephalata* King under laboratory conditions. The study revealed that during the life span of 52 days, *D. cephalata* produced 200 eggs at 27±2°C.

Sharma and Sharma (1989) made observations on the longevity, instar duration, fecundity, growth and embryonic development of *Simocephalus exspinosus* (Koch) under laboratory conditions. During an average life span of 41.16 days *S. exspinosus* passed through 4 pre-adult instars followed by 14 adult instars and produced 265.1 eggs at 20-22°C. He reported a unimodal pattern of egg production in this species.

Malhothra and Langer (1990) studied the biological aspects of *Moina macrocopa* (Straus) under four different temperature ranges viz. 7-10°C, 13-16°C, 25-28°C and 30-32°C. They suggested that egg production and embryonic duration could be influenced by temperature.

Thresiamma *et al.* (1991) studied the influence of different culture media on the production and population density of *Moina micrura* Kurz collected from some local ponds in Kerala under laboratory conditions at 27±1°C. Experiments were conducted using different culture media, with and without addition of unicellular algae *Chlorella* sps. The population attained a peak by 9th day in the most suitable medium and sharply declined. The appearance of ephippial females were recorded subsequent to the attainment of peak production.

Chandini (1991) conducted laboratory investigations on the effects of food and cadmium stress on the reproductive value and residual reproduction value of the two cladocerans such as *Echinisca triserialis* (Brady) and *Daphnia carinata* King. Attempts were also made to test the cost of reproduction hypothesis on whether; the energy invested by organisms at particular time in reproduction could effect their future survival and reproduction.

Singh and Dutta Munshi (1991) conducted laboratory studies on the biology of two cladocerans, *Ceriodaphnia rigaudi* Richard and *Daphnia lumholtzi* Sars collected from river Ganga. The effects of different temperatures on fecundity and longevity were also discussed and results were compared with that of other Indian cladocerans.

Babu and Nayar (1993) conducted investigations on the life cycle of *Ceriodaphnia cornuta* Sars, collected from Kerala, under laboratory conditions. During this study they observed that the animal passed through 2 pre-adult instars followed by 12 adult instars and produced 67.3 eggs within a life span of 16-17 days. Absence of ephippial females and males in the

culture suggested the possibility of parthenogenetic development of unfertilized eggs.

Babu and Nayar (1997) described the life cyle of *Simocephalus serrulatus* (Koch) collected from a pond in Kerala based on laboratory culture. The study indicated that the neonates produced from the same brood pouch may be all females, all males or both male and female. The life cycle was compared with that of other related species of *Simocephalus* from India. The life cycle of the *S. serrulatus* male was also described.

Another important contribution during the period was that of Battachayarya *et al.* (1997) who studied the biology of *Daphnia lumholtzi* from Shillong.

Sureshkumar *et al.* (1999) made a preliminary laboratory observation on the development of *Pleuroxus aduncus* Jurine from a sample collected from Chennai. The study revealed that this species has six distinct developmental stages which follow a similar developmental pattern to that of other cladocerans.

Altaff and Sivakumar (2002) conducted laboratory culture of *Moina micrura* Kurz using poultry manure and mixed algae. The importance of monitoring of physicochemical characteristics and microbial population of the culture medium for a high density production of *Moina micrura* Kurz was discussed. The study revealed that mass culture of *M. micrura* can be done using chicken manure and mixed algae.

Sureshkumar and Altaff (2002) made laboratory observations on the egg production, development and growth of *Macrothrix spinosa* collected

from Chennai. The study showed that during the life span of 18.63 days it produced 97 eggs. Five pre-adult and eleven adult instars were recorded at a temperature of 29±1°C. The total life span, growth rate and the total number of eggs produced were compared with allied tropical and temperate cladocerans.

Nayar and Babu (2002) developed techniques for the mass culture of the cladoceran *Simocephalus serrulatus* (Koch) for use as live feed in aquaculture. Crowding has been found to be an important factor in inducing male production and subsequent ephippia formation. Methods for collection and storage of ephippia were also suggested for future hatching.

Sumitha and Ramanibai (2004) investigated the growth and neonate production of *Moina micrura* Kurz collected from a pond at Chennai. Specimens of *M. micrura* were exposed in the laboratory to different photoperiods (0, 8, 12, 16 and 24 hrs light and dark cycle). Body size and clutch size were gradually increased with long photoperiodic exposures. The neonate production was also very high during 24 hrs light exposure. The study concluded that light is one of the environmental factors which accelerated the neonate production in *M. micrura*.

MATERIALS AND METHODS

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3. 1 Field collections

The specimens for the present study were collected from seasonal rain fed temporary and permanent water bodies of different localities of Thrissur district, Kerala. The collections were made between 7 and 8 a.m. during the period from June 2003 to May 2006.

Water temperature was measured using a mercury thermometer and the hydrogen ion concentration (pH) with a digital pH meter. The water samples were collected in BOD bottles, fixed on the spot and the dissolved oxygen was estimated following Winkler's method (APHA, 1995).

3. 1. 1 Collection Sites

Collections for the present study were made from the following localities in Thrissur district.

Site 1: This is a permanent man-made pond located near Poomkunnam fly-over at Patturakkal in Thrissur town, well protected by walls on all sides with an area of 0.12 km^2. The pond is being used by local people for washing and bathing. Nearly half of the pond contained aquatic macrophytes like *Utricularia, Hydrilla*, and *Pistia.* The collections were made on 5th March 2004 and cladocerans collected from the pond included *Moina micrura, Chydorus sphaericus* and *Diaphanosoma sarsi.*

Site 2: A permanent man-made water body (Vadakkechira pond) located in Thrissur town near North bus stand. The pond has an area of 0.20 km^2 and protected on all sides by walls. The pond was partially covered by aquatic macrophytes comprising mainly *Pistia, Salvinia* and *Hydrilla.* The collection was made on 2nd April 2003 and cladocerans collected from the pond included *Latonopsis australis, Oxyurella singalensis, Pseudosida bidentata, Chydorus sphaericus, Ilyocryptus spinifer, Diaphanosoma exciscum* and *Macrothrix spinosa.* The pond was visited by migratory water fowls, especially Common Teal during this period. The other birds such as white-breasted Kingfisher, Little Cormorant and Darter also visited the site. Another collection was also made on 3rd July 2003 and *Daphnia lumholtzi* and *Diaphanosoma sarsi* were abundantly present along with ephippial females and males.

Site 3: This is a man-made permanent pond located in Ollur, 5 km away from Thrissur town on the side of NH-47. The pond has an area of 0.14 km^2. It is located adjacent to a paddy field and well protected by granite side walls. Local people use this pond for bathing and washing. Aquatic macrophytes were absent and water was green in colour due to the presence of algae like *Chlorella* and *Ankistrodesmus.* The collection was made in 12th August 2003 and cladocerans collected from the pond included *Ceriodaphnia cornuta* and *Moinodaphnia macleayi.*

Site 4: It is a temporary water body formed in an abandoned brick-yard with less human interference located at Parappookkara, 17 km away from Thrissur town. It has an area of 0.22 km^2. Major aquatic macrophytes included *Salvinia, Hydrilla, Pistia, Nymphea* and *Ipomoea.* The water was turbid at the time of collection. The collection was made on 3rd July 2003 and

cladocerans collected include *Scapholeberis kingi* and *Chydorus sphaericus*. Another collection was also made on 18th November 2003 and cladocerans collected included *Simocephalus serrulatus, Macrothrix spinosa, Macrothrix triserialis, Alona costata* and *Chydorus sphaericus*. Further collection was made on 26th January 2004 when the water body started to dry up and cladocerans collected included *Moina micrura, Macrothrix triserialis* and *Chydorus sphaericus*.

Site 5: A shallow man-made pond located at Kalathode, 2.5 km away from Thrissur town. The pond has an area of 0.08 km² and protected on all sides by walls. Aquatic macrophytes were completely absent at the time of collections. The pond is being used by villagers for washing and bathing. Cladocerans collected from this pond in 20th July 2004 included *Ceriodaphnia cornuta* and *Chydorus sphaericus.* The green-coloured water present in the pond was due to dense growth of algae such as *Ankistrodesmus* and *Scenedesmus*.

Site 6: A shallow man-made pond (Kottamkulam temple pond) located at Urakam 12 km away from Thrissur town. The pond has an area of 0.10 km² and is adjacent to a laterite hillock which drained water to the pond during monsoon. It is protected on all sides by walls and several people use this pond for bathing and washing. *Nymphea* and *Hydrilla* were present on one side of the pond. The collection was made in 5th October 2005 and the cladocerans collected from the pond included *Moina micrura, Alona pulchella* and *Ephemeroporus barroisi.*

Site 7: A temporary water body (Chonkulam) with an area of 0.05 km² located adjacent to a paddy field at Mattathurkunnu near Kodakara, 18 kms

away from Thrissur town. Villagers use this water body for bathing and washing. Major aquatic macrophytes included *Salvinia* and *Pistia.* The collection was done using a scoop net. Major cladocerans collected in 22nd January included *Moina brachiata, Diaphanosoma sarsi, Pseudosida bidentata, Macrothrix triserialis, Macrothrix spinosa* and *Ilyocryptus spinifer* while on 2nd March 2005 included *Moina brachiata, Macrothrix triserialis* and *Macrothrix spinosa.* This pond gets completely dried up in summer. Soil samples were also collected on 8th May 2005 for making trials to hatch out resting eggs present in the sample.

Site 8: A man-made pond (Peringamkulam pond) with an area of 0.06 km^2 located near Kodakara. Villagers use this pond for washing. Although, several collections were made during 5th June to 18th September 2004, cladocerans were found to be absent. However, *Notonecta* and aquatic mites were abundantly represented in the sample. The surface of the pond was completely covered by *Salvinia* and *Pistia.*

3. 1. 2 Collection Methods

The collections were made generally with the help of a tow net made of bolting silk of 70μm mesh size with 20 cm mouth diameter. The mouth of the net was tied to a long rope and dragged over a distance of 5 meters at the sub-surface level. In the meanwhile the live animals got trapped in the bottle tied to one end of the net. Care was taken to drag the net at a uniform speed until it was hauled up. This method is mainly employed for the collection of planktonic species.

Collections were also done using 15 cm wide conical scoop net of 70μm mesh size. By means of a handle the net was worked vigorously in beds

of aquatic macrophytes. This was repeated and animals get collected into a bottle tied to one end of the net. The epiphytic species were collected using this method.

The epiphytic cladocerans were also obtained from the above sites by washing the weeds in a bucket of water. The water was again filtered through the bolting silk of 70μm mesh size to obtain the specimens. The specimens associated with the bottom mud were collected using a scoop net after disturbing the bottom. The collected samples were immediately brought to the laboratory in live condition, animals were sorted out for culture and a portion of the sample was preserved for taxonomic study.

3. 1. 3 Preservation and Storage

A portion of the collected sample was preserved in 4-6% formalin and kept in the laboratory. Formalin was found to be suitable for storage because chitin does not become too fragile upon storage. For prolonged storage small amount of sucrose (30-40 g/lit) in 5% formalin was added to prevent carapace distortion and egg-loss from the brood pouch following the methodology of Haney and Hall (1973).

Preparation of Paraffin Mounts

The formalin preserved animal was transferred to a small drop of glycerin placed at the center of a slide. Soft paraffin was melted and applied as a thin border around the specimen using a scalpel. A coverslip was placed supporting on this; and the slide was warmed over a flame. On warming the slide, the paraffin melts and runs in sealing the mount. This slide was labelled and stored for further study.

3.1.4 Identification

Species identification was done using standard keys of Michael and Sharma (1988); Battish (1992); and the monographs of Goulden (1968); Smirnov (1974); Idris (1983); Fryer (1987); Dodson and Frey (1991) and Korovchinsky (1992).

3. 2 Laboratory culture

The first step in cladoceran culture is to ensure a reliable food source. Unicellular algae especially *Chlorella* has been used as food for freshwater cladocerans in laboratory culture and is known to support growth and reproduction (Nandini and Sarma, 2000). The availability of unicellular algae is required for the successful culture of cladocerans.

3. 2. 1 Preparation of Algal culture

An algal stock culture was prepared and maintained in the laboratory to ensure a ready availability of unicellular algae. Water collected from Site 3 containing *Chlorella* sps. was filtered through bolting silk of 70µm mesh size and transferred into an aquarium (40 cm × 20 cm × 20 cm). This water was given a continuous aeration and kept under fluorescent light. Two Gold fishes (*Carassius* sp.) were introduced into this medium so as to maintain the trophic system within the aquarium. These fishes were fed with food pellets available in the market. The algal culture flourished and attained a dark green colour after 15 days. The side walls of the aquarium were always kept clean to ensure sufficient penetration of light. For maintaining this culture for a long period the fish was fed daily, detritus was siphoned out from the bottom and the culture medium was replenished daily by adding aerated-water.

Culture of *Chlorella*

Unicellular algae especially *Chlorella* forms one of the most important food items that can be easily ingested by most of the cladocerans. For developing a culture, the sample taken from the stock culture was centrifuged and algal cells were isolated carefully under a stereoscopic microscope using a micropipette. The isolated *Chlorella* cells were inoculated into the culture medium.

The culture medium was prepared using tap-water aerated for a period of 5 to 6 days; nourished with finely powdered groundnut cake at a rate of 500 mg/litre (Nayar and Babu, 2002). A mixture of super-phosphate (200 mg /litre) and urea (100 mg/litre) was added to this medium as an additional nutrient. The isolated live *Chlorella* cells were inoculated separately into three separate glass jars (30 cm × 15 cm × 15 cm) containing the medium. The inoculum was continuously aerated and kept in the laboratory under fluorescent light of uniform illumination (500 lux). This was again enriched by the addition of 20 ml of the same nutrient medium on every alternate day. James *et al.* (1988) have reported a considerably higher algal production when the medium is slightly acidic (pH 6.5) and the same condition was maintained by adding suitable buffer capsules. The culture was examined on alternate days until the appearance of a dark green colour and the *Chlorella* density was estimated using a haemocytometer. For maintaining this culture, a portion of the culture medium was replaced every day and replenished with the same quantity of water. For prolonged storage without contamination the culture was centrifuged and subsequently transferred to test tubes plugged with cotton. These test tubes were placed in a refrigerator at 10-15°C. It was again re-suspended in water when required for routine feeding.

3. 2. 2 General Rearing Methods

Cladocerans for the biological studies were collected from the field as mentioned in section 3. 1. 2 and reared in laboratory.

(1) ***Pseudosida bidentata*** Herrick, 1884 **var.** ***szalayi*** (Daday, 1898)

This species was obtained from the collection made at Site 7, on 22nd January 2005 when the water temperature was 26°C, pH 7.5 and dissolved oxygen 6.8 mg/litre. The collected population of *P. bidentata* comprised of parthenogenetic females. A stock culture of this species was developed by isolating the parthenogenetic egg-bearing females from this sample. The culture was maintained in the laboratory upto October 2005 for detailed studies on their life cycle.

(2) ***Latonopsis australis*** Sars, 1888

This species was obtained from among the littoral weeds at Site- 2 from the collection made on 2nd April 2003 when the water temperature was 29°C, pH 7.2 and dissolved oxygen 6.6 mg/ litre. The collected population of *L. australis* comprised of only parthenogenetic females. The live specimens were brought to the laboratory and egg-bearing females were isolated for developing a stock culture, which was maintained in the laboratory upto August 2003 for detailed studies on the life cycle.

(3) ***Diaphanosoma sarsi*** Richard, 1895

This species was obtained from the collection made at Site 1, on 5th March 2004 when the water temperature was 28°C, pH 6.2 and dissolved oxygen 4.4 mg/ litre. A good number of adult parthenogenetic females and

ephippial females were present in the sample. A stock culture was developed by isolating the egg-bearing females from this sample. The stock culture was maintained in the laboratory upto November 2004 for life cycle studies.

(4) *Ceriodaphnia cornuta* Sars, 1885

This species was obtained from the collection made at Site 5, on 20th July 2004 when the water temperature was 24°C, pH 6.8 and dissolved oxygen 6.1 mg/ litre. The population of *C. cornuta* comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto February 2005 for the detailed study of life cycle.

(5) *Scapholeberis kingi* Sars, 1903

S. kingi formed a compact aggregation on the surface film at Site 4 and were collected using a scoop net on 3rd July 2003 when the water temperature was 24.5°C, pH 6.0 and dissolved oxygen 7.2 mg/ litre. The aggregation comprised only parthenogenetic females. A stock culture was developed by isolating the egg-bearing females from this sample and was maintained in the laboratory upto February 2004 for life cycle studies.

(6) *Simocephalus serrulatus* (Koch, 1841)

This species was obtained from the collection made at Site 4, on 18th November 2003 when the water temperature was 25°C, pH 5.8 and dissolved oxygen 5.6 mg/litre. The aquatic plants were collected from the site and rinsed in water to collect the specimens into a bottle. The collected population of *S. serrulatus* comprised only parthenogenetic females. A stock culture was

developed by isolating the species from this sample and was maintained in the laboratory from upto May 2005 for detailed studies on life cycle.

(7) ***Moina brachiata*** (Jurine, 1820)

The top soil samples were collected from the Site 7, when the pond got dried up in May 2005. The soil sample was passed through proper sieves to remove the algal matter and other unwanted particles. This sample was dried and stored in polythene packets.

Two litres of the culture medium containing algae was transferred into a glass jar and kept it under mild aeration. A portion of the stored soil sample was introduced into this culture medium and kept in laboratory at 28-30°C, pH 6.8 and dissolved oxygen 7.6 mg/litre. Further, 100 ml of algal culture was added on every alternate day. On 6th day the neonates of *M. brachiata* appeared in the culture. These neonates were sorted out and reared to maturity in separate glass vessels. This was kept as stock culture and maintained in the laboratory upto December 2005 for life cycle studies.

(8) ***Moinodaphnia macleayi*** (King, 1853)

M. macleayi was obtained from the collection made at Site 3, on 12th August 2003 when the water temperature was 25 °C, pH 6.5 and dissolved oxygen 5.4 mg/ litre. The collected *M. macleayi* population comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample, in which a good number of males and ephippial females were developed. The stock culture was maintained in the laboratory upto December 2003 for detailed studies on the life cycle of female.

(9) *Ilyocryptus spinifer* Herrick, 1882

This species was obtained from the collection made at Site 7, on 22nd January 2005 using a scoop net when the water temperature was 26°C, pH 7.5 and dissolved oxygen 6.8 mg/ litre. The *I. spinifer* population collected from the site comprised only of parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto November 2005 for detailed studies on the life cycle.

(10) *Macrothrix triserialis* (Brady, 1886)

M. triserialis was obtained from the collection made at Site 4, on 18th November 2003 when the water temperature was 25°C, pH 6.0 and dissolved oxygen 5.9 mg/ litre. The aquatic plants were collected from the site and rinsed in water to collect the specimens into a bottle. The collected *M. triserialis* population comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto January 2005 for detailed studies on the life cycle.

(11) *Alona pulchella* King, 1853

A. pulchella was obtained from the collection made at Site 6, on 5th October 2005 using a scoop net, when water temperature was 26°C, pH 5.6 and dissolved oxygen 4.2 mg/ litre. A good number of adult parthenogenetic females were present. A stock culture was developed by isolating the egg-bearing females from this sample and was maintained in the laboratory upto May 2006.

(12) *Oxyurella singalensis* (Daday, 1898)

This species was obtained from the collection made at Site 2, on 2nd April 2003 using a scoop net when the water temperature was 29°C, pH 7.2 and dissolved oxygen 6.6 mg/ litre. A good number of adult parthenogenetic females were present in the collection. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory up to October 2003.

Cladoceran Stock culture

The stock culture of the above species was developed under laboratory conditions using the following methodology.

Ten egg-bearing parthenogenetic females of a particular species were sorted out from the field sample using a sterilized pipette under a stereoscopic microscope. These adult females were immediately transferred into a beaker containing filtered pondwater and kept undisturbed for 2-3 days to enable them acclimatize the laboratory conditions.

The egg bearing females were again transferred into an aquarium (40 cm × 20 cm × 20 cm) containing culture medium, prepared by adding finely powdered groundnut cake (500 mg/litre) into 4 litres of aerated tap-water. A mild aeration was given throughout the culture period and kept it in the laboratory. *Chlorella* culture (100 ml) was added daily to ensure a sufficient supply of food and to compensate the water lost by evaporation. The culture was examined periodically. When the culture becomes overcrowded, about one tenth of this stock culture was daily removed to reduce the population density and replenished with an equal amount of water. By this periodic

removal and renewal of water the stock culture could be maintained for a longer duration.

Ephippium collection

The stock culture was kept under constant observation to find out the appearance of ephippial females. When ephippial females appeared, they were carefully collected with the help of a brush and were transferred to separate petri-dishes containing fresh culture medium for further study. Periodic examination is required for the collection of ephippia cast off by these females. The ephippia were collected, dried and stored without damage.

3. 2. 3 Rearing methods for Life cycle studies

Parthenogenetic females

The egg-bearing parthenogenetic females were isolated from the stock culture and introduced individually into beakers containing 100 ml of culture medium. The adult females were removed from the culture soon after the release of the first generation of neonates. These neonates were again reared to maturity in fresh medium until they released the second generation.

Immediately after the release, twenty neonates were individually sorted out and reared in separate glass vessels. Cladocerans such as *P. bidentata, L. australis, D. sarsi, C. cornuta, S. serrulatus, M. brachiata, M. macleayi, M. triserialis* and *O. singalensis* were cultured in test tubes of 10 ml capacity while *S. kingi, I. spinifer* and *A. pulchella* were cultured in petri-dishes of the same capacity.

Each individual was supplied with *Chlorella* (2×10^6 cells ml^{-1}) at an

interval of 24 hrs while changing the culture medium. An equal quantity of the algal medium was added to each test tube. The medium change was necessary to ensure sufficient food as well as for the removal of neonates and the exuvia cast off during moulting. All the studies were made in the laboratory where the water temperature varied from 26-30°C and pH 6.2 to 6.8. All the culture tubes were kept partially immersed in another tray of water to minimize temperature fluctuations.

The moulting, instar duration, egg production, embryonic development, life cycle and life span were individually recorded until death of the last individual. The size measurements were done in each instar to record the growth, the details of which are given in section 3.3. 3.

All the twenty neonates for life cycle studies were reared individually as mentioned above, while another set was also reared simultaneously providing the same conditions for dissections and identification of developmental stages.

Culture of males

The stock culture was examined periodically to find out the appearance of males. When males made their appearance 20 neonates of less than 12 hrs of age were sorted out. Males could be identified mostly based on morphological features and their relative size.

The life cycle was studied by rearing them under the same laboratory conditions provided for the culture of females as mentioned above. The size measurements were done daily and the growth was recorded until death of each individual. The life cycle of four cladoceran males such as *Pseudosida*

bidentata, Moinodaphnia macleayi, Macrothrix triserialis and *Oxyurella singalensis* were studied.

3. 3 Studies on individual species

The following aspects were studied by culturing each species under similar laboratory conditions.

3. 3. 1 Morphology

The general morphology of the three different morphotypes viz. the parthenogenetic female, ephippial female and male were studied under a microscope based on temporary mounts made in glycerol. The characters studied included the body form and size, head morphology, nature of postabdomen, setation in antennae, antennule and the shell.

The dissections were done when needed using tungsten micro-needle fitted on handles. Drawings were made using camera lucida and measurements were done using calibrated micrometers. Microphotographs were taken using stereoscopic zoom-microscope fitted with Sony digital camera of 7.2 mega pixels.

3. 3. 2 Reproduction and Life cycle

Twenty neonates were sorted out and reared in separate glass vessels as mentioned in section 3. 2. 3. The events during the life cycle were followed by periodic observation until death of the last individual.

The mean value of initial size is taken as Size at Birth (SaB). The stage between hatching of a neonate and the moult preceding the first appearance of

eggs in the brood pouch is considered the pre-adult stage. The mean time required for pre-adult stage is taken as Pre-adult Instar Duration (PID).

The time of moulting was determined by examining for the presence of exuvium in the medium. The developmental stage between successive moults is defined as an instar and the time between successive moults as the instar duration. This includes the time required for egg transfer, embryonic development and release of young ones followed by moulting.

Age at First Reproduction (AFR) is the time taken for the completion of pre-adult moults and the attainment of sexual maturity which is calculated in number of days. The first appearance of mature eggs in the brood pouch is considered the initiation of adulthood and the mean size during this stage is expressed as Size at First Reproduction (SFR). This stage is designated as the primiparous stage and the succeeding instars are considered the adult instars.

The period from birth till the release of first generation of neonates is taken as First Generation Time (FGT), which is calculated by adding the mean duration of pre-adult and the primiparous instar (first egg bearing instar). The Relative Length (RL) and Relative Height (RH) were calculated by dividing Size at Birth (SaB) by Size at First Reproduction (SFR). The mean duration of the adult instar is taken as the mean Adult Instar Duration (AID).

Egg production

Egg production in Cladocera is associated with the termination of each adult instar. The number of eggs present in a single brood during an instar is designated as a clutch. The number of eggs/ brood was counted from

the primiparous instar onwards till the end of the life span of an individual. In terms of number it is designated as the clutch size. The maximum clutch size (Emax) is the mean highest number of eggs produced during the life cycle.

Fecundity

Fecundity is defined as the potential reproductive capacity of an organism during its life time measured by the number of eggs produced. In Cladocera, it specifically refers to the number of eggs produced per female, starting from the primiparous instar until the death of the animal. In the present study it is estimated by enumerating the number of eggs in the brood pouch of the parthenogenetic female after dissecting out the eggs using micro-needles and counting them under the microscope. This could also be done by counting the number of eggs in live condition since the eggs are visible through the carapace.

The total number of eggs produced during the life is a measure of reproductive potential and expressed as cumulative fecundity (Σmx). The number of eggs produced per individual per day of adult life was also calculated by dividing the cumulative fecundity by total duration of adult instar in days.

The Rate of Egg Production (REP) is estimated by plotting the cumulative egg production against the instar number following the methodology of Navaneethakrishnan and Michael (1971), in which the angle of slope of regression line is taken as the rate of egg production.

3. 3. 3 Growth

The progressive increase in mean size of the individual during each instar is a measure of growth rate and hence important in studies on biology (Edmondson, 1955). Growth rate is an important life history trait because it is intrinsically linked to and directly affects other life history traits such as Age at First Reproduction (AFR), Size at First Reproduction (SFR), First Generation Time (FGT) and Rate of Egg Production (REP).

The pattern of growth was studied by measuring the size of the individual at each instar. Size of the animal refers to the Total Length (TL) extending from the anterior margin of the head to the tip of the tail spine. The maximum distance between the dorsal and ventral margin of the carapace is taken as the Carapace Height (CH).

Size measurements of each individual were done using calibrated micrometers at regular 24 hrs interval until their death. The mean value of the measurements in each instar and its standard error (SE) was calculated. The growth rate was represented by plotting instar number on x-axis against mean size.

The size increment during each instar is also taken as an index of the growth rate. Size increments of the females were calculated for each individual by subtracting its size at instar-X from its size at instar X +1. The percentage of size increment was calculated with respect to the size at each succeeding instar. Correlation between size and a few selected life history characters were also analyzed.

3. 3. 4 Embryonic Development

The sequence of morphological changes during the embryonic development was documented related to time duration in hours. The deposition of eggs into brood pouch could be regarded as the first event which initiates the embryonic development. The morphological changes in the embryo could be visible through the carapace even when the embryo developed *in vivo.* The end of embryogenesis was determined by the completion of development and subsequent release of a batch of neonates from the brood pouch.

Green (1956) has earlier divided the embryonic development mainly into 3 stages viz: the early, middle and late stages. During the present study attempts were done to find out other clearly identifiable sub-stages. The adult females reared for the study of embryonic development were periodically dissected out under stereoscopic microscope to expose the embryos for photography and measurements. At least 10 embryos were measured to determine the size range.

3. 3. 5 Life span and Survivorship

The life span or longevity is the period of time between the birth and death of an organism. The life span was calculated by recording the duration of life starting from 1st day till the death of the last individual.

The maximum Life span (Lmax) observed in each species is expressed as duration of time in days. The mean life span (Σlx) of each species was also calculated and expressed in terms of days. Handling during the early stages was done with great care since this may increase chances of mortality.

The term survivorship refers to the number of individuals survived after each day. This was estimated by recording the number of individuals survived at an interval of 24 hrs starting from 1st day until death of all the individuals cultured for life cycle studies. The percentage survival was calculated as the percentage of their initial number. Age in days was plotted in x-axis against percentage survival to represent survivorship curves.

ORDER CTENOPODA

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 4

ORDER CTENOPODA

Family Sididae Baird, 1850

4. 1 ***Pseudosida bidentata*** Herrick, 1884

var. ***Szalayi*** (Daday, 1898)

Pseudosida bidentata var. *szalayi* is a littoral cladoceran found in a variety of habitats like ponds, marshes, and rice fields. They were generally collected from among the vegetation and from shallow water bodies. This species was described first by Herrick (1884). Subsequently Daday (1898) described var. *szalayi*. Thomas (1961) reviewed the validity of the name *P. bidentata* and suggested that the species name *bidentata* should be retained. The character that differentiated var. *szalayi* from *P. bidentata* is a spine-like projection on the distal margin of the postabdomen. *P. bidentata* was reported from the shallow water bodies of tropical countries especially from Sumatra, Sri-Lanka, Malaysia, South Africa and Southern United States.

The first record of this species in Kerala is from the collections of Nayar, C.K. G. from Irinjalakuda, reported by Michael and Sharma (1988). A spine-like projection was clearly visible in the specimens collected for the present study and hence described it as *P. bidentata* var. *szalayi*. The biology of *P. bidentata* has not been investigated so far and hence a detailed study of the life cycle of the parthenogenetic female and male have been made.

4. 1. 1 External Morphology

Parthenogenetic female (Plate 3. Fig. B)

Body elongated and oval-shaped, with short head and rostrum. Eye relatively small, situated near the anteroventral corner (Plate 2. Fig. A). Antennules unsegmented, rather long and attached to posteroventral part of the head, with a group of short setae half way along its length and a long sensory seta at the tip (Plate 2. Fig. C). Antennae stout, do not extend beyond the posterior margin of valves; with 2-segmented dorsal ramus and 3-segmented ventral ramus; setation of antenna: (5-10)/ (0-1-3). Dorsal and ventral margin of the valves convex, while the posterior margin rounded. Postabdomen short and broad, with a median projection at its apex (Plate 2. Fig. B); anal denticles absent, lateral surface armed with about 10 clusters of spinules and groups of fine setules; claw long, curved and sharply pointed dorsally; convex surface serrated along the entire length, concave surface with a series of short setules and 3 basal spines; groups of short spinules on the ventral and lateral side of the basal spine; proximal end of the postabdomen has 2 long natatorial setae.

Mean size: 1.925 × 0.908 mm

Male (Plate 3. Fig. C)

Male smaller than female. Antennule elongated (Plate 2. Fig. E). First thoracic leg modified to form a curved hook. Postabdomen with two sperm ducts, one on each side (Plate 3. Fig. D). Mean size: 1.266 × 0.616 mm.

Ephippial female (Plate 3. Fig. E)

Ephippial female similar to parthenogenetic female in external morphology. Most often each female carries 2 to 4 separate ephippia on either side; each enclosing single egg (Plate 3. Fig. E). The ephippium oval, comparatively small, white in colour without pigmentation; anterior broad end with an air space, and a few spines on its surface (Plate 3. Fig. F).

Mean size of ephippial female: 1.386 × 0.750 mm. Mean size of ephippium: 0.366× 0.291 mm

4. 1. 2 Reproduction

The population developed during the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. The parthenogenetic females produced male and female neonates from a single clutch. Thus the individuals produced from a single clutch were all females, all males or both males and females. Among this the parthenogenetic females dominated the culture.

The appearance of males was followed by the production of ephippial females in the culture. The ephippial females appeared when the stock culture was crowded. These ephippial females were produced during their early instars, which resumes parthenogenetic reproduction after 1-2 generations. Although, each ephippial female produced two ephippia at a time, each ephippium enclosed single egg within it. Some of the ephippia were also produced without eggs within it. The ephippium was released into the medium during moulting after being completely detached from the carapace.

Life cycle of male

Males appeared in the culture before the production of ephippial females and were produced from the parthenogenetic females. The males could be identified early in life by the presence of their relative smaller size and elongated antennule. Twenty male neonates were sorted out and individually reared in 10 ml test tubes as cited in section 3. 2. 3.

The male neonates had mean Size at Birth (SaB) of 0.672 × 0.236 mm. The 1st and 2nd moulting occurred in same duration of 30.5 hrs each, while the 3rd moulting took place after an interval of 37 hrs. The first pre-adult neonates had mean TL of 0.683 and attained TL of 0.975 mm after 1st moulting. The CH was 0.241 and 0.341 mm respectively during first and second instars. They attained mean size of 1.142 × 0.440 mm during 3rd instar and became mature (Table 4). They underwent 3 moults in a total pre-adult duration of 98.0 hrs.

The maximum increment in TL (42.75%) and CH (41.49%) was recorded after the 1st moulting (Table 4). The increment after 2nd moulting was TL (23.08%) and CH (26.10%) respectively. The increment of TL and CH decreased after the third moulting (5th day) and there was no moulting further till death. The maximum increment of growth occurred during the pre-adult instars (Fig. 1 b). The mean life span is calculated as 10.43 days while the maximum life span of *P. bidentata* male observed in the present study (Lmax) was 22.5 days (Fig. 1 e).

Life cycle of parthenogenetic female

The population developed during laboratory culture of *P. bidentata* comprised a sufficient number of parthenogenetic females. The reproduction and life cycle of *P. bidentata* female was studied by culturing them individually following the methodology given in section 3. 2. 3.

The features characteristic of the reproduction and life cycle are given as follows.

Pre-adult instar

The neonates produced from the parthenogenetic females had an average Size at Birth (SaB) of 0.697 × 0.246 mm. Both the first and second moulting occurred in a uniform duration of 21.5 hrs while the third moulting took place after an interval of 29.0 hrs. The total pre-adult instar duration was 72.0 hrs and the mean Pre-adult Instar Duration (PID) was 24.0 hrs.

Attainment of maturity

Although, the development of ovary in the parthenogenetic female started very early in the life cycle the ovary became conspicuous at 68±1.2 hrs of life (Plate 3. Fig. A). The ovary appeared as elongated structures on either side of the alimentary canal. They started to bear eggs after completion of 3rd moult at 72 hrs; and hence the Age at First Reproduction (AFR) was 3.0 days. The Size at First Reproduction (SFR) was 1.300 × 0.533 mm (Table 1). The Relative Length (RL) and Relative Height (RH) of neonates were 0.420 and 0.349 mm respectively.

Egg production

Eggs were deposited into brood pouch after completion of three moults. The eggs very soon attained an elongated shape with slight yellow-green colour. There was an accumulation of yolk and lipid droplets after deposition into the brood pouch and the eggs measured a length of 0.300 mm. During this primiparous instar (4^{th} instar) egg production started with a mean of 4.37 eggs/ brood. The primiparous instar was completed in 38.0 hrs and the First Generation Time (FGT) is calculated as (72hrs + 38hrs) = 110.0 hrs.

During the subsequent instars there was a steady increase in egg production to 15.2 eggs/ brood. This maximum clutch size (Emax) was attained in the 11^{th} instar (Table 1). The egg production of *P. bidentata* showed a single peak with maximum egg production in the 11^{th} instar followed by a steady decrease (Fig. 1 c). The egg production continued upto 15^{th} instar followed by 2 instars without egg production.

The female underwent moulting towards the end of each instar. Each clutch in the early adult instar consisted of 1-2 rows of eggs on either side of the brood pouch, which were very clearly observed in live animals. The adult instar duration varied from 36.0 to 45.0 hrs, with a mean Adult Instar Duration (AID) of 38.42 hrs. The relationship of egg production with instar number is represented in Fig. 1 c.

Fecundity

The range of egg production of a single female varied from 2 to 16 with an egg production of 4.83 eggs/day of adult life. The cumulative number

of eggs produced (Σmx) during the entire life span was 108.13 (Table 1). Twelve broods were produced during the entire life with a mean of 9.0 eggs/ brood. The cumulative egg production (Σmx) was linearly correlated with instar number (Fig. 1 d) to obtain the Rate of Egg Production (REP). The angle of slope of regression line gives the rate of egg production and hence the REP of *P. bidentata* is calculated as 9.0919.

4. 1. 3 Growth

The first pre-adult neonates had a mean Total Length (TL) of 0.721 mm and the 2nd instar had TL of 0. 808 mm. During the 3rd instar it attained TL of 1.100 mm. Primiparous stage was attained during the 4th instar when the mean TL was 1.300 mm. The maximum mean TL of 2.046 mm was attained at the end of 17th instar (Table 2).

The mean Carapace Height (CH) during the first pre-adult instar was 0.250 mm and attained CH of 0.285 mm at second instar. During the third instar it attained CH of 0.391 mm. They attained a mean CH of 0.533 mm during the primiparous condition. Maximum mean CH was attained in 16th instar with CH of 0.906 mm which remained unchanged during 17th instar (Table 2). During the life span each individual has undergone three pre-adult and fourteen adult moults.

The increment of TL and CH during each instar is given in Table 2. The maximum growth increment recorded during the life cycle was in 3rd instar; however the most significant decrease in growth increment occurred only after the 8th instar. The relationship between TL, CH and instar

number of *P. bidentata* is represented in Fig. 1 a. The correlation coefficients of life history characters in *P. bidentata* are given in Table 3. The TL and CH are positively correlated ($r = 0.997$).

4. 1. 4 Embryonic Development

The stages of embryonic development of *P. bidentata* are represented in Plate 4. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of spherical eggs which are yellow in colour. Mean duration: 0.5 hrs. Mean size: 0.310 mm

Stage II: The embryo becomes pear-shaped with yellow colour. The yolk granules are centrally placed.

Mean duration: 2.4 hrs. Mean size: 0.332 mm

Stage III: The embryo is more elongated during this stage. The outer transparent area of the embryo shows cellular division and the inner area appeared granular with the centrally placed fat globule.

Mean duration: 8.1 hrs. Mean size: 0.398 mm.

Stage IV: During this stage the cellular divisions are distinguishable and the embryo is more elongated in anterio-posterior axis. The rudiments of head and antennae are visible during this stage.

Mean duration: 9.5 hrs. Mean size: 0.465 mm

Stage V: The rudiments of head, antennule, antennae, and postabdomen appear. The yolk granules are conspicuous and appeared green coloured. Mean duration**:** 4.3 hrs. Mean size: 0.508 mm

Stage VI: This stage can be recognized by the presence of pink eyes. The rudiments of antennae antennules and the postabdomen are clearly visible. Mean duration: 4.5 hrs. Mean size: 0.510 mm.

Stage VII: This stage can be recognized by the presence of black eyes. The rudiments of antennae antennules, thoracic legs and postabdomen are distinct. Mean duration: 6.5 hrs. Mean size: 0.546 mm.

Stage VIII: This stage can be recognized by the presence of black eyes and ocellus. The antennae antennules, thoracic legs and the postabdomen are distinct. Some movements are also initiated in the embryo.Mean duration: 1.7 hrs. Mean size: 0.558 mm

Release of neonates: The neonates are released to the exterior by the jerking movements of the mother. This could be regarded as the final event of embryonic development. The embryonic development of primiparous instar of *P. bidentata* was completed in 37.5 hrs.

4. 1. 5 Life Span and Survivorship

The survivorship curve (Fig.1 e) indicated the relationship of age (days) and percentage survival of *P. bidentata.* The mean life span (Σlx) of female is calculated as 9.85 days while the maximum life span (L max) of female observed during the present study was 25.41 days.

4. 2 *Latonopsis australis* Sars, 1888

L. australis was described first by Sars (1888) from Australia. Harding and Petkovski (1963) after examining a number of important characters concluded that *L. australis* was the only valid name; and considered name *L. occidentalis* Birge as a synonym. The first report of this species from India is by Biswas (1971) from Rajasthan and subsequently recorded from Tamil Nadu by Michael and Sharma (1988) and Venkataraman (1993). Babu and Nayar (2004) reported this species from Thekkady, Kerala.

The available literature shows that the biology of *L australis* has not been studied so far and hence an investigation of the life cycle of the parthenogenetic female has been made.

4. 2. 1 External Morphology

Parthenogenetic female (Plate 5. Fig. C)

Body elongated; short and thick head, indistinctly separated from the rest of body. Eye located very near to anterior margin of head, ocellus minute (Plate 9. Fig. A). Antennule segmented with a long flagellum beset with sensory setae (Plate 9. Fig. B). Antennae short and broad about half the maximum length of the body, with 2-segmented dorsal ramus and 3-segmented ventral ramus; setation of antenna: (4-7)/ (0-1-4). Valves slightly convex dorsally and broadly rounded ventrally; ventral margin with a series

of long and movable setae; 3 long plumose setae at posteroventral corner along with other setae decreasing in length dorsally (Plate 9. Fig. A). Postabdomen short, with 8-9 marginal spines and claw with 2 long basal spines (Plate 9. Fig. C). Mean size: 1.125×0.642 mm.

Male (Plate 5. Fig. D)

Male smaller than female. Head short and thick, visually not separated from the body. Antennules long, attached to the anteroventral corner of head; with club-shaped series of setae on the proximal end; segmentation in antennule not clearly visible (Plate 9. Fig. D). Postabdomen short with two long sperm ducts; lateral surface armed with a series of 4-5 denticles (Plate 9. Fig. E). Mean size: 0.825×0.418 mm.

Ephippial female (Plate 5. Figs. E & F)

The general features of ephippial female similar to parthenogenetic female. Ephippium oval, relatively small, white in colour and located dorsally; most often carry 1-2 ephippia; each enclosing single egg; anterior broad end with an air space and a few spines on its surface (Plate 5. Fig. G). Mean size : 1.03×0.717 mm. Size of ephippium: 0.316×0.250 mm

4. 2. 2 Reproduction

The population developed during the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. Among this the parthenogenetic females dominated the culture. Ephippial females were found to be produced either directly from primiparous individuals

or from parthenogenetic individuals who have already undergone 1-2 generations of parthenogenetic instars. Ephippial females appeared soon after the production of males. 1-2 ephippial eggs are produced at a time and released after completion of development, each enclosed single egg containing abundant yolk (Plate 5. Fig. G). They also produced ephippium without enclosing egg within it (Plate 5. Fig. H). The air space, present at the upper surface of ephippia enables floating. When the eggs get dried up, they float on the surface for some time and most often get attached to other objects in water.

Life cycle of parthenogenetic female

The features characteristic of the reproduction and life cycle of *L. australis* are given as follows.

Pre-adult instar

The neonates produced from the parthenogenetic females had an average birth size (SaB) of 0.512 × 0.208 mm. The first moulting occurred in an interval of 26.0 hrs while both the second and third moulting took place in a uniform duration of 28.0 hrs. The total pre-adult instar duration was 82.0 hrs and the mean PID was 27.33 hrs.

Attainment of maturity

Although, the development of ovary in the parthenogenetic female started very early in the life cycle, the ovary was clearly visible at 74±2.5 hrs (Plate 5, Fig. B). They started to bear eggs after completion of 3rd moult at

82.0 hrs; and hence the AFR was 3.42 days. The SFR was 1.104×0.472 mm. The RL and RH of neonates were 0.464 and 0.441 mm respectively.

Egg production

The eggs were deposited into the brood pouch after the completion of three moults. Soon after the deposition into the brood pouch there was a rapid increase in size due to the accumulation of lipid droplets and yolk. The eggs thus attained an elongated shape with green colour and measured a mean length of 0.208 mm. During this primiparous instar (4^{th} instar) egg production started with a mean of 4.2 eggs/ brood. The primiparous instar was completed in 31.50 hrs and the first generation time (FGT) is calculated as 113.5 hrs.

During the subsequent instars there was a steady increase in egg production to 13.4 eggs/ brood. This maximum clutch size (Emax) was attained in the 8^{th} instar (Table 5). The egg production of *L. australis* showed a single peak with maximum egg production in 8^{th} instar followed by a steady decrease (Fig. 2 b). The egg production continued upto 12^{th} instar towards the end of life span. Each clutch in the early adult instar consisted of 1-2 rows of eggs placed on either side of the brood pouch. The female underwent moulting towards the end of each adult instar. The adult instar duration varied from 32.0 to 50.0 hrs (Table 5), with a mean AID of 42.85 hrs.

Fecundity

The relationship of egg production with instar number is represented in Fig. 2 b. The range of egg production of a single female varied from 2 to 15

with an egg production of 4.40 eggs/day of adult life. The cumulative number of eggs produced (Σmx) during entire life span was 73.1 (Table 5). Nine broods were produced during the entire life with a mean of 8.12 eggs/ brood. The cumulative egg production (Σmx) is linearly correlated with adult instar number (Fig. 2 c). The rate of egg production (REP) of *L. australis* is calculated as 7.9021.

4. 2. 3 Growth

The first pre-adult neonates had a mean TL of 0.608 mm and 2nd instar had TL of 0. 728 mm. During the 3rd instar it attained a TL of 0.888 mm. Primiparous stage was attained during the 4th instar when the mean TL was 1.104 mm. The maximum mean TL of 2.016 mm was attained at the end of 12th instar (Table 6). The mean CH during the first pre-adult instar was 0.256 mm and attained CH of 0.312 mm at 2nd instar. During the 3rd instar it attained CH of 0.356 mm. A mean CH of 0.472 mm was attained during the primiparous instar. Maximum mean CH of 0.840 mm was attained in 12th instar (Table 6). During the life span each individual has undergone three pre-adult and nine adult moults.

The size increment during each instar has been represented in Table 6. Maximum growth increment recorded during the life cycle was in 6th instar; and decreased during the subsequent instars. The relationship between TL, CH and instar number of *L. australis* is represented in Fig. 2 a. The correlation coefficients of life history characters are given in Table 7. Positive correlation was obtained between TL and CH ($r = 0.996$).

4. 2. 4 Embryonic Development

The embryonic stages are described based on Plate 6 and the conspicuous characters are mentioned below. The embryonic development of primiparous instar of *L. australis* was completed in 30.5 hrs.

Stage I: This stage is recognized by the presence of oval-shaped egg which appears green in colour. The inner zone of the egg appeared granular and contains abundant yolk granules. Mean duration: 1.0 hrs. Mean size: 0.229 mm

Stage II: The embryo shows elongation. The outer transparent zone becomes more distinct. Fat globules are centrally placed in green coloured yolk granules. Mean duration: 1.5 hrs. Mean size: 0.246 mm

Stage III: The embryo is more elongated during this stage. The outer transparent zone of embryo shows cellular division. Mean duration: 4.6 hrs. Mean size: 0.269 mm

Stage IV: During this stage the embryo is more elongated in the anterio-posterior axis. The head lobe appears during this stage. Mean duration: 2.2 hrs. Mean size: 0.302 mm

Stage V: The cellular divisions are visible and rudiments of head appear at the anterior region. Mean duration: 4.0 hrs. Mean size: 0.307 mm.

Stage VI: The head lobe becomes more distinct with the appearance of complete segmentation over the outer transparent area. Mean duration: 3.5 hrs. Mean size: 0.317 mm

Stage VII: The rudiment of antennae is more distinctly visible. The posterior region shows distinct cellular divisions with initiation of the development of thoracic legs and postabdomen. Mean duration: 1.2 hrs. Mean size: 0.317 mm

Stage VIII: The outer membrane is cast off and the rudiments of head antennae and thoracic legs are more clearly visible. Mean duration: 3.40 hrs. Mean size: 0.347 mm

Stage IX: The presence of eye is noticed for the first time during this stage. The head, antennules, rudiments of legs and postabdomen are very distinct. The amount of yolk gets decreased. Mean duration: 4.8 hrs. Mean size: 0.370 mm.

Stage X: The eye and postabdomen become distinct. Setae appear on the antennules, antennae and thoracic legs. Yolk completely disappears. Movements are also initiated in the embryo during this stage. Mean duration: 4.3 hrs. Mean size: 0.586 mm.

4. 2. 5 Life Span and Survivorship

The survivorship curve (Fig. 2 d) indicates the relationship of age (days) and percentage survival of *L. australis*. Survival was higher near the age of maturity and declined steadily further after maturity. The mean life span (Σlx) of female is calculated as 7.95 days; while the maximum life span (Lmax) observed in the present study was 19.49 days.

4. 3 *Diaphanosoma sarsi* Richard, 1894

Diaphanosoma sarsi is a planktonic cladoceran which often dominates the limnetic region of lakes, man made reservoirs, marshes and rice fields. This species was described first by Richard (1894). *D. sarsi* was first reported in India from Bihar (Gurney, 1907) and later from Rajasthan (Biswas, 1971); Meghalaya (Patil, 1976); West Bengal (Sharma, 1978) and Delhi (Michael and Sharma, 1988). Raghunathan (1989a) made the first record of *D. sarsi* from Wynad, Kerala. The first report of the males of this species from India is that of Babu and Nayar (2004) collected from Thekkady, Kerala.

4. 3. 1 External Morphology

Parthenogenetic female (Plate 7. Fig. B)

The body elongated and highly transparent. Head small, without rostrum. Eye relatively large (Plate 9. Fig. G). Antennules small, cigarette-shaped with terminal setae. Antennae large but not reaching the posterior margin of valves; dorsal ramus 2-segmented and ventral ramus 3-segmented (Plate 9. Fig. F). Thoracic legs six pairs, of similar structure. Valves with varying number of denticles followed by a series of fine setules (Plate 9. Fig. H); ventral margin inflexed to form a broad flap. Postabdomen narrow, without anal spines; claw with 3 long and sharply pointed basal spines (Plate 9. Fig. I). Mean size: 1.022×0.404 mm

Male (Plate 7. Fig. C)

Smaller than female, characterized by the presence of long whip-like antennule (Plate 9. Fig. J). Postabdomen with two long sperm ducts. Endopodite of first thoracic leg modified to form a sickle-shaped hook. Mean size: 0.690 mm.

Ephippial female (Plate 7. Fig. D)

Ephippial female similar to parthenogenetic female in external morphology. Most often each female carried 2 to 4 ephippia, on either side, each enclosing single egg. The ephippium sphere-shaped, comparatively small, white in colour, having finger like outgrowths over the surface (Plate 7. Fig. E). Mean size: 0.730 mm. Size of ephippium: 0.180 mm

4. 3. 2 Reproduction

The population developed in the laboratory comprised asexually reproducing females, ephippia bearing females and males. Ephippial females were produced directly from individuals during their early adult instars, and resumed parthenogenetic reproduction after 1-4 sexual generations. The ephippia were spherical, comparatively smaller, with finger shaped outgrowths on their surface and released during moulting. The number of ephippia produced by each ephippial female ranged from 2 to 4. They are placed on either side, each enclosed single egg. The outgrowths present on the surface of ephippium helps to float on the water surface or clinging to the vegetation.

Males appeared just before the production of ephippial females. However, the parthenogenetic reproduction was dominant during its life.

Life cycle of parthenogenetic female

Parthenogenetic population dominated in the *D. sarsi* culture throughout the study period. The parthenogenetic females were individually cultured to study the following features characteristic of the life cycle.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean SaB of 0.522 × 0.184 mm. The first and second moulting occurred in duration of 24.0 hrs and 24.5 hrs respectively. The total pre-adult instar duration was 48.5 hrs and the mean PID was 24.25 hrs.

Attainment of maturity

The ovary appeared as elongated structures on either side of the alimentary canal and was clearly visible at 39±2 hrs of life (Plate 7. Fig. A). However, they started to bear eggs after completion of 2nd moult at 48.5 hrs; and hence the AFR was 2. 02 days. The SFR was 0.809 ×0.302 mm. The RL and RH of neonates were 0.522 and 0.609 mm respectively.

Egg production

Eggs were deposited into the brood pouch after the completion of two moults. The egg production started during the primiparous instar (3rd instar) with a mean number of 4.2 eggs/ brood. The amount of yolk and lipid

droplets was very low even after deposition into brood pouch and hence the eggs appeared transparent. The eggs measured a mean length of 0.299 mm during this stage; and having an elongated shape with slight green colour. The primiparous instar was completed in 32.0 hrs and the FGT is calculated as 80.5 hrs.

During the subsequent instars there was a steady increase in egg production to 13.0 eggs/ brood (Table 8). This maximum clutch size (Emax) was attained in the 9th instar. The egg production of *D. sarsi* showed a single peak with maximum egg production in 9th instar (Fig. 3 b). Although, egg production decreased subsequently; there was the production of a uniform number of eggs in each brood. The egg production continued throughout the life span up to the 15th instar (Table 8). Each clutch produced in the early adult instar consisted of 1-2 rows of eggs on either side of the brood pouch. The adult instar duration varied from 30.0 to 39.0 hrs, with a mean AID of 35.04 hrs.

Fecundity

The relationship between egg production and instar number is represented in Fig. 3 b. The range of egg production of a single female varied from 4 to 16 with an egg production of 6.17 eggs/day of adult life. The cumulative number of eggs produced (Σmx) during the entire life span was 117.3 (Table 8). Thirteen broods were produced during the entire life with a mean of 9.02 eggs/ brood. The cumulative eggs produced (Σmx) is linearly

correlated with instar number (Fig. 3 c). The rate of egg production (REP) of *D. sarsi* is calculated as 9.2632.

4. 3. 3 Growth

The first pre-adult neonates had a mean TL of 0.552 mm and the 2nd instar had TL of 0.732 mm. The percentage of increment in TL during the pre-adult stage was 32.60%. The primiparous stage was attained during the 3rd instar when the mean TL was 0.809 mm. The maximum mean TL of 1.320 mm was attained at the end of 15th instar (Table 9).

The mean CH during the first pre-adult instar was 0.184 mm and attained CH of 0.248 mm at 2nd instar. The percentage of increment in CH after the completion of pre-adult instar was 34.78%. During the 3rd instar it attained the primiparous condition with a mean CH of 0.302 mm. The maximum mean CH of 0.486 mm was attained in 15th instar (Table 9). During the life span each individual has undergone two pre-adult and thirteen adult instars. Maximum growth increment recorded during the life cycle was in 2nd instar and further decreased during the subsequent instars (Table 9).

The relationship between TL, CH and instar number of *D. sarsi* is represented in Fig. 3 a. The correlation coefficients of life history characters in *D. sarsi* are given in Table 10. The TL and CH shows positive correlation ($r = 0.985$).

4. 3. 4 Embryonic Development

The stages of embryonic development of *D. sarsi* are represented in Plate 8. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of oval-shaped eggs containing yolk and fat globule. Mean duration: 1.2 hrs. Mean size: 0.303 mm

Stage II: The embryo is more elongated during this stage. Yolk granules are centrally placed. Mean duration: 4.8 hrs. Mean size: 0.314 mm.

Stage III: The outer transparent area of the embryo shows cellular division. Head rudiment appears and yolk gets concentrated towards the centre. Mean duration: 5.2 hrs. Mean size: 0.324 mm.

Stage IV: The embryo is more elongated in anterio-posterior axis and the cellular divisions at the posterior region become more distinct during this stage. The rudiments of head, antennules and antennae appeared. Mean duration: 3.5 hrs. Mean size: 0.365 mm

Stage V: This stage is recognized by the presence of pink eye. The rudiments of antennae and thoracic legs are more visible. Mean duration: 3.2 hrs. Mean size: 0.412 mm

Stage VI: This stage is recognized by the presence of black eye. The rudiments of antennae, antennules, thoracic legs and postabdomen become more distinct. Mean duration: 5.6 hrs. Mean size: 0.426mm.

Stage VII: During this stage setae appear on the antennae, antennules, thoracic legs. Postabdomen become more visible. The amount of yolk gets decreased. Mean duration: 3.8 hrs. Mean size: 0.460 mm

Stage VIII: The eye, antennules, antennae, thoracic legs, postabdomen and valves are well developed during this stage. Movements are also observed in the embryo. Mean duration: 3.5 hrs. Mean size: 0.506 mm.

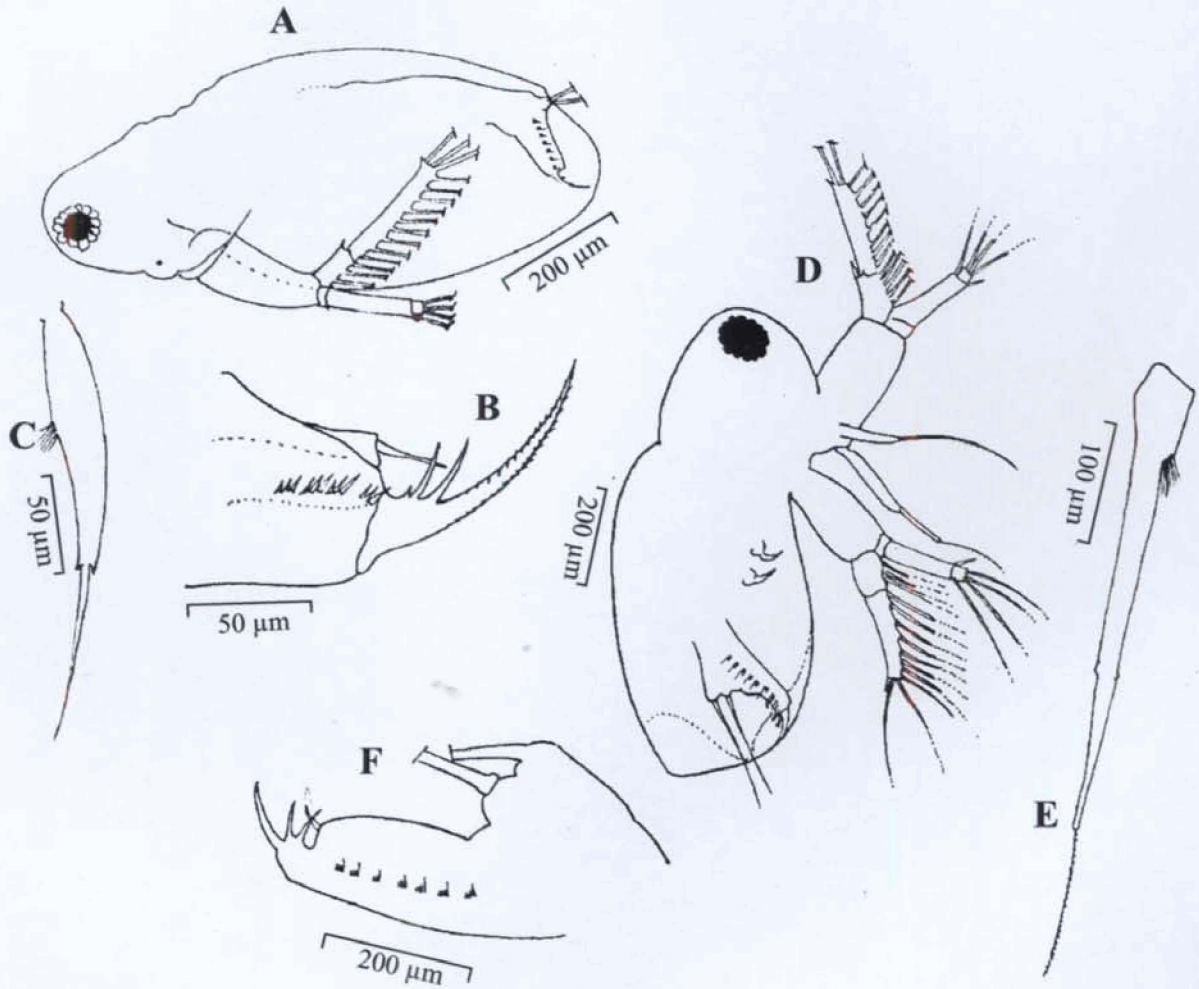
Release of neonates

The embryonic development of primiparous instar of *D. sarsi* was completed in 30.8 hrs and the neonates were released from the brood pouch prior to moulting.

4. 3. 5 Life Span and Survivorship

The survivorship curve (Fig. 3 d) indicates the relationship of age (days) and percentage survival of *D. sarsi*. As evident from the data survival was higher near the age of maturity and declined slowly further after maturity. The mean life span (Σlx) of female is calculated as 9.0 days; while the maximum life span (Lmax) observed during the present study was 21.0 days.

Plate 2

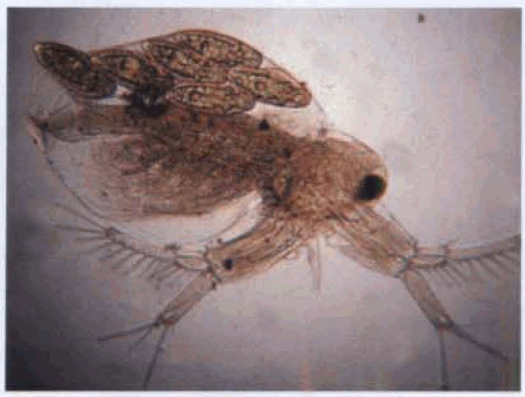


Pseudosida bidentata (Daday)

Fig. **A.** Female, **B.** Postabdomen of female, **C.** Antennule of female, **D.** Male, **E.** Antennule of male, **F.** Postabdomen of male.

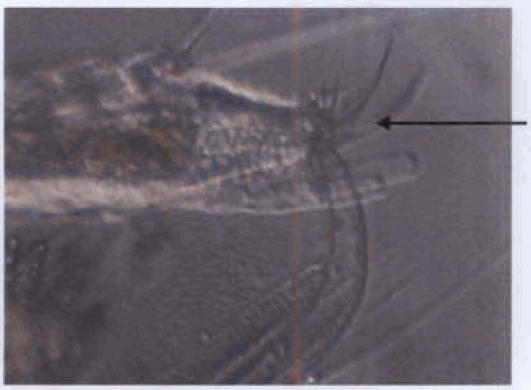
Plate 3

A



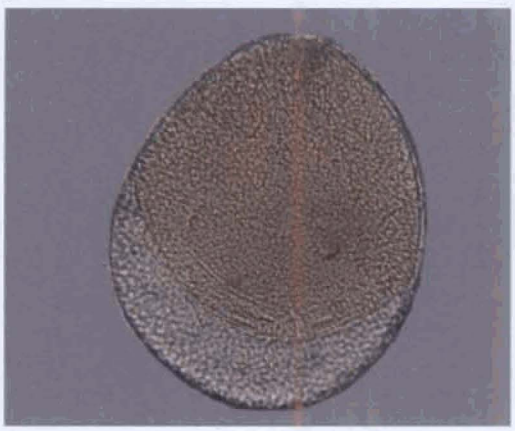
B

C



D

E



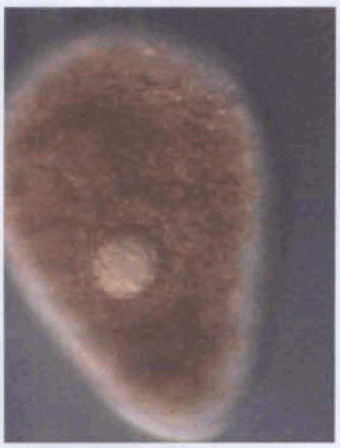
F

Pseudosida bidentata

Fig. **A.** Female with ovary (1.028 mm), **B.** Parthenogenetic female with embryo (1. 436 mm), **C.** Male (1.004 mm), **D.** Postabdomen of male enlarged **E.** Ephippial female (1.372 mm), **F.** Ephippium with egg (0.366 mm).

Plate 4

Stage-I (0.316 mm)



Stage-II (0.336 mm)

Stage-III (0.408 mm)

Stage-IV (0.469 mm)

Stage-V (0.506 mm)

Stage-VI (0.510 mm)

Stage-VII (0.546 mm)

Stage-VIII (0.556 mm)

Pseudosida bidentata Embryonic development

Plate 5

A

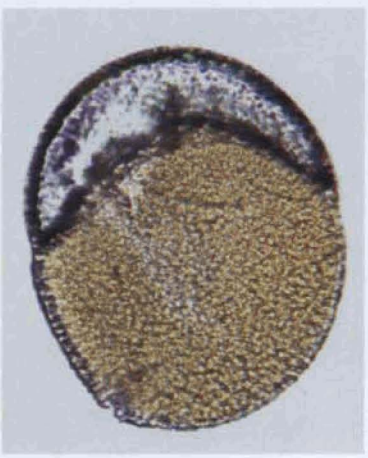
B

C

D

E

F



G

H

Latonopsis australis

Fig. **A.** Pre-adult (0.598 mm) **B.** Female with ovary (0.988 mm),
C. Parthenogenetic female (1.130 mm),
D. Male (0.822 mm), **E.** Ephippial female (1.08 mm), **F.** Ephippial female ventral view (1.16 mm)
G. Ephippium with egg (0.316 mm), **H.** Ephippium without egg (0.266 mm).

Plate 6

Stage-I (0.226 mm)

Stage-II (0.244 mm)

Stage-III (0.268 mm)

Stage-IV (0.296 mm)

Stage-V (0.306 mm)

Stage-VI (0.318 mm)

Stage-VII (0.320 mm)

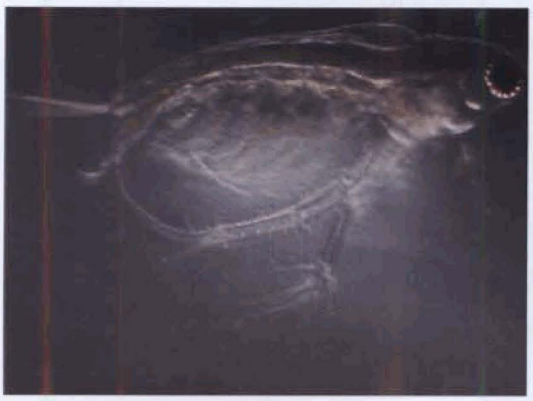
Stage-VIII (0.342 mm)

Stage-IX (0.384 mm)

Stage-X (0.602 mm)

Latonopsis australis Embryonic development

Plate 7

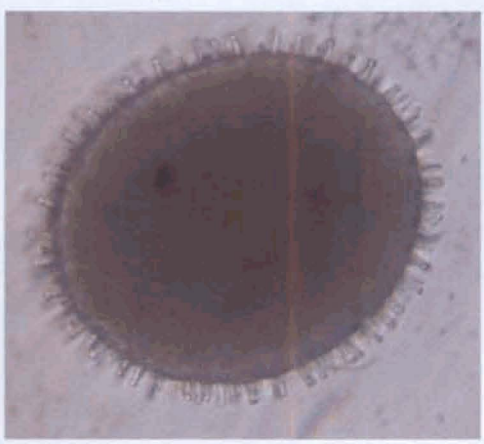


A

B

C

D



E

Diaphanosoma sarsi

Fig. **A.** Pre-adult (0.698 mm) **B.** Parthenogenetic female (0.946 mm)
C. Male (0.686 mm) **D.** Ephippial female 0.766 mm **E.** Ephippium (0.188 mm)

Plate 8

Stage-I (0.306 mm)

Stage-II (0.313 mm)

Stage-III (0.325 mm)

Stage-IV (0.368 mm)

Stage-V (0.418 mm)

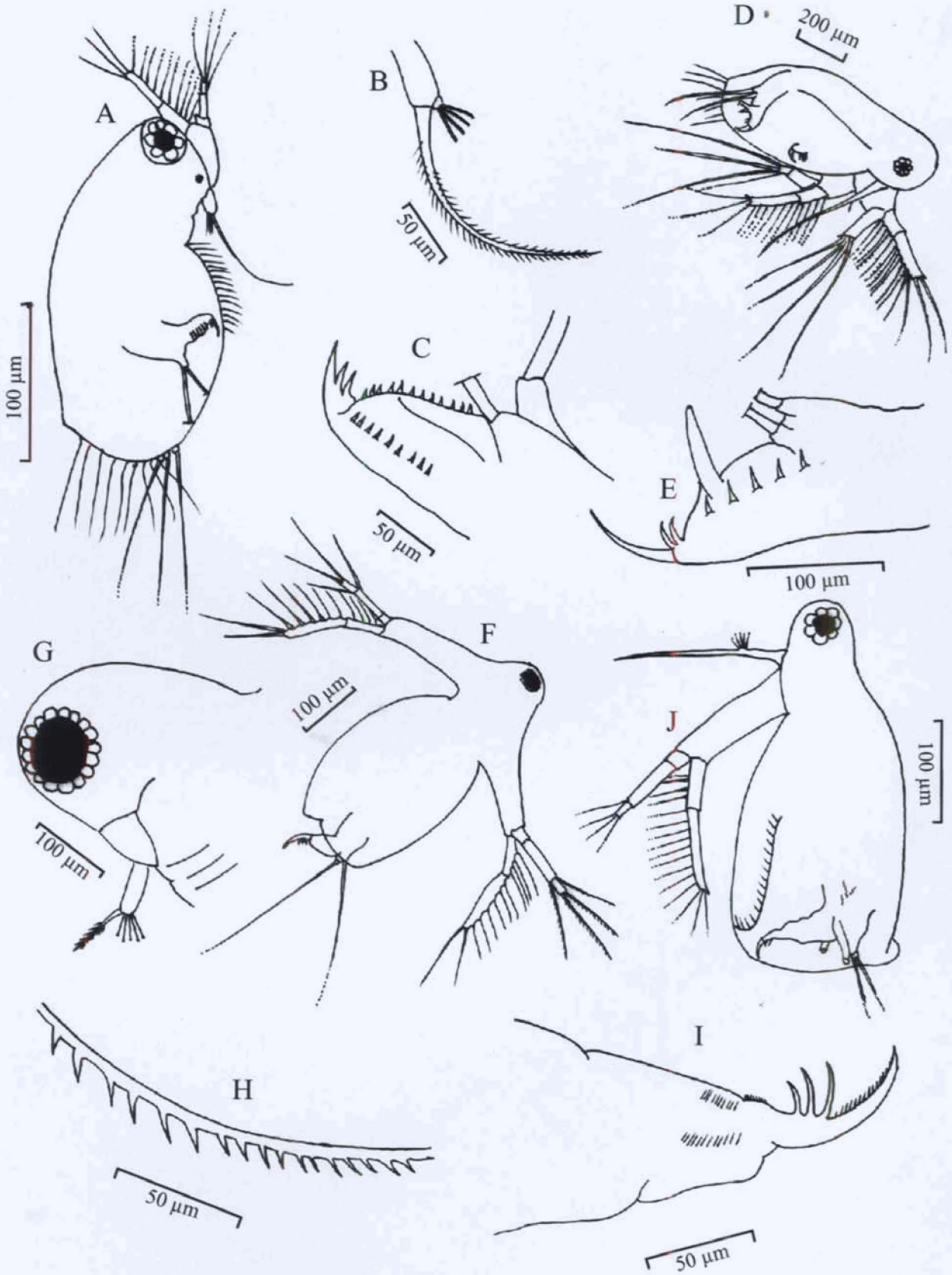
Stage-VI (0.426 mm)

Stage VII (0.462 mm)

Stage-VIII (0.502 mm)

Diaphanosoma sarsi Embryonic development

Plate 9



Latonopsis australis Sars Fig. **A.** Female, **B.** Antennule of female.
C. Postabdomen of female, **D.** Male, **E.** Postabdomen of male
Diaphanosoma sarsi Richard **F.** Female, **G.** Head with antennule,
H. Posteroventral margin of shell, **I.** Postabdomen of female. **J.** Male.

Pseudosida bidentata

Fig.1 a Relationship between Total length (TL), Carapace height (CH) and instar number in female

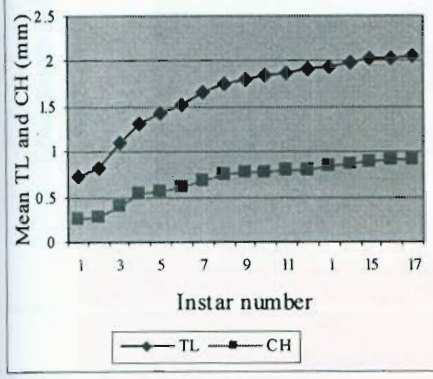


Fig. 1 b Relationship between Total length (TL), Carapace height (CH) and age in male

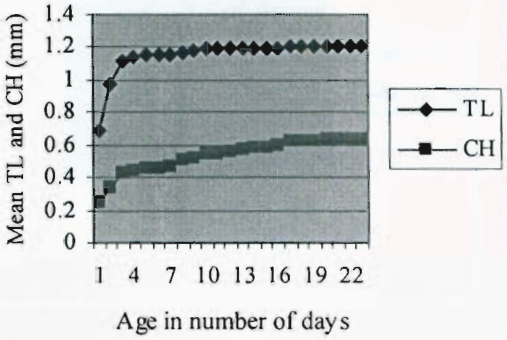


Fig. 1. c. Egg production in relation to instar number

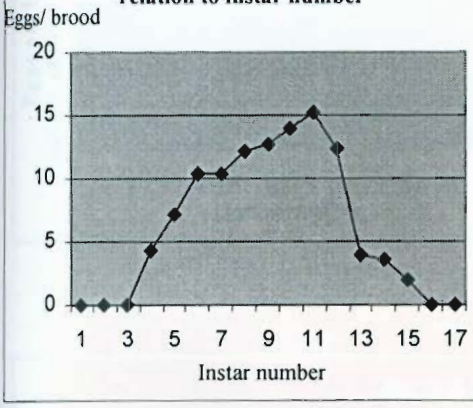


Fig. 1 d. Cumulative egg production related to adult instar number

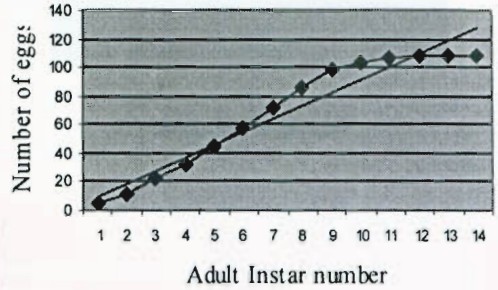
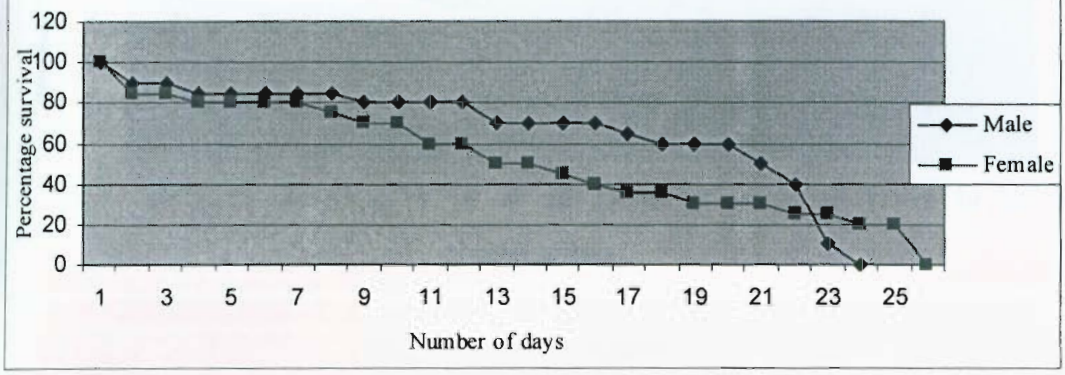


Fig. 1. e. Survivorship curve



Latonopsis australis

Fig. 2 a Relationship between Total length (TL), Carapace height (CH) and instar number.

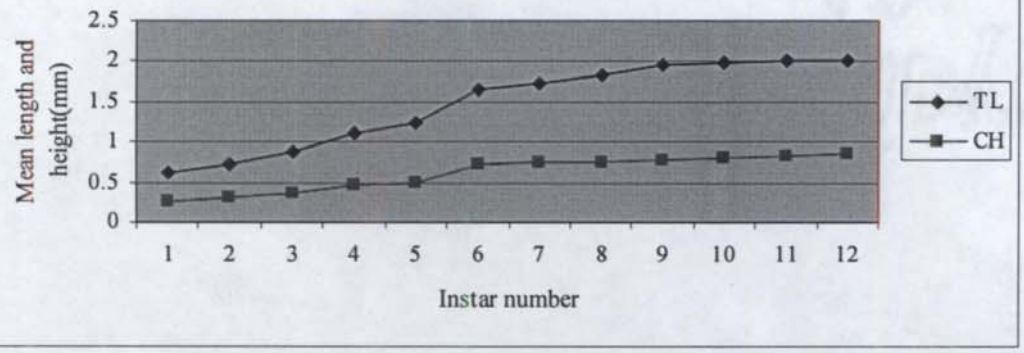


Fig. 2 b Egg production in relation to instar number

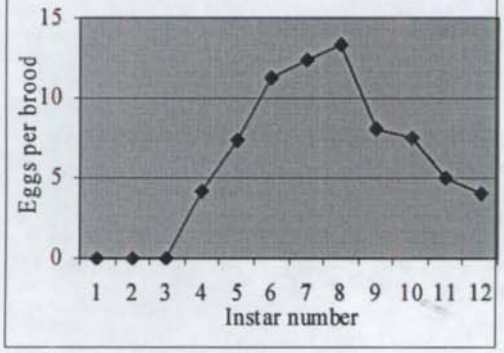


Fig. 2 c Cumulative egg production related to adult instar number

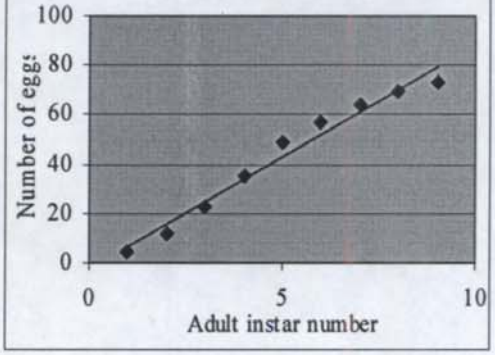
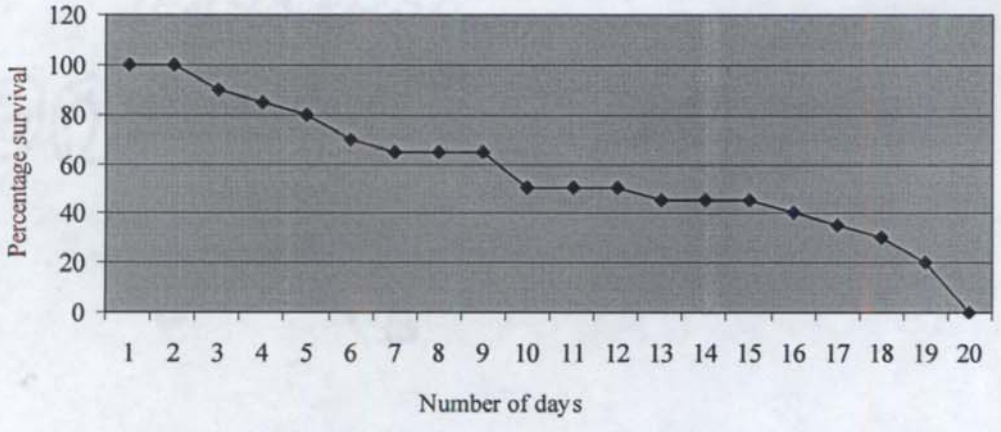


Fig. 2 d Survivorship curve



Diaphanosoma sarsi

Fig. 3 a Relationship between Total length (TL), carapace height (CH) and instar number

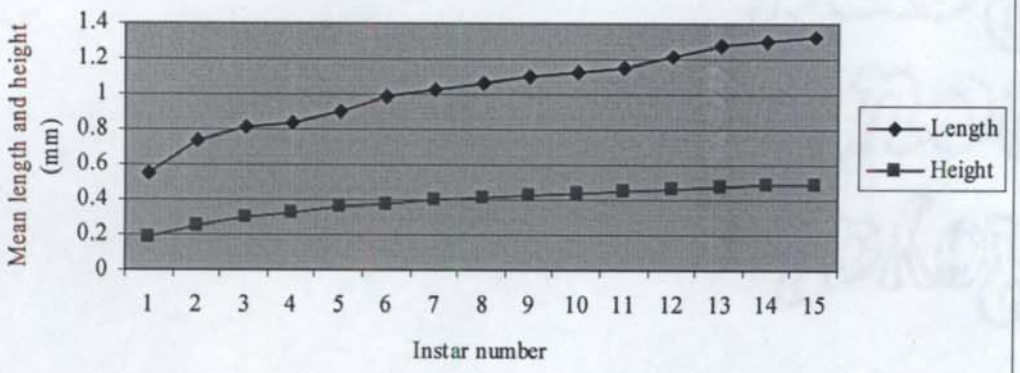


Fig. 3 b Egg production in relation to instar number

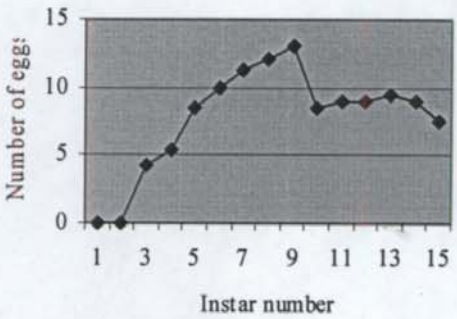


Fig. 3 c Cumulative egg production related to instar number

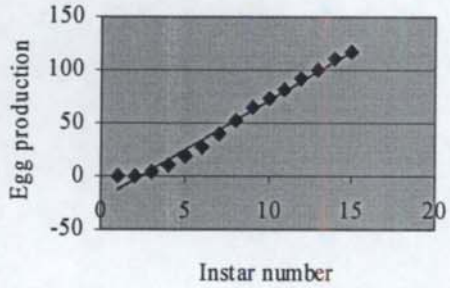


Fig. 3 d Survivorship curve

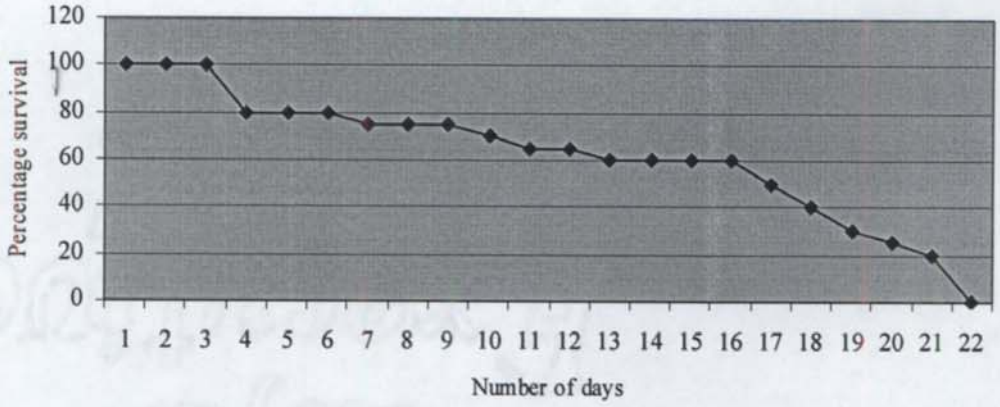


Fig. 4 Growth increment in Sididae

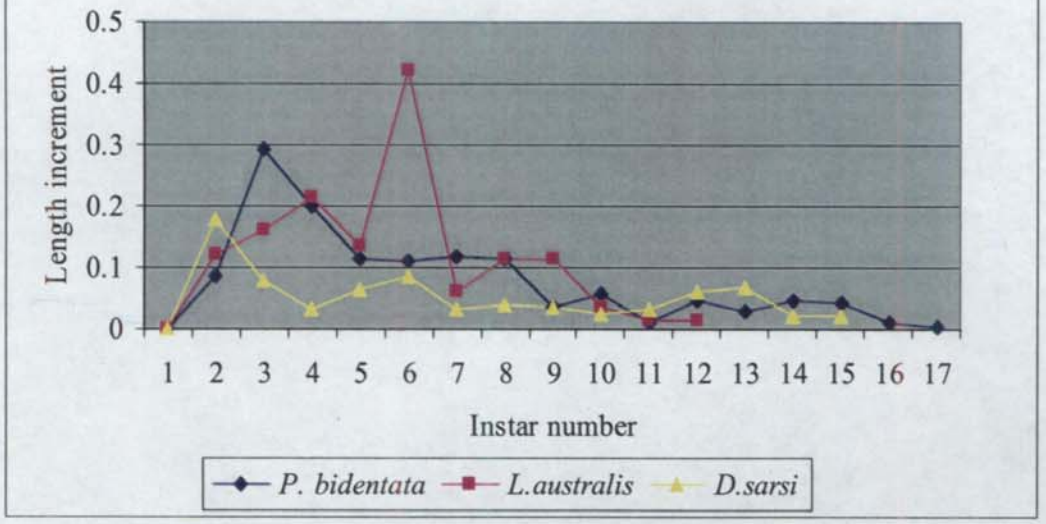
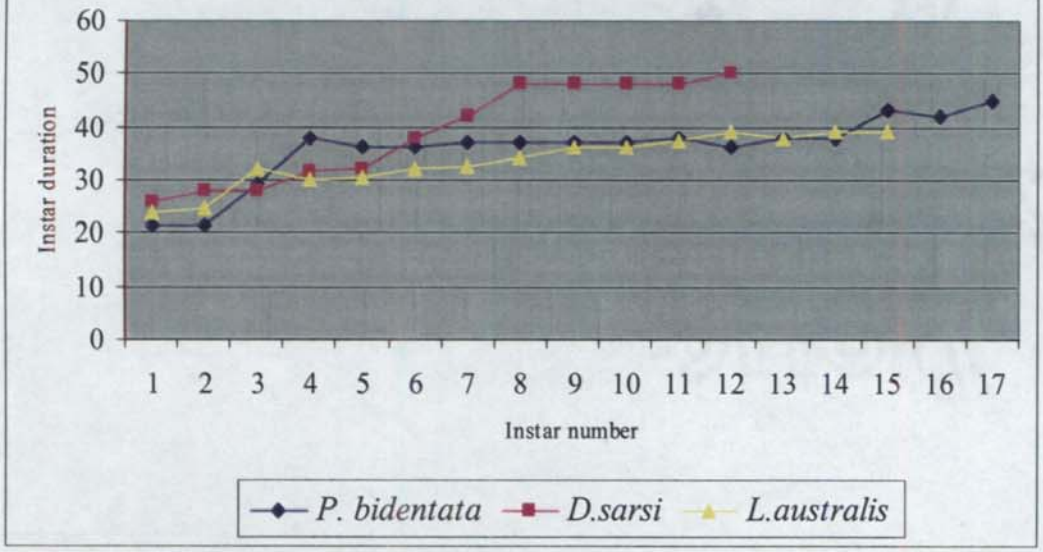


Fig. 5 Instar duration in Sididae



ORDER ANOMOPODA

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 5

ORDER ANOMOPODA

Family Daphniidae Straus, 1820

5. 1 *Ceriodaphnia cornuta* Sars, 1885

Ceriodaphnia cornuta, a common littoral species found in the freshwater ponds of India (Gurney, 1906, 1907; Arora, 1931; Brehm, 1936) is considered a synonym of *C. rigauadi* Richard by Rzoska (1956) and Nayar (1971). This species was also reported from Meghalaya (Patil, 1976); Bihar (Nasar, 1977); West Bengal (Sharma, 1978; Karnataka (Patil and Gouder, 1988), Chennai, Tamil Nadu (Raghunathan, 1990); Kerala (Michael and Sharma, 1988); Lake Kolleru, Andhra Pradesh (Durga Prasad and Padmavathi 2003a) and Thekkady, Kerala (Babu and Nayar, 2004).

Studies on the biology of *C. cornuta* were initiated in India by Michael (1962) from West Bengal; Murugan (1975b) from Tamil Nadu; Kanaujia (1988a) from Orissa and Babu and Nayar (1993) from Kerala. Although, the above papers give good accounts of the life history of parthenogenetic females; information about the ephippia bearing females and males of this species is scanty. Hence a detailed laboratory study on the life cycle of this species has been made.

5. 1. 1 External Morphology

Parthenogenetic female (Plate 10. Fig. C)

Body oval-shaped; head small, distinctly separated from rest of the body by a conspicuous cervical sinus, ventral margin of the head produced into a short rostrum (Plate 12. Fig. A). Eye large, ocellus small. Antennules short, fusiform and not extending beyond the tip of rostrum, with a central sensory seta somewhat distal to the middle and a group of sensory setae on the apex. The antennae well developed, with 4-segmented dorsal ramus and 3-segmented ventral ramus; setation of antenna: (0-0-1-3)/ (1-1-3). Valves with distinct polygonal marking (Plate 12. Fig. A), margins smooth, posterodorsal corner produced into a blunt process. Postabdomen short, with 5-6 sharply pointed anal spines; claws smooth, without basal spine (Plate 12. Fig. B). Mean size: 0.533×0.395 mm.

Male (Plate 10. Fig. F)

Smaller than female; body quadrangular in outline with somewhat straight dorsal margin; antennule longer than that of female, and with two sensory hairs (Plate 12. Fig. D). The first thoracic leg with a prehensile hook and a long jointed filament emerging through the ventral margin of valves (Plate 12. Fig. C). Mean size: 0.362 × 0.216 mm.

Ephippial female (Plate 10. Fig. D)

The general features of ephippial female similar to that of parthenogenetic female except in their smaller size and more rounded body. Ephippium dorsally placed, oval, single and white in colour. Ephippium

forms a part of the carapace, made up of honey-comb pattern of ornamentation (Plate 10. Fig. E). The egg is single within the ephippium with a mean size of 0.204 × 0.140 mm. Mean size of ephippial female: 0.468× 0.280 mm. Size of ephippium: 0.341× 0.264 mm.

5. 1. 2 Reproduction

The population developed in the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. Among this the parthenogenetic females dominated the culture throughout the period of study.

The ephippial females appeared in the stock culture during January 2005 when the mean water temperature was 22-24°C and the culture became crowded. They were formed from the parthenogenetic females in the stock culture. These ephippial females again resumed parthenogenetic reproduction after 3-4 days. The carapace of the ephippial females get modified as ephippium and are released into the medium along with moulting. The ephippium floated on the surface for a few minutes and afterwards get adhered to the side walls of the container or sank to the bottom. The ephippium enclosed a single egg with a mean size of 0.204 × 0.140 mm. The development of ephippial egg as well as the ephippium took place simultaneously. In the absence of synchronization of these two processes, some of the ephippia were released without eggs within it.

The sudden appearance and subsequent disappearance of males and ephippial females were observed in stock culture. Although, males are rarely

obtained from the field, they appeared in stock culture when the stock culture became crowded.

Life cycle of parthenogenetic female

The features characteristic of the reproduction and life cycle of *C. cornuta* are given as follows.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean birth size (SaB) of 0.232×0.120 mm. The first moulting occurred at an interval 23.10 hrs, while the second moulting took place after an interval of 23.0 hrs. The total pre-adult duration was 46.1 hrs and the mean pre-adult duration (PID) was 23.05 hrs.

Attainment of maturity

Although, the ovary was clearly visible at 32 ± 1.2 hrs of life towards the end of 2nd pre-adult instar (Plate 10. Fig. B), they attained maturity and started to bear eggs after completion of 2nd moult at 46.1 hrs (Table 11). Hence the age at first reproduction (AFR) was 1.91 days. The SFR was 0.426× 0.288 mm.

Egg production

The eggs were deposited into the brood pouch after the completion of two moults. There was a gradual increase in the size of eggs and attained a mean size of 0.152 mm, due to the accumulation of yolk. They became oval shaped with green colour. During this primiparous instar (3rd instar) the eggs

were produced with an initial number of 2.0 eggs/ brood. The primiparous instar was completed in 24.2 hrs and the first generation time (FGT) is calculated as 70.3 hrs.

The females continued reproduction during the succeeding instars; and there was a steady increase in egg production to 9.3 eggs/ brood. This maximum clutch size (E max) is attained in the 8^{th} instar (Table 11). The egg production in *C. cornuta* showed a single peak with maximum production in 8^{th} instar (Fig. 6 b).

Subsequently there was a gradual decline in the rate of egg production upto 11^{th} instar. There was a sharp fall in egg production immediately after this instar and showed variation till the penultimate instar (16^{th}). However, the last instar (17^{th}) was without egg production; and they continued moulting until their death (Table 11).

Each clutch produced in the adult instar consisted of 1 to 2 rows of eggs on either side of the brood pouch; with more number of eggs occupying in the upper row. The adult instar duration varied from 24.2 to 34.6 hrs (Table 11) with a mean AID of 30.0 hrs.

Fecundity

The relationship of egg production with instar number is represented in Fig. 6 b. The range of egg production of a single female was from 2 to 12 with an egg production of 3.36 eggs/day of adult life. The cumulative number of eggs produced (Σmx) during the entire life span was 63.0 (Table 11). Fourteen broods were produced during the entire life with an average of

4.5 eggs /brood. The Σmx of *C. cornuta* is linearly correlated with instar number (Fig. 6 c). The rate of egg production (REP) of *C. cornuta* is calculated as 4.8039.

5. 1. 3. Growth

The first pre-adult neonates had a mean TL of 0.232 mm and the 2nd instar had TL of 0.352 mm. Primiparous stage was attained during 3rd instar when the mean TL was 0.426 mm. The maximum mean TL of 0.696 mm was attained at 17th instar (Table 12).

The mean CH during the first pre-adult instar was 0.120 mm and attained CH of 0.248 mm at 2nd instar. They attained a mean CH of 0.288 mm during the primiparous condition. Maximum CH was attained in 17th instar with CH of 0.498 mm (Table 12). During the life span each individual has undergone two pre-adult and fifteen adult moults.

The increment of TL and CH during each instar is given in Table 12. Maximum growth increment recorded during the life cycle was in 2nd instar with TL of 51.72 % and CH of 106.67 % respectively. Although, growth increment decreased subsequently, the most significant decrease in growth increment occurred only after 7th instar.

The relationship between TL, CH and instar number of *C. cornuta* has been represented in Fig. 6 a. The correlation coefficients of life history characters are given in Table 13. The TL and CH shows positive correlation ($r = 0.990$).

5. 1. 4 Embryonic Development

The stages of embryonic development of *C. cornuta* are represented in Plate 11. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of oval-shaped egg which is yellow-green in colour with a central fat droplet. Mean duration: 1.05 hrs. Mean size: 0.158 mm.

Stage II: The embryo is more elongated. The outer area of the embryo remains transparent; while the inner granular area is opaque due to the centrally placed yolk. Mean duration: 1.25 hrs. Mean size: 0.168 mm.

Stage III: The embryo shows cellular divisions. The anterior region is elongated. Mean duration: 4.7 hrs. Mean size: 0.172 mm.

Stage IV: During this stage head become more distinct with the appearance of rudiment of antennae. The outer area of embryo remains transparent, while the inner area is opaque due to the presence of yolk. Mean duration: 2.6 hrs. Mean size: 0.182 mm.

Stage V: During this stage head became more distinct with appearance of rudiments of antenna and thoracic legs. Mean duration: 2.0 hrs. Mean size: 0.187 mm

Stage VI: This stage can be recognized by the presence of a pink eye. The yolk granules start to disappear and the rudiments of antennae, thoracic

legs and postabdomen are clearly visible. Mean duration: 1.5 hrs. Mean size: 0.206 mm

Stage VII: This stage is recognized by the presence of a black eye. The yolk granules were completely absent and the antennae, thoracic legs and postabdomen are more distinct. Mean duration: 7.9 hrs. Mean size: 0.216 mm

Stage VIII: During this stage eye gets enlarged, antennae gets segmented, postabdomen and shell become more distinct. Mean duration: 2.4 hrs. Mean size: 0.243 mm.

Release of neonates

The neonates are released from the brood pouch after completion of the embryonic development by the intermittent movements of its postabdomen. After this the female underwent moulting within duration of 40.0 minutes. The embryonic development of primiparous instar of *C. cornuta* was completed in 23.4 hrs.

5. 1. 5 Life span and Survivorship

The survivorship curve (Fig. 6 d) indicates the relationship of age (days) and percentage survival in *C. cornuta*. This shows that the survival was higher during the pre-reproductive phase and declined during the reproductive period.

The mean life span (Σlx) of female is calculated as 8.23 days, while the maximum life span (L max) of females observed during the present study was 20.66 days.

5. 2 *Scapholeberis kingi* Sars, 1903

Scapholeberis kingi is a hyponeustonic cladoceran often found associated with the surface films of shallow water bodies. *S. kingi* is a darkly pigmented cladoceran which is considered a distasteful species (Dodson and Frey, 1991). This species was described first by O. F. Müller (1776) from Denmark. However, the name *Scapholeberis* was coined by Schoedler (1858).

The first report of this species from India is that of Gurney (1907) from Culcutta. Subsequently reported from Kashmir and Nilagiri Hills, (Brehm, 1936); Dharwad, (Patil and Gouder, 1989); and Andhra Pradesh, (Durga Prasad and Padmavathy, 2003a).

The only available report on the biology of this species is that of Murugan and Sivaramakrishnan (1976) from Tamil Nadu.

5. 2. 1. External Morphology

Parthenogenetic female (Plate 13. Fig. C)

Body quadrangular, broadly rounded dorsally, maximum height behind the middle. Head relatively small and slightly depressed, rostrum rounded and slightly projecting ventrally (Plate 12. Fig. F). Eye rather large; small ocellus, situated closer to the rostrum than to the eye. Antennules short, attached to the posterior margin of the rostrum, with long sensory setae on the anterior surface and a group of 5-7 sensory setae on the apex. Antennae with 3-segmented dorsal ramus and 3-segmented ventral ramus; setation of antenna: (1-1-3)/ (0, 1, 3). Valves with faint reticulations, posterior margin straight

vertically with distinct posterodorsal corner; ventral margin straight horizontally with a series of sub-marginal branched setae; the posteroventral corner of each valve produced into a short spine (Plate 13. Fig. A). Postabdomen short and broad, dorsal distal margin rounded with 5 marginal denticles, decreasing in size proximally (Plate 12. Fig. G); lateral surface has rows of spinules; claw slightly curved dorsally, with a series of spinules on the concave surface. Mean size: 0.591 × 0.383 mm

Ephippial female (Plate 13. Fig. D)

The body length and carapace height of ephippial female of almost same dimensions. Ephippium dorsally placed, with straight posterodorsal margin and semi-circular ventral margin, having several rows of honeycomb ornamentation; darkly pigmented; enclosing one resting egg with elliptical shape (Plate 13. Fig. E; Plate 33. Fig. C). Ephippial females were also seen with ephippia having no eggs within it. Mean size: 0.648 × 0.609 mm. Mean size of ephippium: 0.478×0.303 mm.

5. 2. 2 Reproduction

S. kingi formed an aggregation in the surface film of water when collected from Site 4, as well as when cultured in the laboratory indicating that they are adapted for hyponeustonic life. The presence of specialized setae on the ventral surface of the valves assists their movements in the surface water film (Plate 12. Fig. F).

The population developed during the laboratory culture comprised asexually reproducing females and ephippia bearing females, while the males

were not observed. Among this the parthenogenetic females dominated the culture. Ephippial females appeared in December 2003 when the water temperature was 23°C and the population became crowded. The presence of ephippial females could be identified due to the dark pigmentation of ephippium. The ephippium is placed dorsally with a straight posterodorsal margin and a semicircular ventral margin (Plate 13.Fig. D). Ephippial females disappeared suddenly from the stock culture after a brief period of appearance.

Life cycle of parthenogenetic female

The parthenogenetic reproduction was the dominant method of reproduction in *S. kingi* throughout the period of study. The features characteristic of the reproduction and life cycle are given as follows.

Moulting

S. kingi underwent moulting towards the termination of each instar with casting off old carapace as exuvium. The detachment of old carapace is initiated from the posteroventral region of the body. During this event the animal remained in the water surface with its ventral side facing upwards to enable movement through the surface film. The casting off the exuvium is aided by the jerky movements of the body. These movements resulted in the detachment of old carapace (exuvium) first from the postabdomen and head; and subsequently from the remaining portion attached to the ventral side of the body and finally from the posterodorsal part. The exuvium is shed as a whole and the moulting process is completed in a mean duration of

2.0 minutes. There was an increase in size followed by each moult indicating that growth in *S. kingi* is associated with moulting.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean birth size (SaB) of 0.290 × 0.187 mm. The first and second moulting occurred in an interval of 32.0 hrs each. The total pre-adult instar duration was 64.0 hrs and the mean PID was 32.0 hrs.

Attainment of maturity

The ovary of the parthenogenetic females were conspicuous as a pair of orange coloured elongated sacs on either side of the alimentary canal at 48 ± 2.0 hrs of life during the 2nd pre-adult instar (Plate 13. Fig.B). The ovary appeared orange coloured due to the accumulation of lipid prior to egg production. However, they started to bear eggs after the second moult, and hence the AFR was 2.7 days. The SFR was 0.483× 0.283 mm.

Egg production

The mature females bear eggs after the 2nd moulting. In the primiparous instar (3rd instar), there is a lower egg production. The eggs were subsequently deposited into the brood pouch which measured a mean length of 0.160 mm. Soon after the deposition into the brood pouch there is an increase in egg size due to the initiation of embryonic development. The primiparous instar was completed in 34.5 hrs. The time required for the release of the first batch of neonates (FGT) is 98.5 hrs.

There was a steady increase in egg production during the subsequent instars and attained a maximum clutch size of 14 eggs/ brood. This maximum clutch size (Emax) was attained in the 10^{th} instar (Table 14). The egg production of *S. kingi* showed a single peak with maximum egg production in 10^{th} instar followed by a gradual decline (Fig. 7 b). The egg production continued throughout the life span (Table 14).

Each clutch in the early adult instar consisted of 1 to 2 rows of eggs which were deposited on either side of the brood pouch. The female continued moulting till the end of life span. The adult instar duration varied from 34.5 to 39.5, with a mean adult duration (AID) of 36.68 hrs.

Fecundity

The relationship between egg production and instar number is represented in Fig. 7 b. The range of egg production of a single female varied from 1 to 16 with an egg production of 4.85 eggs/day of adult life. The cumulative number of eggs (Σmx) produced during the entire life was 81.5 (Table 14). Eleven broods were produced during the entire life with a mean of 7.40 eggs/ brood. The cumulative egg production (Σmx) is linearly correlated with instar number (Fig.7 c). The rate of egg production (REP) of *S. kingi* is calculated as 7.3434.

5. 2. 3 Growth

The first pre-adult neonates had a mean TL of 0.290 mm and 2^{nd} instar had TL of 0.376 mm. Primiparous stage was attained during the 3^{rd} instar

when the mean TL was 0.483 mm. The maximum mean TL of 0.731 mm was attained at the end of 13th instar (Table 15).

The mean CH during the first pre-adult instar was 0.187 mm and attained CH of 0.226 mm at 2nd instar. They attained a mean CH of 0.283 mm during the primiparous instar. Maximum CH was attained in 13th instar with CH of 0.496 mm (Table 15). During the life span each individual has undergone two pre-adult and eleven adult moults.

The increment of TL and CH during each instar is given in Table 15. Maximum growth increment recorded during the life cycle was in 2nd instar with TL of 29.66% and in 3rd instar with CH of 25.22% respectively, and the growth increment decreased in the succeeding instars.

The relationship between TL, CH and instar number of *S. kingi* is represented in Fig. 7 a. A higher growth rate is noticed during the early instars than in the later phase of life cycle. The correlation coefficients for some life history characters are given in Table 16. The TL and CH are positively correlated (r = 0.990).

5. 2. 4 Embryonic Development

The transfer of egg from the ovary into the brood pouch could be regarded as the first event of embryonic development. In *S. kingi* the egg transfer took place after 2nd moult within a short duration of 48-50 minutes. A similar pattern was also observed in the succeeding instars.

The stages of embryonic development in *S. kingi* are represented in Plate 14. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of oval-shaped eggs with yellow-green colour. A fat globule was centrally placed. Mean duration: 1.4 hrs. Mean size: 0.116 mm

Stage II: This stage is recognized by the elongated embryo with yellow-green colour. The outer area of the embryo is transparent while the inner area is granular with a centrally located fat globule. Mean duration: 1. 2 hrs. Mean size: 0.146 mm

Stage III: The anterior region of embryo is elongated and cellular divisions are initiated. The inner granular zone of the embryo contained abundant fat droplets.

Mean duration: 6.2 hrs. Mean size: 0.198 mm.

Stage IV: The embryo has elongated anterio-posteriorly and shows cellular divisions. The head lobe and antennary rudiments appear. The yolk is concentrated in the centre during this stage.

Mean duration: 10.6 hrs. Mean size: 0.210 mm

Stage V: This stage can be recognized by the presence of pink eye. The head lobe, antennae and rudiments of thoracic legs become more clearly visible.

Mean duration: 3.20 hrs. Mean size: 0.214 mm

Stage VI: The eye, head, antennae, postabdomen and valves become more distinct.

Mean duration: 7.0 hrs. Mean size: 0.216 mm

Stage VII: This stage is characterized by the presence of distinct eye, antennae and postabdomen. The antennae are segmented and bear small setae.

Mean duration: 3.0 hrs. Mean length: 0.232 mm

Stage VIII: This stage is characterized by the presence of distinct eye, antennules, antennae, thoracic legs and postabdomen.

Mean duration: 1.10 hrs. Mean length: 0.242 mm

Release of neonates:

The embryonic development of primiparous instar of *S. kingi* was completed in 33.7 hrs. The neonates were released from the brood pouch by the jerking movements of postabdomen and they resembled the adult with a mean length of 0.290 mm.

5. 2. 5 Life span and Survivorship

The survivorship curve (Fig.7 d) indicates the relationship of age (days) and percentage survival of *S. kingi*. The mean life span (Σlx) of female is calculated as 8.14 days; while the maximum life span (Lmax) observed during the present study was 19.47 days.

5. 3 *Simocephalus serrulatus* (Koch, 1841)

Simocephalus serrulatus is a large cladoceran most often found among the littoral weeds and sediments of ponds. The first report of this species from India is by Michael and Sharma (1988) from Meghalaya and Tamil Nadu; and later recorded from West Bengal and Southern Tamil Nadu by Venkataraman (1995, 1999).

Our information on the biology and life cycle of *Simocephalus* is based on the studies by Murugan and Sivaramakrishnan (1973) and Murugan (1977) on *S. acutirostratus* from Tamil Nadu; further by Kanaujia (1987, 1988b) on *S. vetulus* from Orissa and Sharma and Sharma (1989) on *S. exspinosus* from Shillong, Meghalaya. Subsequently Babu and Nayar (1997) made a study on the life cycle of male and parthenogenetic females of *S. serrulatus* collected from Kerala. The present study is a detailed investigation on the life cycle of *S. serrulatus.*

5. 3. 1 External Morphology

Parthenogenetic female (Plate 16. Fig. B)

Body rhomboidal and slightly widened behind (Plate 15. Fig. A.); dorsal margin of carapace evenly arched while ventral margin bulging in middle, with a blunt posterior protuberance slightly above median axis of body. Head projecting and acute anteriorly with serrations on the apex; rostrum short (Plate 15. Fig. B). Eye comparatively large, ocellus small and

having an oval or triangular shape, situated closer to the apex of rostrum than to the eye. Antennules short, serrated on the anterior margin and with a group of terminal sensory setae (Plate 15.Fig. B). Antennal formula: (0-0-1-3)/ (1-1-3). Valves with faint reticulations. Postabdomen broad, post-anal margin armed with 8 anal denticles, decreasing in size proximally up to the pre-anal corner, lateral surface covered with scattered groups of short spinules; claw long and slender with two groups of spinules on its concave surface (Plate 15. Fig. C). Mean size: 1.730 × 1.150 mm.

Male

The body smaller than female, quadrangular shaped, without distinct posterior protuberance (Plate 15. Fig. D). The antennules bear two sensory setae on the middle (Plate 15. Fig. E). Postabdomen with reduced pre-anal margin, post-anal margin armed with 3 anal denticles, decreasing in size proximally up to the pre-anal corner (Plate 15. Fig. F). Mean size: 0.680 mm.

Ephippial female (Plate 16. Fig. C)

The ephippial females smaller than parthenogenetic females; and without the blunt posterior spine of carapace. The ephippium triangular, orange-yellow coloured, with honeycomb ornamentation, darkly pigmented, enclosing a single egg (Plate 16. Fig. D).

The egg distinctly orange coloured, measured a size of 0.390 × 0.200 mm, and encased by an inner transparent and an outer thick leathery membrane (Plate16. Fig. E). Size of ephippial female: 1.55 × 1.21 mm. Size of ephippium: 0.975 × 0.650 mm.

5. 3. 2 Reproduction

The population developed during the laboratory culture comprised asexually reproducing females, males and ephippia bearing females. Among this the parthenogenetic females dominated the culture. The number of ephippial females increased in the stock culture during December 2004 when the water temperature was 22-24°C.

Production of ephippial female

The ephippial females appeared in the stock culture from December 2004 to February 2005 when the culture became crowded. Generally the ephippial production is initiated immediately after the pre-adult moults. The development of egg and the ephippium most often took place simultaneously and the eggs were deposited into the ephippium. However, due to absence of synchronization of egg release and ephippial development some of the ephippia were found to be produced without eggs within it (Plate 16. Fig. F).

The ephippium appeared as triangular structure with melanin pigmentation (Plate 16. Fig. D). Two membranes were found to enclose the ephippial egg (Plate 16. Fig. E). The ephippial development was completed in duration of 65-70 hrs. When the ephippial females were transferred to fresh culture medium, many of them cast off their ephippia along with moult. The newly released ephippia floated on the surface of the medium for some time and then sank to the bottom or get adhered to the side walls of the container. After the release of the first ephippium the ephippial females continued production of ephippia for 2-3 generations and then resumed parthenogenetic reproduction.

Life cycle of parthenogenetic female

In the laboratory culture the females were generally found clinging on to the side walls of the container with the help of their antennal hooks. The detritus getting attached to their body is continuously removed by the movements of well developed postabdomen. They continued water filtration and feeding even when they remained stationary or while clinging to the substratum. Defecation also took place frequently.

The population of *S. serrulatus* developed during laboratory culture comprised an abundant number of parthenogenetic females. The features characteristic of the reproduction and life cycle are given as follows.

Moulting

The stages of moulting are represented in Plate 16. Figs. G-I. *S. serrulatus* underwent moulting towards the termination of each instar with casting off old carapace as exuvium. The detachment of old carapace is initiated from the posteroventral region of the body. The casting off the exuvium is aided by the intermittent jerky movements of the body, which is initiated from the extension of postabdomen. These movements resulted in the detachment of exuvium first from the postabdomen and head; and subsequently from the remaining portion attached to the ventral part of the body and finally from the posterodorsal part. The exuvium is shed as a whole and the moulting was completed in a mean duration of 2.5 minutes. There was an increase in size followed by each moult (Table 18), which indicates that in *S. serrulatus* growth is associated with moulting.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean birth size (SaB) of 0.576 ×0.296 mm. The first, second and third moulting occurred in a uniform duration. The total pre-adult instar duration was 130.0 hrs and the mean pre-adult instar duration (PID) was 43.33 hrs. After the third moulting they attained sexual maturity and started reproduction.

Attainment of maturity

The ovaries of parthenogenetic females were clearly visible at 110 ±3 hrs of life. In mature females ovaries are seen as a pair of elongated sacs on each side of alimentary canal (Plate 16. Fig. B). After completion of pre-adult instar the contents of ovaries are discharged into the brood pouch through the opening present in the posterior end. This discharged mass soon attains a spherical shape to form the eggs, after completion of the 3rd moult in 130.0 hrs (Plate 16. Fig. B). Hence the AFR was 5.42 days. The size at first reproduction (SFR) was 1.144 × 0.880 mm.

Egg production

The females attain the primiparous instar (4th instar), and starts the egg production with an initial clutch size of 5.2 eggs/brood (Table 17). The eggs were deposited into the brood pouch after completion of three moults which contained yolk. The eggs were yellow-green coloured and measured a mean size of 0.225 × 0.172 mm. The duration of primiparous instar was 44.0 hrs and the first generation time (FGT) is calculated as 174.0 hrs.

During the subsequent instars there was a steady increase in egg production to 26.2 eggs/ brood. This maximum clutch size (Emax) was attained in 11th instar (Table 17). The egg production of *S. serrulatus* showed a single peak with maximum egg production in the 11th instar followed by a sharp fall and continued throughout life (Fig. 8 b).

Each clutch in the early adult instars consisted of 1 to 3 rows of eggs placed on either side of brood pouch with higher number of eggs in each upper row. The female underwent moulting towards the end of each instar until death. The adult instar duration varied from 44.0 to 52.2 hrs with a mean AID of 48.04 hrs (Table 17).

Fecundity

The relationship of egg production with instar number is represented in Fig. 8 b. The range of egg production was from 4 to 30 with a mean 5.81 eggs per day of adult life. The cumulative number of eggs (Σmx) produced during the entire life span was 151.3 (Table 17). Thirteen broods were produced during the entire life with a mean of 11.63 eggs\ brood.The Σmx is linearly correlated with instar number (Fig. 8 c). The rate of egg production (REP) of *S. serrulatus* is calculated as 12.2752.

5. 3. 3 Growth

The first pre-adult neonates had a mean TL of 0. 576 mm and the 2nd instar had TL of 0. 600 mm. During the 3rd instar it attained a TL of 1.108 mm. Primiparous stage was attained during the 4th instar when the mean TL

was 1.144 mm. The maximum mean TL of 2.280 mm was attained at the end of 12th instar and no further growth during succeeding instars (Table 18).

The mean CH during the first pre-adult instar was 0.296 mm and attained CH of 0.344 mm at 2nd instar. During the 3rd instar it attained a mean CH of 0.664mm. They attained a mean CH of 0.880 mm during the primiparous condition. Maximum CH was attained in 14th instar with CH of 1.586 mm and no further growth during succeeding instars (Table 17). During the life span each individual has undergone three pre-adult and thirteen adult moults.

The increment of TL and CH during each instar is given in Table 18. Maximum growth increment recorded during the life cycle was in 3rd instar with TL of 84.67% and CH of 93.02% respectively (Table 18).

The correlation coefficients of the life history characters studied in *S. serrulatus* are given in Table 19. The TL and CH are positively correlated (r = 0.988). The relationship between TL, CH and instar number of *S. serrulatus* has been represented in Fig. 8 a. A higher growth is noticed during the pre-reproductive instars than the reproductive phase of the life cycle.

5. 3. 4 Embryonic Development

The stages of embryonic development of *S. serrulatus* are represented in Plate 17. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of spherical egg which is yellow-green in colour, containing yolk granules. Mean duration: 1.2 hrs. Mean size: 0.216 mm.

Stage II: This stage is recognized by the appearance of two distinct zones in the embryo. The outer zone becomes transparent while the inner region becomes opaque due to the accumulation of yolk towards the centre. Mean duration: 3.3 hrs. Mean size: 0.234 mm.

Stage III: The embryo is slightly elongated during this stage and shows cellular divisions. The divisions were clearly seen in the inner area due to the presence of yellow coloured yolk which contains abundant fat globules. Mean duration: 9.5 hrs. Mean size: 0.280 mm

Stage IV: The cellular divisions become more distinct and the embryo is more elongated in the anterio-posterior axis during this stage. Mean duration: 6.5 hrs. Mean size: 0.292 mm.

Stage V: During this stage the rudiments of head and antennae starts to differentiate. The embryo is covered by a membrane. Mean duration: 3.5 hrs. Mean size: 0.302 mm.

Stage VI: The embryonic membrane is cast off during this stage. This is followed by the appearance of pink eyes. The rudiments of head, antennae and legs become more distinct. Mean duration: 4.5 hrs. Mean size: 0.307 mm.

Stage VII: This stage could be recognized by the presence of black eye. The thoracic legs and postabdomen are visible. The segmentation of antennae is more distinct. Mean duration: 8.0 hrs. Mean size: 0.420 mm.

Stage VIII: This stage could be recognized by the disappearance of yolk and appearance of terminal setae in antennules, antennae and thoracic legs. Mean duration: 2.3 hrs. Mean size: 0.456 mm.

Stage IX: This stage could be recognized by the complete disappearance of yolk and appearance of distinct antennae, antennules, thoracic legs and postabdomen. Movements are also observed in the embryo during this stage. Mean duration: 1.7 hrs. Mean size: 0.496 mm.

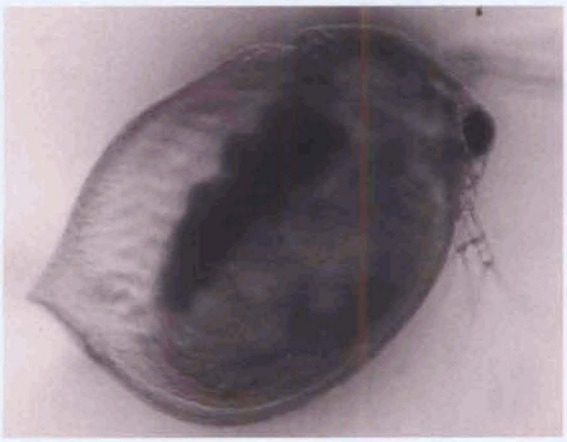
Release of neonates: After completing the embryonic development the neonates were released from the brood pouch of the female by the jerking movements of its postabdomen. The neonates are also capable of movement and come out through the posteroventral region of the carapace. They swim in water by the movements of their antennae immediately after release. The embryonic development of primiparous instar of *S. serrulatus* was completed in duration of 40.5 hrs followed by subsequent moulting. Moulting was completed within duration of 3.5 minutes and it cast off the exuvia by the intermittent jerky movements of the body (Plate 16. Fig. I).

5. 3. 5 Life span and Survivorship

The survivorship curve (Fig. 8 d) indicates the relationship of age (days) and percentage survival of *S. serrulatus*. As evident from the data survival was higher near the age of maturity and declined slowly further after maturity. The mean life span (Σlx) of female is calculated as 13.37 days; while the maximum life span (Lmax) observed during the present study was 31.44 days.

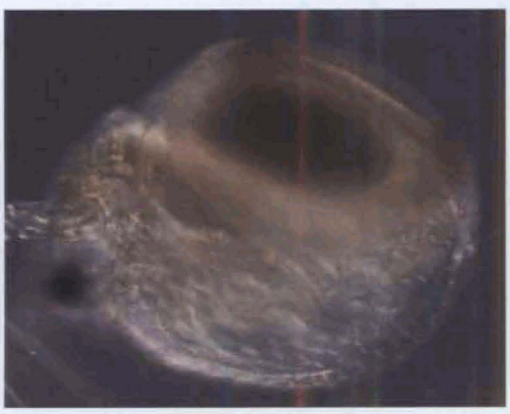
Plate 10

A



B

C



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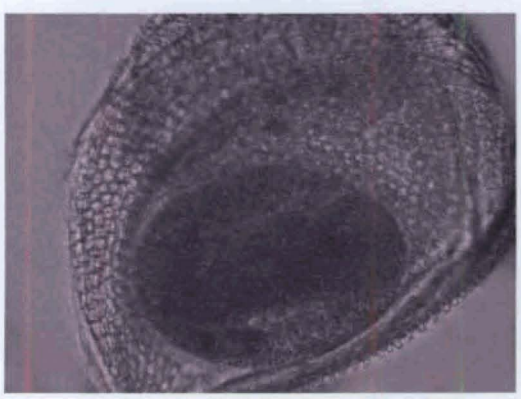


E

F

Ceriodaphnia cornuta

Fig. **A.** Pre-adult (0.367 mm), **B.** Female with ovary (0.387 mm), **C.** Parthenogenetic female (0.432 mm), **D.** Ephippial female (0.465 mm), **E.** Ephippium with egg (0.343 mm), **F.** Male (0.358 mm).

Plate 11

Stage-I (0.156 mm)

Stage-II (0.162 mm)

Stage-III (0.176 mm)

Stage-IV (0.180 mm)

Stage-V (0.188 mm)

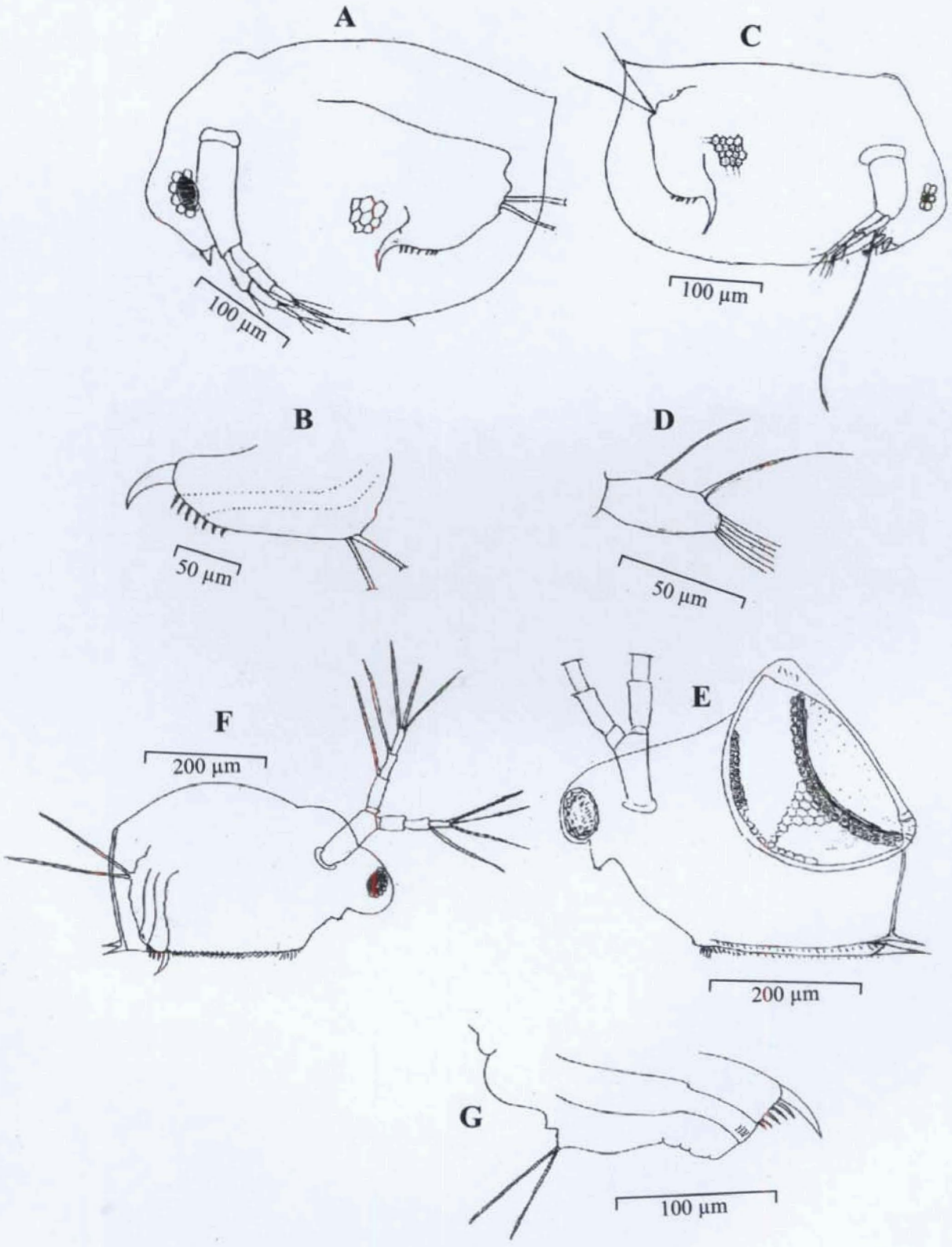
Stage-VI (0.202 mm)

Stage-VII (0.212 mm)

Stage-VIII (0.230 mm)

Ceriodaphnia cornuta Stages of embryonic development

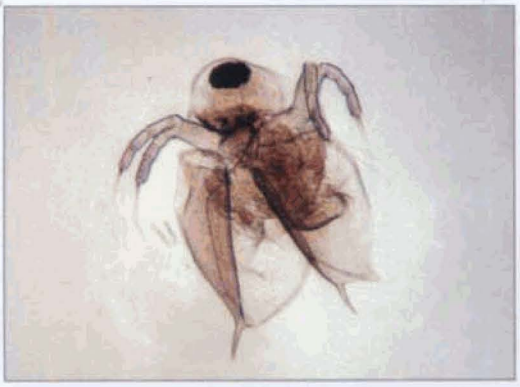
Plate 12



Ceriodaphnia cornuta Sars Fig. **A.** Female, **B.** Postabdomen of female, **C.** Male, **D.** Antennule of male.

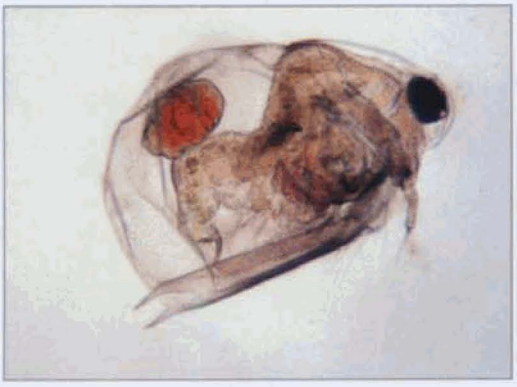
Scapholeberis kingi Sars **E.** Ephippial female, **F.** Female, **G.** Postabdomen of female.

Plate 13



A

B



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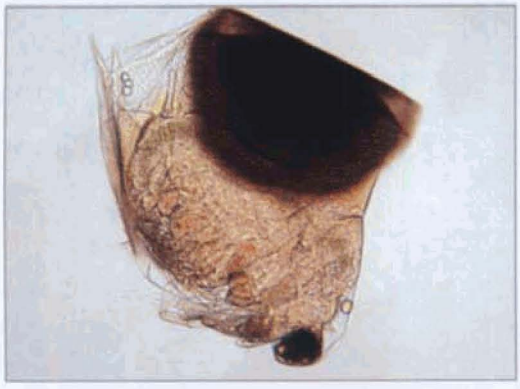

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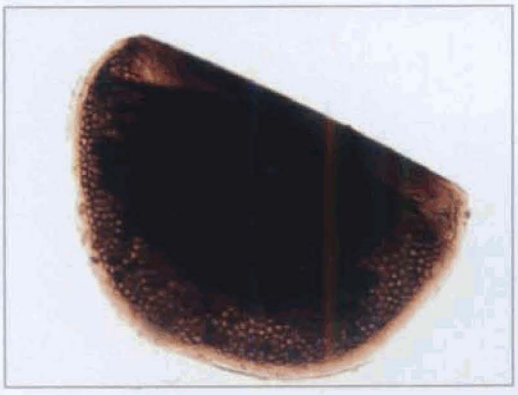


E

Scapholeberis kingi

Fig. **A.**Neonate - Ventral view (0.330 mm), **B.** Pre-adult (0.518 mm),
C. Parthenogenetic female with egg (0.768 mm),
D. Ephippial female (0.642 mm), **E.** Ephippium (0.482 mm)

Plate 14

Stage-I (0.114 mm)

Stage-II (0.138 mm)

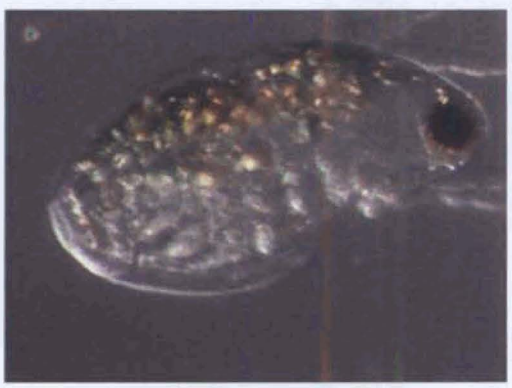
Stage-III (0.204 mm)

Stage-IV (0.212 mm)

Stage-V (0.212 mm)

Stage-VI (0.216 mm)

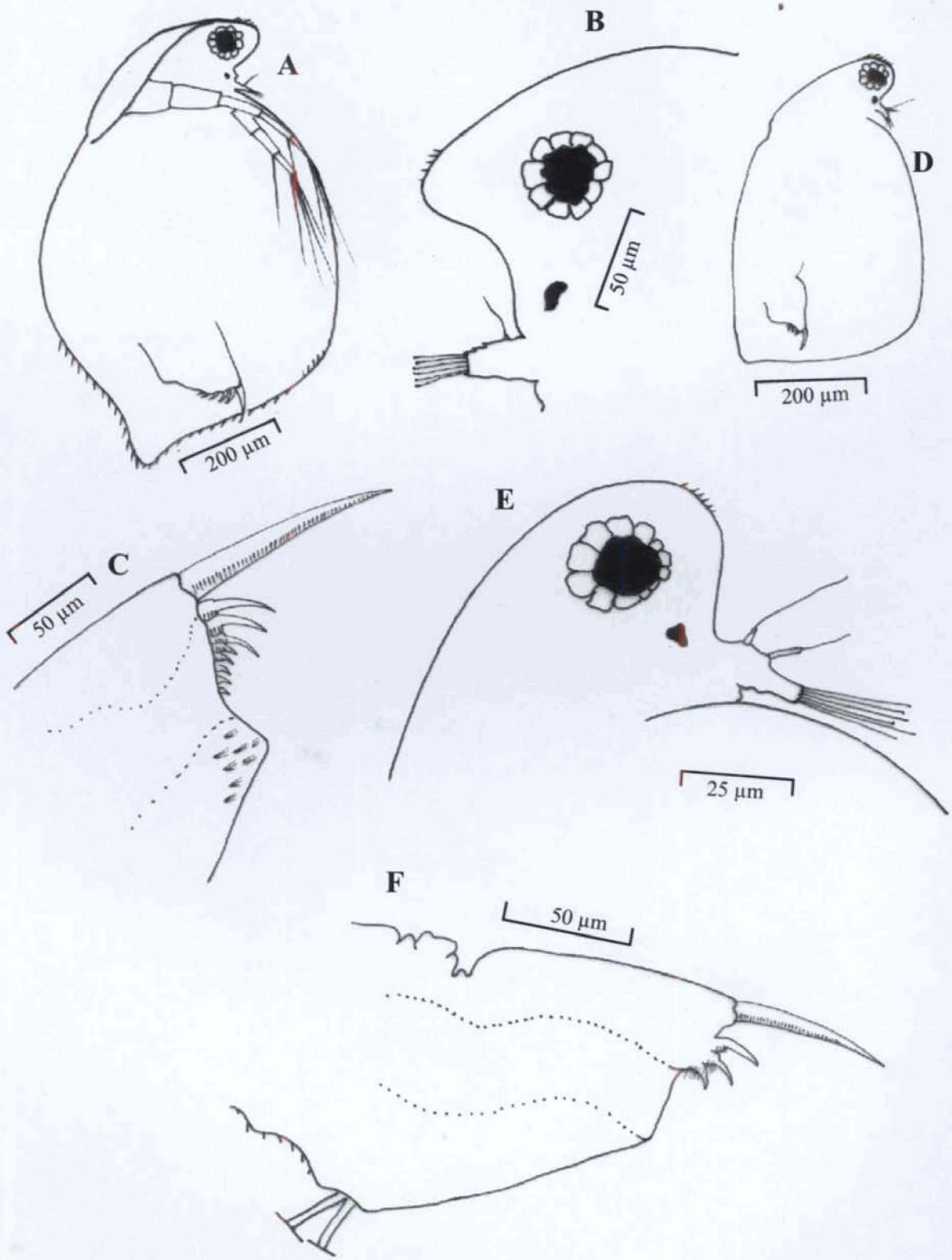
Stage-VII (0.228 mm)



Stage-VIII (0.252 mm)

Scapholeberis kingi Embryonic development

Plate 15



Simocephalus serrulatus (Koch)

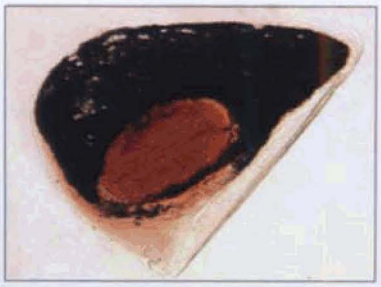
Fig. **A.** Female **B.** Head of female **C.** Postabdomen of female
D. Male **E.** Head of male **F.** Postabdomen of male

Plate 16

A

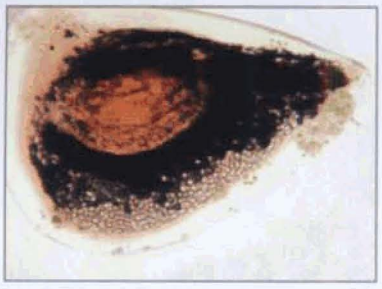
B

C



D

E



F

G

H

I

Simocephalus serrulatus

Fig. **A.** Female with ovary (1.102 mm) **B.** Parthenogenetic female (1.680 mm)
C. Ephippial female (1.558 mm) **D.** Ephippium with egg (0.988 mm)
E. Ephippial egg enlarged (0.390 mm) **F.** Ephippium without egg (0.920 mm)
G. Initiation of moulting H. Detachment of carapace
I. Casting off exuvium

Plate 17

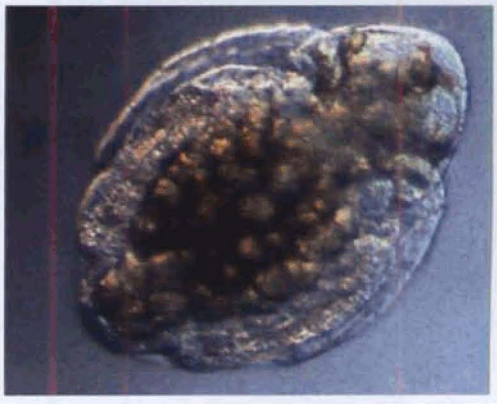
Stage I 0.220 mm)

Stage II (0.226mm)

Stage III (0.286 mm)

Stage IV (0.296 mm)

Stage V (0.298 mm)



Stage VI (0.304 mm)

VII (0.424 mm)

Stage VIII (0.457 mm)

Stage IX (0.487 mm).

Simocephalus serrulatus Embryonic development

Ceriodaphnia cornuta

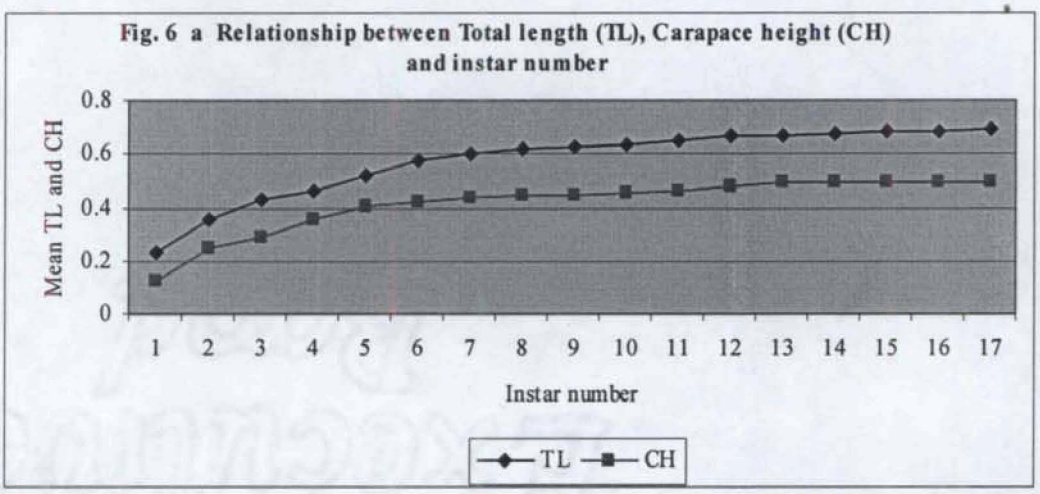


Fig. 6 a Relationship between Total length (TL), Carapace height (CH) and instar number

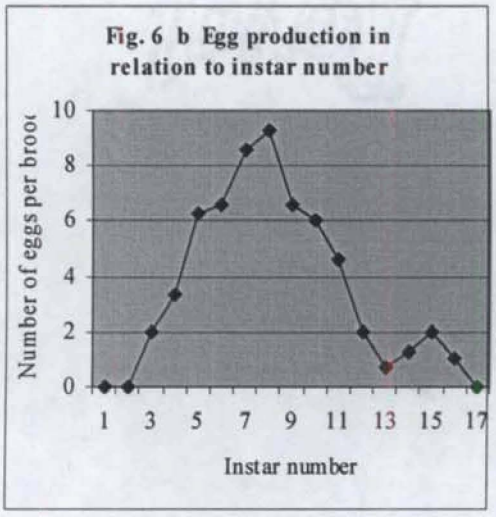


Fig. 6 b Egg production in relation to instar number

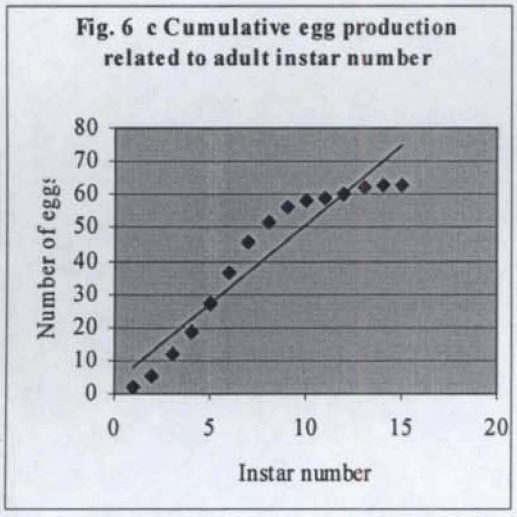


Fig. 6 c Cumulative egg production related to adult instar number

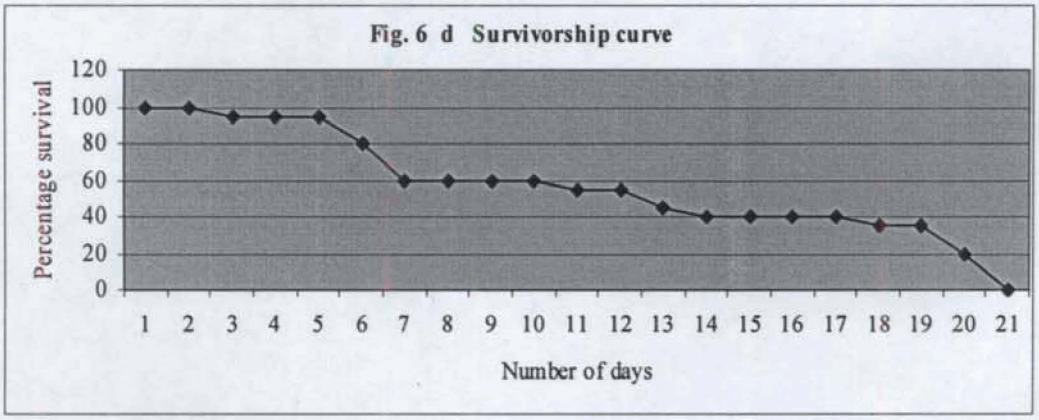


Fig. 6 d Survivorship curve

Scapholeberis kingi

Fig. 7 a Relationship between Total length (TL), Carapace height (CH) and instar number

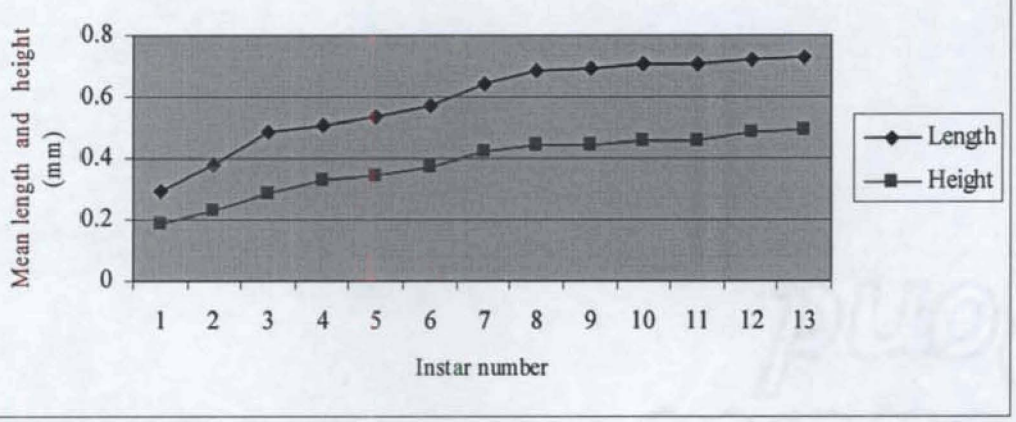


Fig. 7 b Egg production in relation to instar number

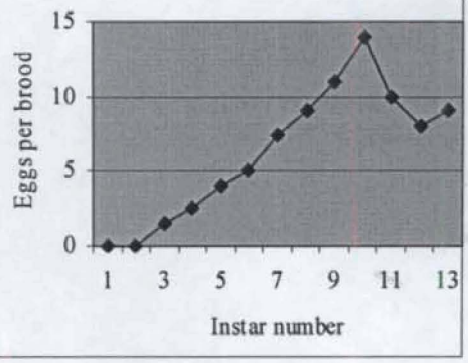


Fig. 7 c Cumulative egg production related to adult instar number

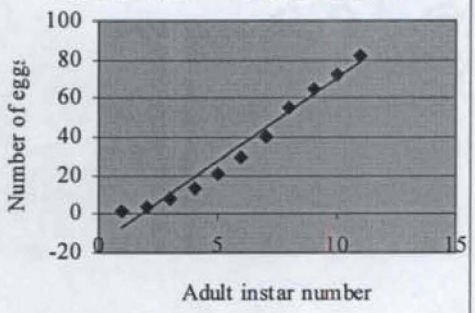
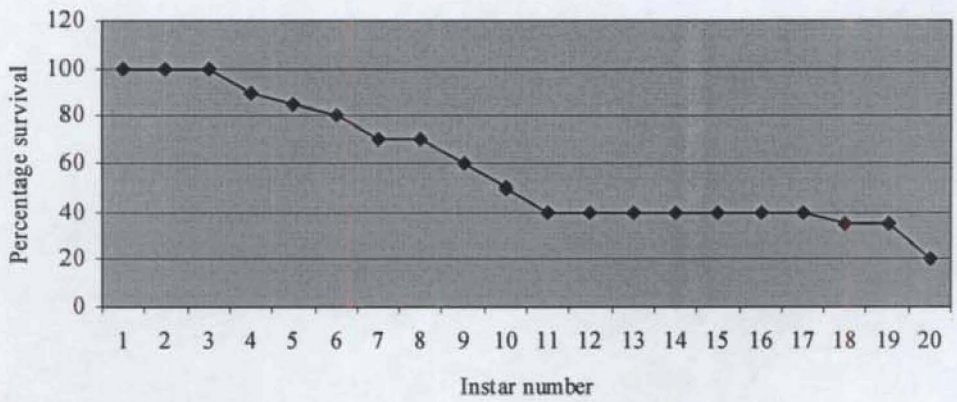


Fig.7 d Survivorship curve



Simocephalus serrulatus

Fig. 8 a Relationship between Total length (TL), Carapace height (CH) and instar number

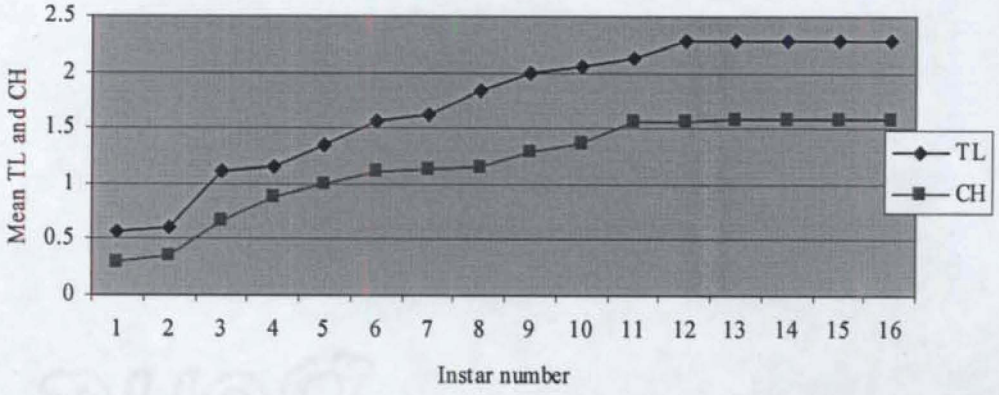


Fig. 8 b Egg production in relation to instar number

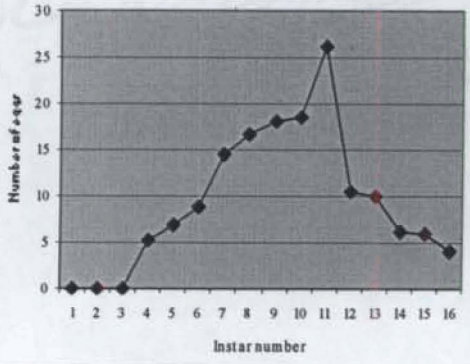


Fig. 8 c Cumulative egg production related to adult instar number

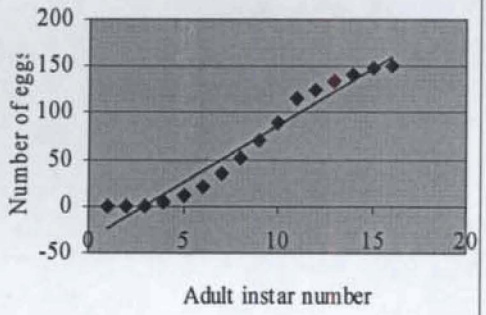


Fig. 8 d Survivorship curve

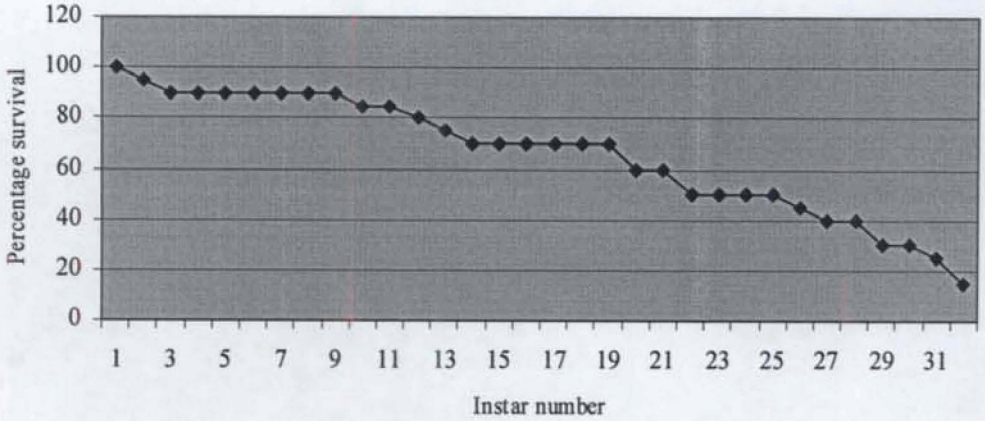


Fig. 9 Growth increment in Daphniidae

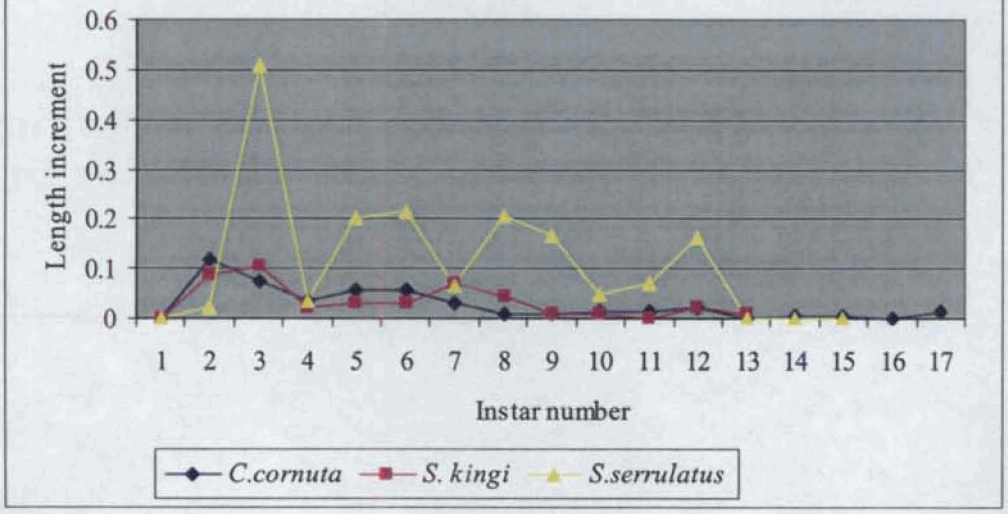
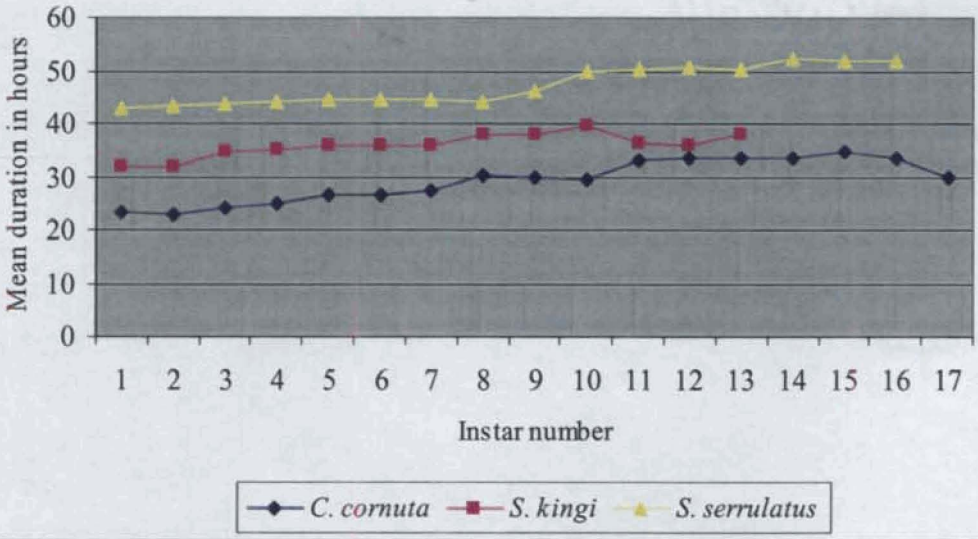


Fig. 10 Instar duration in Daphniidae



Family Moinidae Goulden, 1968

5. 4 *Moina brachiata* (Jurine, 1820)

Moina brachiata is primarily an inhabitant of temporary ponds and ditches. This species was described first by Jurine (1820) from Switzerland as *Monoculus brachiatus*. The first report of this species in India is by Brehm (1936) from Kashmir. Subsequent reports are that of Biswas (1971) and Nayar (1971) from Rajasthan, Patil (1976) from Meghalaya and Chandrasekhar and Kodarkar (1994) from Andhra Pradesh.

Our information on the biology and life cycle of *Moina* is based on the studies made in *Moina micrura* by Murugan (1975a) from Tamil Nadu and Thresiamma *et al.* (1991) from Kerala. Information on the biology of *M. brachiata* is scanty; hence made an investigation on its life cycle.

5. 4. 1. External Morphology

Parthenogenetic female (Plate 18. Fig. A)

Head rather broad; supraocular depression shallow. Eye situated near the dorsal margin of head; ocellus absent (Plate 20. Fig. A). Antennules long and thin (Plate 18. Fig. B), with several setae arranged in rings; long sensory seta at 1/3rd distance from head, and with 8 terminal setae (Plate 20. Fig. B). Valves nearly spherical, with granulated surface, ventral margin with

38-40 setae. Antennae well developed, with 4-segmented dorsal ramus and 3-segmented ventral ramus; antennary setation: (0-0-1-3)/ (1-1-3). Postabdomen with 11 feathered teeth, and one long bident tooth; claw with pecten and few hairs on the convex surface (Plate 20. Fig. C). Mean size: 1.158×0.710 mm.

Male (Plate 18. Fig. C)

Oblong and rectangular body, antennules long, bent about 1/3rd distance from the base with two sensory setae originating near the bend (Plate 18. Fig. D). The distal end of antennules with 6 long curved hooks that are grouped together. The 1st thoracic leg with a long hook. Size : 0.816 mm.

Ephippial female

Ephippial female similar to parthenogenetic female. Ephippium bright yellow, with distinct reticulations along the posterior and ventral margins (Plate 18. Fig. E). Egg single, situated in a spherical depression. Mean size of ephippium: 0.512 mm.

5. 4. 2 Reproduction

A laboratory culture of *M. brachiata* was prepared by hatching the resting eggs present in the dried soil sample collected from Site-7 as mentioned in section 3.2.2 (7). The neonates appeared on 6th day, indicating hatching of the resting eggs present in the soil sample. Several parthenogenetic females appeared in the culture from which a stock culture was developed for further studies.

Life cycle of parthenogenetic female

The population developed in the laboratory comprised only parthenogenetic females. Males and ephippial females appeared after one month. The sporadic appearance and disappearance of population was one of the interesting aspect observed in this species during the study. The features characteristic of the reproduction and life cycle of *M. brachiata* is given as follows.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean birth size (SaB) of 0.496 × 0.256mm. Both the first and second moulting occurred in same duration of 11.0 hrs each. The total pre-adult instar duration was 22.0 hrs. The mean duration of pre-adult instar (PID) was 11.0 hrs.

Attainment of maturity

The ovary became conspicuous during the middle of 2nd pre-adult instar at 14.0±1 hrs. However, they started to bear eggs after completion of 2nd moult at 21.0 hrs; and hence age at first reproduction (AFR) was 0.92 days. The size at first reproduction (SFR) was 0.758 ×0.508 mm.

Egg production

The eggs were deposited into the brood pouch within 10 minutes after completion of 2nd moult. The size of the egg gets increased immediately after its deposition. During this primiparous instar (3rd instar) egg production started with a mean number of 6.4 eggs/ brood. The primiparous instar was completed in 21.0 hrs and the FGT is calculated as 43.0 hrs.

During the subsequent instars egg production gets almost doubled to attain 12.4 eggs/ brood in the 4th instar. The egg production increased further and attained a peak of 15.6 eggs/ brood (Table 20). This maximum clutch size (E max) was attained in 5th instar (Fig. 11 b). The egg production in *M. brachiata* showed two peaks with maximum egg production during the 5th and 10th instar. The egg production decreased further and continued till the end of life span. The duration of adult instars steadily increased throughout the life cycle from 21.0 to 35.0 hrs. The mean duration (AID) was 25.85 hrs.

Fecundity

The relationship between egg production and instar number is shown in Fig. 11 b). The range of egg production of a single female varied from 6 to 20 with 10.78 eggs/day of adult life. The cumulative number of eggs (Σmx) produced during the entire life span was 116.3 (Table 20). Ten broods were produced with a mean of 11.63 eggs /brood. The cumulative egg production (Σmx) of *M. brachiata* is linearly correlated with instar number (Fig.11 c). The rate of egg production (REP) of *M. brachiata* is calculated as 11.771.

5. 4. 3 Growth

The first pre-adult neonates had a mean TL of 0.528 mm and the 2nd instar had TL of 0.636mm. Primiparous stage was attained during the 3rd instar when the mean TL was 0.758 mm, with an increment of 19.18%. The maximum mean TL of 1.302 mm was attained at the end of 12th instar (Table 21). The mean CH during the first pre-adult instar was 0.272 mm and

attained CH of 0.332 mm at second instar. They attained a mean CH of 0.508 mm during the primiparous condition, with an increment of 53.01%. Maximum CH was attained in 12th instar with CH of 0.752 mm (Table 21). During the life span each individual has undergone two pre-adult and ten adult moults.

The size increment during each instar has been represented in Table-21. Maximum growth increment recorded during the life cycle was with CH of 53.01% in 3rd however the growth increment decreased further after 5th instar. The relationship between TL, CH and instar number of *M. brachiata* is represented in Fig. 11 a. The correlation coefficients of life history parameters given in Table 22, shows a positive correlation between TL and CH ($r = 0.952$).

5. 4. 4 Embryonic Development

The stages of embryonic development in *M. brachiata* are represented in Plate 19. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of spherical egg which appears granular. Mean duration: 3.0 hrs. Mean size: 0.133 mm

Stage II: The embryo is elongated in anterio-posterior axis and shows cellular divisions. The embryo is covered by membrane. Mean duration: 5.0 hrs. Mean size: 0.191 mm

Stage III: The head lobe and antennary buds made their first appearance during this stage. Mean duration: 3.70 hrs. Mean size: 0.206 mm.

Stage IV: The head lobe and antennary bud become more distinct during this stage. The cellular divisions are more clearly seen. Mean duration: 2.30 hrs. Mean size: 0.208 mm.

Stage V: During this stage the head lobe and the rudiment of antennae become more distinct. The eye, thoracic legs and postabdomen started to differentiate. Mean duration: 1.50 hrs. Mean size: 0.310 mm.

Stage VI: The embryonic membrane is cast off just before to this stage. The embryo can be recognized by the presence of eye and segmented antennae and thoracic legs. Mean duration: 2.10 hrs. Mean size: 0.426 mm

Stage VII: Setae appear on the antennae, antennules and thoracic legs. The valves become distinct. Mean duration: 1.50 hrs. Mean size: 0.446 mm

Stage VIII: During this stage the development is completed. The neonates resembled the adult in external appearance and were released within a short duration. Mean duration: 1.20 hrs Mean size: 0.496 mm.

Release of neonates: The embryonic development of primiparous instar of *M. brachiata* was completed in a mean duration of 20.3 hrs, followed by moulting.

5. 4. 5 Life span and Survivorship

The survivorship curve (Fig. 11 d) indicates the relationship of age (days) and percentage survival of *M. brachiata.* The mean life span (Σlx) of female is calculated as 5.63 days; while the maximum life span (L max) observed during the present study was 11.69 days.

5. 5 *Moinodaphnia macleayi* (King, 1853)

Moinodaphnia macleayi was described first by King (1853); while the name *Moinodaphnia* was coined by Herrick (1887). *M. macleayi* is considered a "missing link" by Herrick (1887) between *Moina* and the other daphniids; because it possessed an ocellus and a supposed abdominal process.

Although, *M. macleayi* is widely distributed in tropics; it was reported first from Kerala by Brehm (1953) in a pond at Jagady, Thiruvananthapuram. Subsequent reports are from West Bengal (Sharma, 1978) and Lake Kolleru, Andhra Pradesh (Durga Prasad and Padmavathy, 2003a). There is no report of the studies on biology of this species from the country and hence made the present study on reproduction, life cycle, growth and embryonic development of both males and females reared in the laboratory.

5. 5. 1 External Morphology

Parthenogenetic female (Plate 21. Fig. C)

Body broadly rounded in outline. Head small, triangular with a flat ventral margin. Eye large and fills the tip of the head, presence of a slight supra-ocular depression above the eye; ocellus distinct (Plate 20. Fig. D). Antennules long and thin, arise from the ventral margin just behind the eye; with a long lateral seta and a group of nine terminal setae (Plate 20. Fig. E). Antennae thin, dorsal ramus 4-segmented and ventral ramus 3-segmented; setation of antenna: (0-0-1-4)/ (1-1-3). Shell broadly rounded and slightly

reticulated; valves with series of short marginal spines. Postabdomen well developed, distal end elongated, having 11 feathered setae and one bident tooth; claw straight, with fine setae on concave margin (Plate 20. Fig. F). Mean size: 0.825× 0.592 mm

Male (Plate 21. Fig. D)

Smaller than female. Head elongated, with large eye; ocellus small (Plate 20. Fig. G). Antennules long and curved inward; sensory seta originate about 1/3rd distance away from head, terminal setae comprise one long seta and 4-6 short setae. (Plate 20. Fig. H). First leg with large curved hook. Postabdomen similar to female with 6 feathered teeth (Plate 20. Fig. I). Mean size: 0.600 × 0.416 mm

Ephippial female (Plate 21. Fig. E)

Ephippial female similar to that of parthenogenetic female, except for the presence of reticulations in the valves. Ephippium white, occupying a central position in between the valves (Plate 21. Fig. F). Egg single (Plate 21. Fig. H), surrounded by polygonal ornamentation (Plate 21. Fig. G). Mean size: 0.840 × 0.496 mm. Mean size of ephippium: 0.565× 0.541 mm

5. 5. 2 Reproduction

The population developed during the laboratory culture comprised asexual females, ephippia bearing females and males. The parthenogenetic and sexual reproduction was observed throughout the period of study. The neonates produced from a clutch were all females, all males or both males and females. However, parthenogenetic females dominated the stock culture.

The appearance of males was followed by the production of ephippial females in the culture. The carapace of ephippial female gets modified as an ephippium which was subsequently released into the medium during moulting. The ephippium floated on the surface for a few minutes and then sank to the bottom upon getting wet. During the course of development the egg changed from an oval to spherical shape. The egg had mean initial size of 0.260×0.162 mm. The ephippia were produced by the females during their early part of life cycle. After completing 1-2 ephippial generations they resumed the parthenogenetic reproduction.

Life cycle of Male

M. macleayi male was reported for the first time from India by Venkataraman (1995). Males were not obtained from the field collection during the present study. However, a good number of males were produced in the laboratory culture throughout the period of study. Males could be distinguished shortly after birth, due to the presence of a prominent curved antennule and their relative smaller size. Twenty neonates of less than 12 hrs of age were sorted out and reared individually for life cycle studies following the methodology cited in section 3. 2. 3. .

The neonates had a mean birth size (SaB) of 0.380 × 0.218 mm. *M. macleyai* male during the entire lifespan, underwent only 2 moults. The 1st and 2nd moulting occurred in the same duration of 42 hrs each. The neonates attained TL of 0.568 and CH of 0.272 mm after first moulting. The total pre-adult duration was 84 hrs (Table 25).

After the 2nd moulting they became sexually mature as indicated by the presence of yellow coloured testis located just below the alimentary canal. The size after second moult was 0.668 × 0.320 mm. The maximum growth was observed during 2nd, 3rd and 4th day respectively and decreased further (Table 25). There was no moulting after attainment of maturity and growth was retarded further. The maximum size was 0.762 × 0.376 mm recorded on 12th day (Table 25). The percentage increment of TL and CH during each day is represented in Table 25. The relationship between TL, CH and instar number is given in Fig.12 b. The mean life span (Σlx) of male is calculated as 5.60 days, while the maximum life span observed during the present study (L max) was 12.19 days.

Life cycle of parthenogenetic female

The features characteristic of the reproduction and life cycle of *M macleayi* is given as follows.

Moulting

The neonates underwent moulting towards the end of each instar followed by an increase in size, especially during the early instars. Moulting in adult instars was followed by the release of young ones. Soon after this the eggs released from the ovary are deposited into the brood pouch.

Pre-adult instar

The neonates produced from the parthenogenetic females had a birth size (SaB) of 0.480 × 0.296 mm. The first moulting occurred at an interval of

23.26 hrs while the second moulting occurred at 23.56 hrs duration. The total pre-adult duration was 46.82 hrs and the mean PID was 23.41 hrs.

Attainment of maturity

The ovaries of parthenogenetic females were clearly visible at 32± 0.5 hrs of life as green coloured, elongated sacs on either side of the alimentary canal (Plate 21. Fig. B). However, the eggs were deposited into the brood pouch after the completion of 2nd moulting and the age at first reproduction (AFR) was 1.95 days. The size at first reproduction (SFR) was 0.628× 0.432 mm.

Egg production

The parthenogenetic females started to bear eggs after completion of the 2nd moult at 63.82± 1 hour. The eggs attained a spherical shape soon after its deposition into the brood pouch and measured a size of 0.148 × 0.096 mm. Egg production started during the primiparous instar (3rd instar) with the production of 2.2 eggs/ brood. The primiparous instar had a mean duration of 29.3 hrs and the first generation time (FGT) is calculated as 76.0 hrs.

During the subsequent instars there was a sharp increase in egg production to 19.6 eggs/ brood. This maximum clutch size (Emax) was attained in the 12th instar (Table 23). The egg production of *M. macleayi* showed a single peak with maximum egg production in 12th instar followed by a sudden decrease (Fig. 12 c).

Each clutch in the early adult instar consisted of 1 to 4 rows of eggs on either side of brood pouch with 3 to 4 eggs in each row. The egg production continued throughout the life span.

Fecundity

Fig. 12 c indicates the relationship between egg production and instar number. The range of egg production of a single female varied from 2.0 to 26.0 with an egg production of 7.02 eggs per day of adult life. The cumulative number of eggs (Σmx) produced during entire life was 150.9 (Table 23). Fifteen broods were produced during the entire life span with a mean of 10.06 eggs/ brood. The cumulative egg production (Σmx) of *M. macleayi* is linearly correlated with adult instar number (Fig. 12 d). The rate of egg production (REP) of *M. macleayi* is calculated as 11.4968.

Instar duration

Aithough, they had a uniform duration of pre-adult instars with an average (PID) of 23.41 hrs. The primiparous instar had a mean duration of 29.3 hrs. The duration of adult instars varied from 29.3 to 38.0 with a mean (AID) of 34.43 hrs (Table 23). *M. macleayi* passed through fifteen adult instars during the life span. Fig. 14 indicates the relationship between instar number and instar duration.

5. 5. 3 Growth

The first pre-adult neonates had a mean TL of 0.480 mm and 2nd instar had TL of 0.584 mm. Primiparous stage was attained during the 3rd instar

when the mean TL was 0.628 mm; with an increment of 7.53%. The maximum mean TL of 1.104 mm was attained at the end of 16th instar and no growth observed during 17th instar (Table 24).

The mean CH during the first pre-adult instar was 0.296 mm and attained CH of 0.312 mm at second instar. They attained a mean CH of 0.432 mm during the primiparous condition; with an increment of 38.46%. Maximum CH was attained in 16th instar with CH of 0.786 mm (Table 24). During the life span each individual has undergone two pre-adult and fifteen adult moults.

The size increment during each instar is given in Table 24. Maximum growth increment recorded during the life cycle was with TL of 21.67% in 2nd instar and with CH of 38.46% in 3rd instar; and the growth increment decreased in the succeeding instars. The relationship between TL, CH and instar number of *M. macleayi* has been represented in Fig. 12 a. The correlation coefficients of the life history characters are given in Table 26, which shows a positive correlation between TL and CH (r = 0.992).

5. 5. 4 Embryonic Development

The stages of embryonic development in *M. macleayi* are represented in Plate 22. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of spherical egg, with less amount of yolk. Mean duration: 3.60 hrs. Mean size: 0.136 mm

Stage II: During this stage the embryo is elongated anterio-posteriorly. The elongation of the anterior region is clearly visible. Cellular divisions are initiated in the embryo. Mean duration: 5.10 hrs. Mean size: 0.153 mm.

Stage III: The head lobe appears and cellular divisions continued. Mean duration: 3.50 hrs. Mean size: 0.157 mm.

Stage IV: The head lobe is clearly distinct with the formation of rudimentary structures. Mean duration: 2.00 hrs. Mean size: 0.229 mm.

Stage V: The presence of pink eyes is noticed for the first time during this stage. The rudiments of antennae become distinct. The embryo contained less amount of yolk and was enclosed in a membrane. Mean duration: 2.4 hrs. Mean size: 0.241 mm.

Stage VI: The eyes undergo fusion and become black in colour. The rudiments of thoracic legs become distinct during this stage. Mean duration: 6.20 hrs. Mean size: 0.283 mm

Stage VII: The embryonic membrane is cast off just before this stage and the antennae get segmented followed by the development of terminal setae. The eye gets enlarged followed by the appearance of a small ocellus. The rudiments of thoracic legs and postabdomen become more distinct during this stage. Mean duration: 4.6 hrs. Mean size: 0.312 mm

Stage VIII: Yolk completely disappears and some movements are initiated in the embryo. The antennae, antennules, thoracic legs and postabdomen become distinct and the embryo resembles the adult in external morphology. Mean duration: 1.5 hrs. Mean size: 0.401 mm

Release of neonates

The hatching could be regarded as the culmination of embryonic development. The neonates were released by the opening of the brood pouch by the extension of postabdominal processes. The total duration of embryonic development during the primiparous instar of *M. macleayi* was 28.90 hrs.

5. 5. 5 Life span and Survivorship

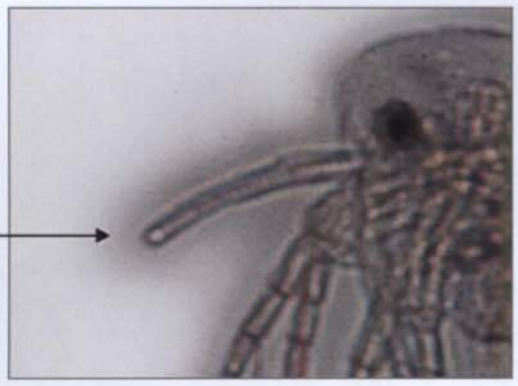
The survivorship curve (Fig. 12 e) indicates the relationship of age (days) and percentage survival of *M. macleayi*. The the mean life span (Σlx) observed during the present study is 9.23 days while the maximum life span observed during the present study (Lmax) for the female was 23.45 days.

Plate 18

A

B

C



D

E

Moina brachiata

Fig. **A.** Parthenogenetic female (1.128 mm), **B.** Female head enlarged, **C.** Male (0.726 mm), **D.** Male head enlarged (→ indicates the elongated antennule), **E.** Ephippium (0.504 mm).

Plate 19

Stage-I (0.112 mm)

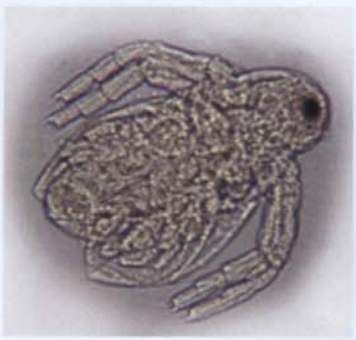
Stage-II (0.195 mm)

Stage-III (0.204 mm)

Stage-IV (0.208 mm)

Stage-V (0. 306 mm)

Stage-VI (0.424 mm)

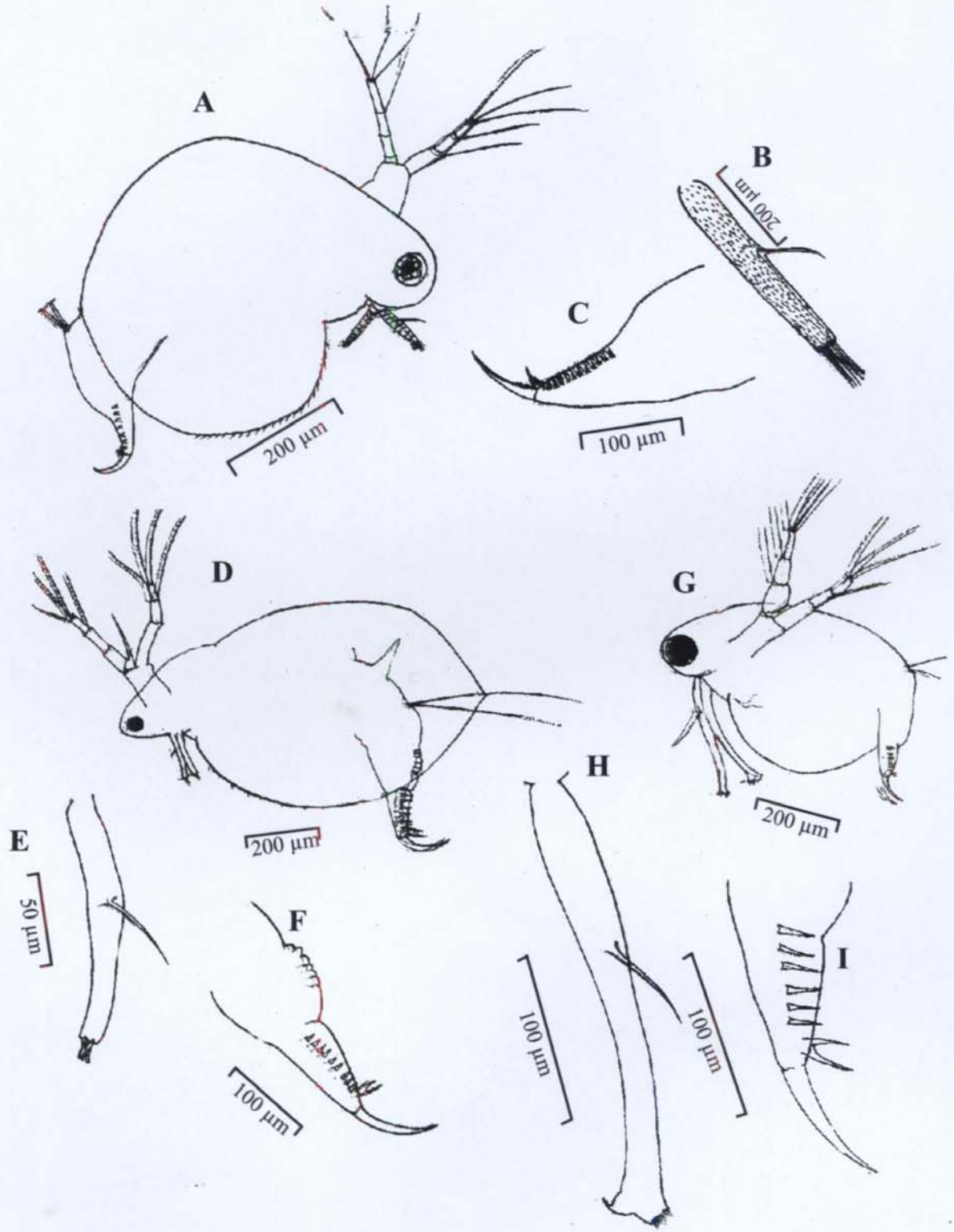


Stage-VII (0.450 mm)

Stage-VIII (0.492 mm)

Moina brachiata Embryonic development

Plate 20

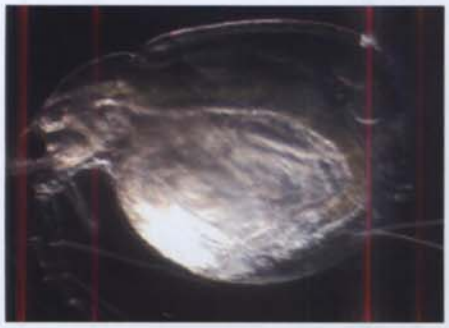


Moina brachiata (Jurine) Fig. **A.** Female, **B**. Antennule of female, **C**. Postabdomen of female
Moinodaphnia macleayi (King) **D.** Female, **E**. Antennule of female, **F.** Postabdomen of female,
G. Male, **H**. Antennule of male, **I**. Postabdomen of male.

Plate 21

A

B



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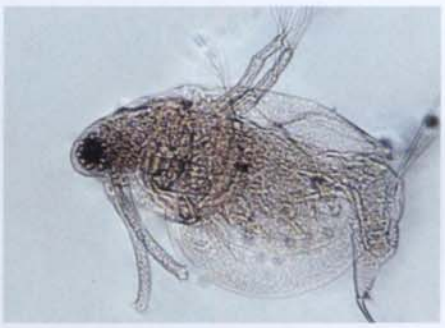

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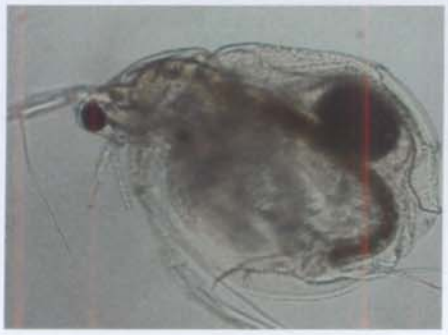

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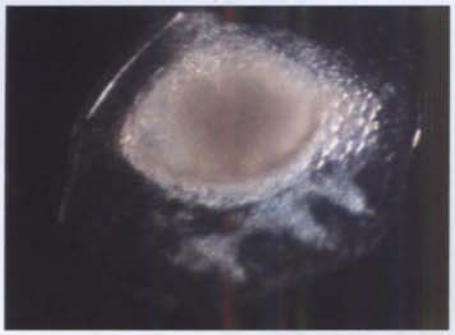
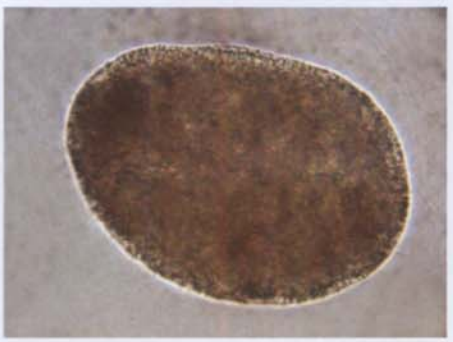


F

G



H

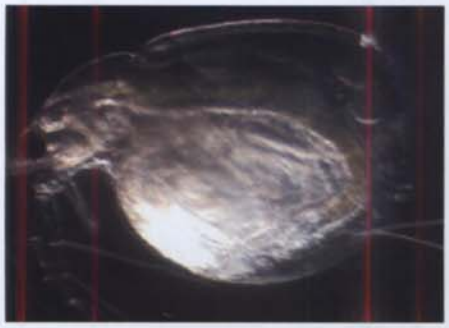
Moinodaphnia macleayi

Fig. **A**. Pre-adult (0.526 mm), **B**. Female with ovary (0.580 mm), **C**. Parthenogenetic female with egg (0.857 mm), **D**. Male (0.604 mm), **E**. Ephippial female (0.710 mm), **F**. Ephippium (0.457 mm), **G**. Ephippium enlarged, **H**. Ephippial egg (0.265 mm).

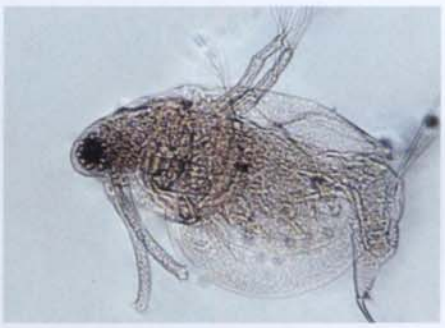
Plate 21

A

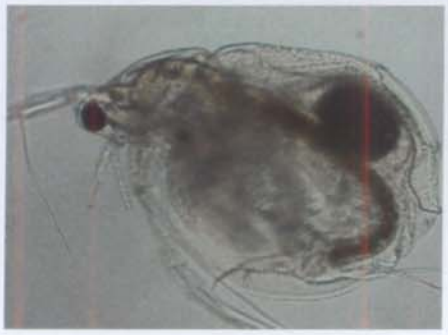
B



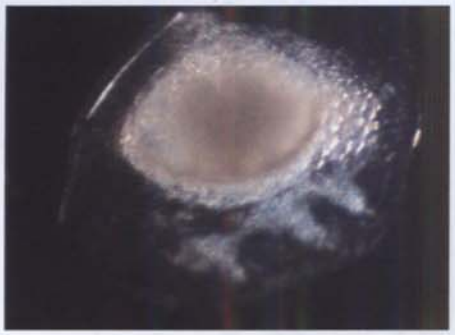
C



D

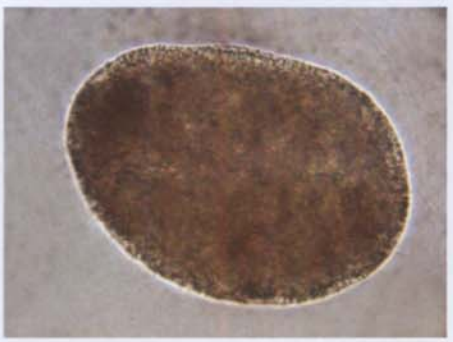


E



F

G



H

Moinodaphnia macleayi

Fig. **A**. Pre-adult (0.526 mm), **B**. Female with ovary (0.580 mm), **C**. Parthenogenetic female with egg (0.857 mm), **D**. Male (0.604 mm), **E**. Ephippial female (0.710 mm), **F**. Ephippium (0.457 mm), **G**. Ephippium enlarged, **H**. Ephippial egg (0.265 mm).

Plate 22

Stage-I (0.138 mm)

Stage-II (0.153 mm)

Stage-III (0.158 mm)

Stage-IV (0.228 mm)

Stage-V (0.240 mm)

Stage-VI (0.284 mm)

Stage-VII (0.312 mm)

Stage-VIII (0.398 mm)

Moinodaphnia macleayi Embryonic development

Moina brachiata

Fig.11 a Relationship between Total length (TL), Carapace height (CH) and instar number

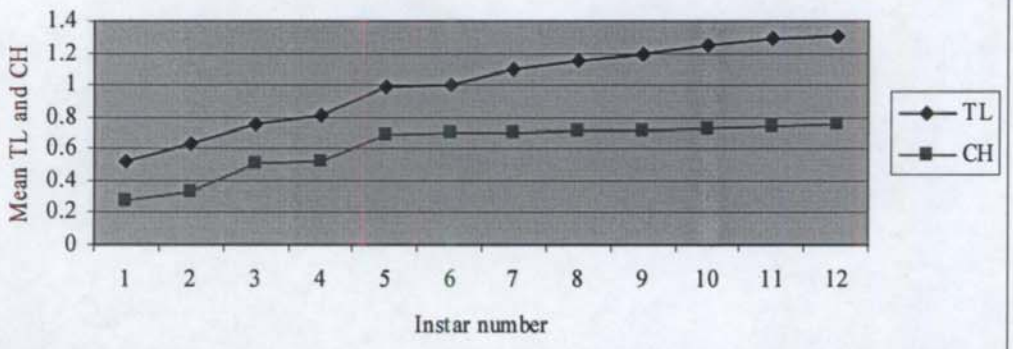


Fig. 11 b Egg production in relation to instar number

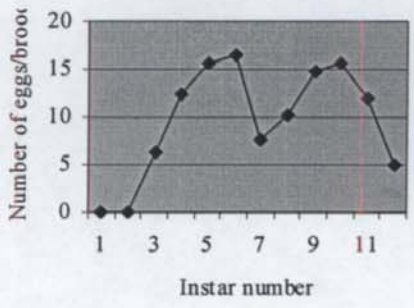


Fig.11 c Cumulative egg production related to adult instar number

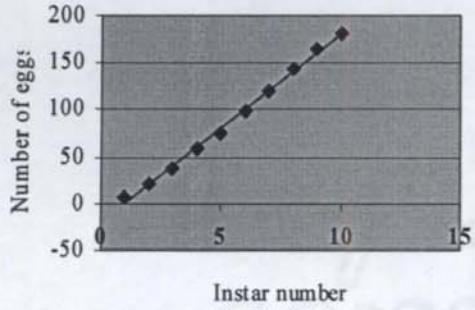
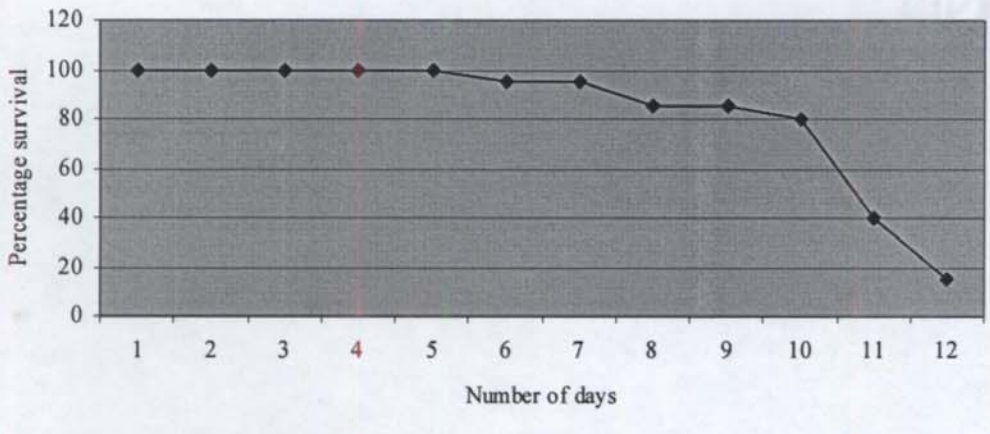


Fig. 11 d Survivorship curve



Moinodaphnia macleayi

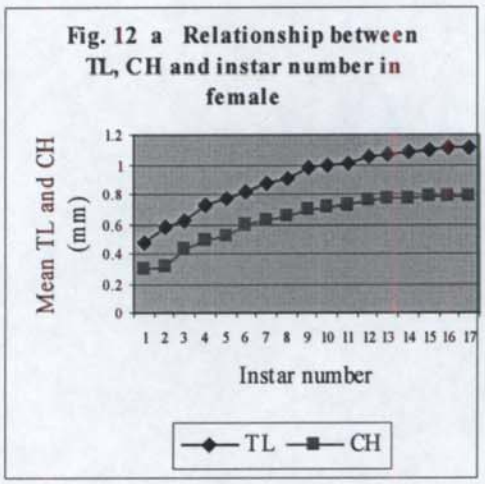


Fig. 12 a Relationship between TL, CH and instar number in female

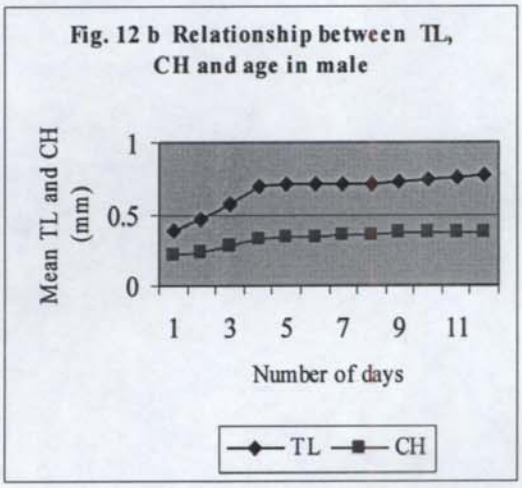


Fig. 12 b Relationship between TL, CH and age in male

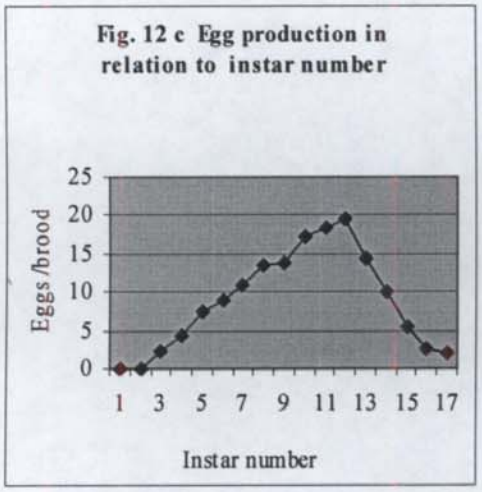


Fig. 12 c Egg production in relation to instar number

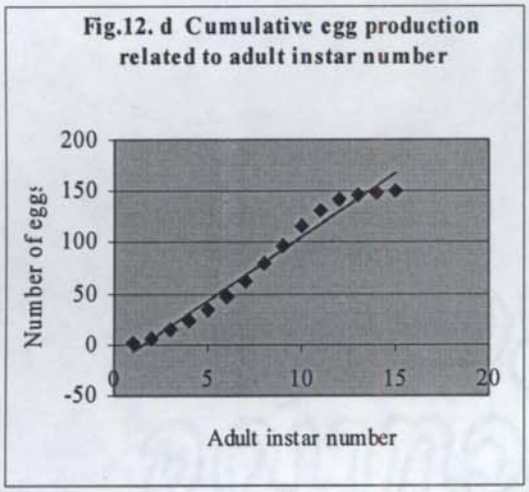


Fig.12. d Cumulative egg production related to adult instar number

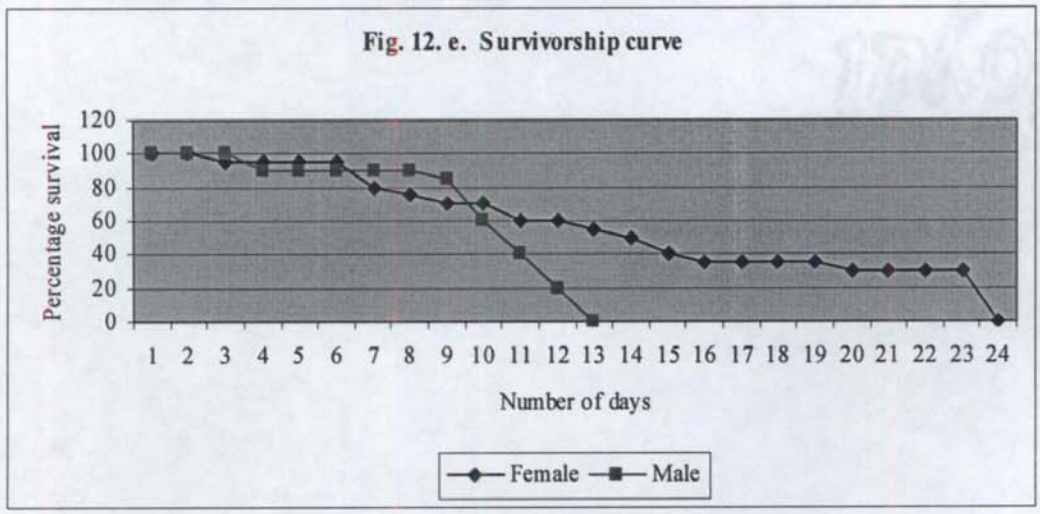


Fig. 12. e. Survivorship curve

Fig. 13 Growth increment in Moinidae

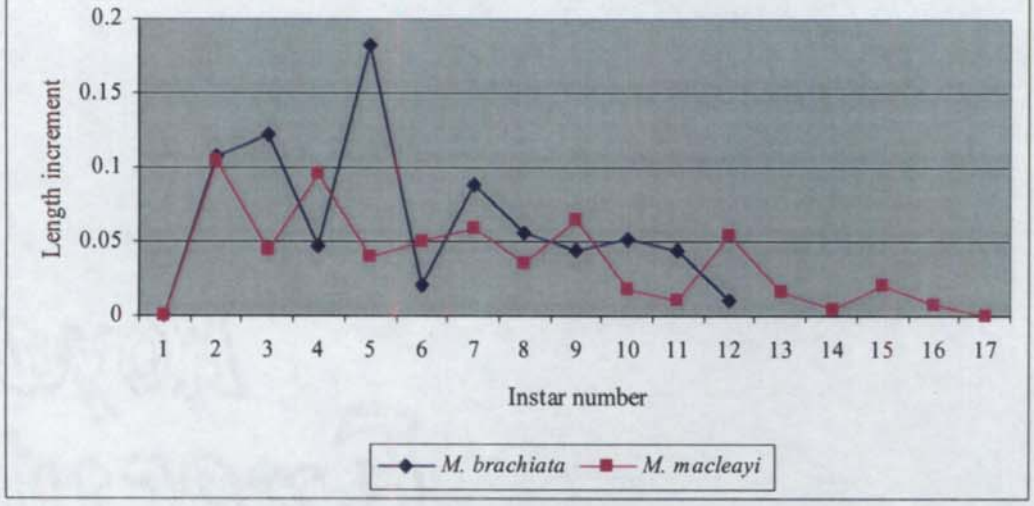
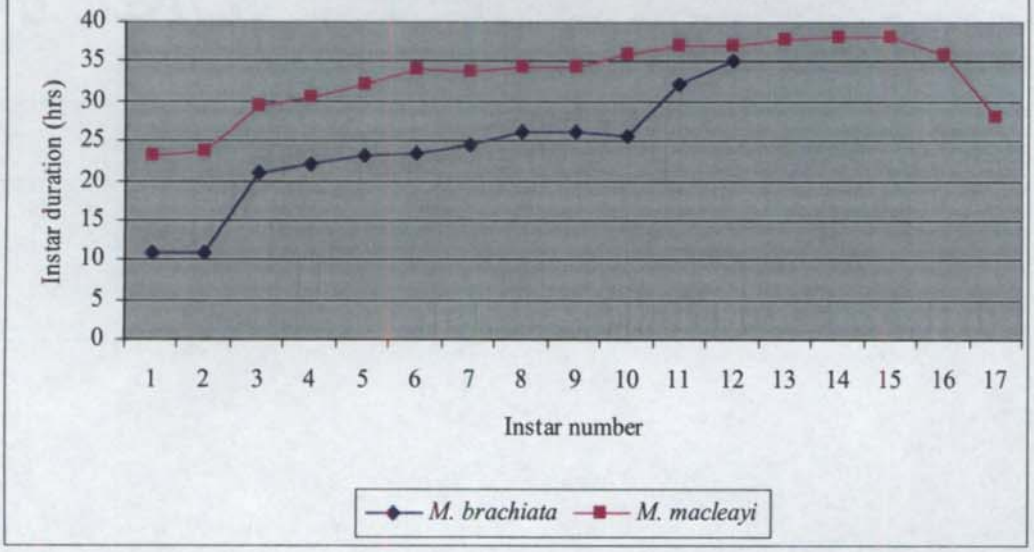


Fig. 14 Instar duration in Moinidae



Family Macrothricidae Norman and Brady, 1867

5. 6 ***Ilyocryptus spinifer*** Herrick, 1882

Ilyocryptus spinifer is an inhabitant of freshwater bodies which often prefers the muddy substratum. This species was first described by Herrick (1882) from USA. *I. spinifer* is also reported from Australia (Henry, 1922), Africa (Kořínek, 1984) and Asia (Rajapaksa and Fernando, 1982).

Many authors consider *I. spinifer* a pan-tropical species occurring throughout tropical and sub-tropical regions (Birge, 1918; Harding, 1957). Smirnov (1976) included it in a separate Family Ilyocryptidae.

I. longiremis Sars was considered a synonym of *I. spinifer* Herrick by Gurney (1907) who reported this species from Calcutta. Although, *I. spinifer* has been reported from different states in India, this species is reported first from Kerala by Michael and Sharma (1988). Subsequently, Patil and Gouder (1988) reported it from Karnataka, Durga Prasad and Padmavathy (2003a) from Andhra Pradesh and Babu and Nayar (2004) from Thekkady, Kerala.

The biology of *I. spinifer* has not been investigated so far and hence made a detailed description of its parthenogenetic reproduction.

5. 6. 1 External Morphology

Parthenogenetic female (Plate 23. Fig. C)

Body oval shaped, with deeply arched ventral margin, length greater than width (Plate 25. Fig. A). Head small, slightly bluntly pointed anteroventrally, appears triangular in lateral view (Plate 23. Fig. D). Eye relatively large; ocellus small, situated about half way between eye and base of antennules. Antennules elongated and slender, 2-segmented, attached to posteroventral margin of the head, with a group of terminal sensory setae (Plate 25. Fig. C). Valves with straight dorsal margin, ventral and posterior margins evenly rounded, with a series of long plumose setae (Plate 25. Fig. B); hexagonal reticulations throughout shell surface. Antennae with 4-segmented dorsal ramus and 3-segmented ventral ramus; antennary setation: (0-0-0-3)/ (1-1-3). Postabdomen bilobed, with 25 marginal denticles and long anal spines; claw slender with two unequal basal spines (Plate 23. Fig. F; Plate 25. Fig. D); anal aperture opens in the middle of postabdomen. The carapace of the field animals were found profusely covered with detritus. Mean size: 0.708 × 0. 586 mm.

Ephippial female (Plate 23. Fig. G)

Ephippial female similar to parthenogenetic female in all respects except reticulation of the shell. Ephippium released with a major portion of carapace and has hexagonal reticulations. The ventral margin bears several setae. The ephippium encloses two eggs of almost same size (Plate 23. Fig. H). Mean size of ephippial female: 0.570 mm. Size of ephippium: 0.506 mm.

5. 6. 2 Reproduction

The population developed during the laboratory culture comprised only asexually reproducing females. Males were not observed during the present study.

Life cycle of parthenogenetic female

Parthenogenetic reproduction was observed throughout the period of study. The neonates produced by parthenogenetic reproduction were completely asexual females. The features characteristic of the reproduction and life cycle of *I. spinifer* is given as follows.

Pre-adult instar

The neonates produced from parthenogenetic females had a birth size (SaB) of 0.304× 0.208 mm. The newly hatched young ones were found to avoid light and burrow into the bottom. Moulting was not observed during pre-adult instar and they retained their of old carapace. The period between the birth and first appearance of eggs inside the brood pouch is taken as the pre-adult instar duration of the species. The mean pre-adult instar duration (PID) was 68.0 hrs.

Attainment of maturity

Although, the development of ovary started very early in life, they were clearly visible at 52.0 ±1.5 hrs. The ovary appeared as elongated sacs on either side of the alimentary canal with dark-brown colour (Plate 23. Fig. B).

They started to bear eggs after 68.0 hrs. The AFR was 2.83 days and the SFR was 0.584× 0.448 mm

Egg production

One of the important features noted during the life cycle was the delayed deposition of each clutch of eggs into the brood pouch. The eggs were first deposited into brood pouch after 13.0 hrs during the primiparous instar. The same pattern of delayed egg production was observed in the successive instars.

The eggs were oval-shaped and measured a mean size of 0.195× 0.126 mm. During this primiparous instar (2^{nd} instar) egg production started with an average of 2.0 eggs/ brood. The primiparous instar was completed in 52.0 hrs and the first generation time (FGT) is calculated as 120.0 hrs.

During the subsequent instars there was a sharp increase in egg production to 12.0 eggs/ brood (Table 27). The maximum clutch size (Emax) was attained in the 6^{th} instar, with production of 12.0 eggs/ brood. However, the egg production of *I. spinifer* showed a bimodal pattern with two peaks. The egg production declined after 6^{th} instar and again increased further to produce 7.5 eggs in 10^{th} instar (Fig. 15 b). In *I. spinifer* egg production continued throughout the life span.

Each clutch during the adult instars consisted of 1 to 2 rows of eggs placed on either side of the brood pouch. Due to the absence of moulting the adult instar duration is taken as the time interval between the releases of successive batches of neonates. This includes the time for egg transfer,

completion of embryonic development and subsequent release of young ones. The adult instar duration varied from 42.0-50.0 hrs. The mean adult duration (AID) is calculated as 45.96 hrs (Table 27).

Fecundity

The relationship between egg production and instar number is represented in Fig. 15 b. The range of egg production of a single female was from 2 to 14 with an egg production of 2.69 eggs/ day of adult life. The cumulative number of eggs produced (Σmx) during the entire life span was 61.5 (Table 27). Twelve broods were produced during the entire life with a mean of 5.12 eggs/ brood. The cumulative egg production (Σmx) is linearly correlated instar number Fig. 15 c. The rate of egg production of *I. spinifer* is calculated as 5.7637.

5. 6. 3 Growth

The first pre-adult neonates had a mean TL of 0.336 mm. The primiparous stage was attained during the 2nd instar when the mean TL was 0.584 mm. The maximum mean TL of 0.830 mm was attained at the end of 13th instar (Table 28).

The mean CH during the first pre-adult instar was 0.238 mm.. The mean CH of 0.448 mm was attained during the primiparous condition (2nd instar). Maximum mean CH of 0.662 mm was attained in 13th instar (Table 28). During the entire life span each individual has undergone one pre-adult and twelve adult instars.

The increment of TL and CH during each instar is given in Table 28. Maximum growth increment recorded during the life cycle was in 2nd instar with TL of 73.81% and CH of 88.24% respectively. This indicates maximum growth during the early (pre-reproductive) phase of life cycle than during the late (reproductive) phase.

The relationship between TL, CH and instar number of *I. spinifer* has been represented in Fig. 15 a. The correlation coefficients of life history characters are given in Table 29, which shows a positive correlation between TL and CH (r= 0.989).

5. 6. 4 Embryonic Development

The stages of embryonic development of *I. spinifer* are represented in Plate 24. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of an oval-shaped egg which is brown in colour.

Mean duration: 1.0 hrs. Mean size: 0.188 mm

Stage II: The embryo has two distinct areas. The outer area of the embryo appears transparent while the inner area is granular and opaque due to the concentration of yolk.

Mean duration: 3.5 hrs. Mean size 0. 190 mm

Stage III: The embryo started to elongate, the outer transparent area become more distinct and shows cellular divisions. Mean duration: 8 .0 hrs. Mean size 0. 202 mm

Stage IV: This stage is characterized by the appearance of rudiments of head and antennae.

Mean duration: 4.0 hrs. Mean size: 0. 218 mm

Stage V: This stage is characterized by the presence of rudiments of head and antennae. The embryo also shows rudiments of thoracic legs and postabdomen.

Mean duration: 7.5 hrs. Mean size: 0. 224 mm

Stage VI: This stage is recognized by the presence of pink eye. The antennae become segmented and thoracic legs become distinct.

Mean duration: 4.0 hrs. Mean size: 0. 258 mm.

Stage VII: This stage is characterized by the presence of black eye. The antennae, antennules, thoracic legs and postabdomen become more distinct.

Mean duration: 9.0 hrs. Mean size: 0. 288 mm

Stage VIII: During this stage the segmentation of antennae and development of setae is complete. The valves and the postabdomen become distinct. The embryo resembles the adult in external morphology.

Mean duration: 2.0 hrs. Mean size: 0.304 mm

Release of neonates

The adult parthenogenetic females released neonates from the brood pouch by the opening of valves and movement of postabdomen. The total mean duration of primiparous instar was 52 hrs; out of which 13.0 hrs needed for the deposition of eggs and 39.0 hrs for the embryonic development. The delayed deposition of eggs (13.0-14.0 hrs) as observed in the present study may be due to the absence of moulting in this species.

Retention of Carapace

The carapace was found profusely covered with algae (*Chlorella*) in the laboratory culture. Absence of true moulting enables these animals to retain its old carapace without renewing encrustation. These retained carapaces appear as concentric rings over the shell, which appears as successive growth lines (Plate 23. Fig. E).

The retained carapaces along with encrustation provide an excellent camouflage. The slow movements exhibited by the species are due to this algal encrustation.

5. 6. 5 Life Span and Survivorship

The survivorship curve (Fig. 15. d) indicated the relationship of age (days) and percentage survival of *I. spinifer*. The mean life span (Σlx) is calculated as 11.43 days, while the maximum life span (L max) of female observed during the study (Lmax) was 25.81 days.

5. 7 *Macrothrix triserialis* (Brady, 1886)

Macrothrix triserialis is a littoral cladoceran most often found among submerged vegetation in water bodies especially ponds, paddy fields and marshes. This species was described first by Brady (1886) from Sri-Lanka. *M. triserialis* resembles *M. rosea* (Lievin, 1848) which is commonly recorded in Europe and North America.

Among Cladocera, Macrothricidae is one of the least studied families and hence many species have recently been re-described (Kotov, 1999; Silva-Briano *et al.* 1999). Recently Dumont *et al.* (2002) revised the group *Macrothrix rosea-triserialis*.

In India *M. triserialis* is reported from Bihar (Gurney, 1907). Later from Rajasthan (Biswas, 1971; Michael and Sharma, 1988), West Bengal (Michael and Sharma, 1988) and Karnataka (Patil and Gouder, 1988). The first report of this species from Kerala is that of Michael and Sharma (1988) based on the collections of Nayar, C.K.G from Irinjalakuda. Further report of this species is by Raghunathan (1989a) from Wynad and recently from Thekkady by Babu and Nayar (2004).

The biology of *M. triserialis* has not been studied so far and hence made a detailed description of the life cycle of parthenogenetic female and male.

5. 7. 1 External Morphology

Parthenogenetic female (Plate 26. Fig. A)

Body nearly oval; dorsal margin slightly arched, ventral margin deeply arched. Head large, separated from rest of body by a cervical depression (Plate 25. Fig. E); rostrum slightly pointed, labral plate serrated and almost straight posteriorly. Eye relatively large; ocellus small, situated nearer to the apex of the rostrum than to the eye. Antennules slender, cylindrical, with series of spinules, distal part armed with 3 spinules and a few terminal setae (Plate 25. Fig. F). Antennae relatively short, the longest seta of the antenna have a series of short setules and 3 spines in the middle. Shell produced into a sharp pointed angle posteriorly; ventral margin serrated and with a series of rather long setae. Postabdomen bilobed, with several anteriorly directed spines along its dorsal margin, lateral spines in rows; claw short, slightly curved dorsally, without basal spine; distal segment of natatorial setae short, with a group of long and fine setules attached to them (Plate 25. Fig. G). Mean size: 0.892 × 0.533 mm.

Male (Plate 26. Fig B)

Males smaller than female. Antennules elongated, with one strong bristle at the base anteriorly and 4 rows of rigid hairs in its proximal portion, distal part armed with 3 spines and a few terminal setae (Plate 25. Fig. H). The first thoracic leg bears a curved hook. Postabdomen relatively small, dorsal side forms a cylindrical tube, transversely truncated at the tip and with ejaculatory duct opening dorsally (Plate 25. Fig. I). Mean size: 0.517× 0.308 mm.

Ephippial female (Plate 26. Fig. C)

Ephippial female similar to the parthenogenetic female except reticulations in the shell. The ephippium appears transparent with striations, enclosing two eggs. Ephippium shed along with a major portion of the valve (Plate 26. Fig. D). Eggs large, spherical, brown coloured and granular containing abundant yolk (Plate 26. Fig. E). Mean size: 0.766 ×0.516 mm. Mean size of ephippium: 0.450×0.508 mm.

5. 7. 2 Reproduction

The population developed during the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. The life cycle is represented in Plate 27.

The neonates produced from a single clutch during the parthenogentic reproduction consist of males and females. They were found to be all females, all males or both males and females. The males could be distinguished during early stages due to their relative smaller size and elongated antennules. Production of *M. triserialis* males are often followed by the generation of ephippial females leading to sexual reproduction.

The ephippial females develop a transparent ephippium which encloses two eggs inside it. Detachment of ephippium always occurs during moulting, and the ephippium is released into the medium. The ephippia then sink to the bottom or most often get attached to the sides of the container. The ephippial females after completion of 2-3 generations resumed the parthenogenetic reproduction.

Population growth

The population density in the laboratory culture was estimated from 28-11-2004 to 24-12-2004. Numerical study was made by taking a subsample of 1 ml from the container. A counting chamber of 1 ml capacity was used for counting and calculated the number of individuals/ litre of water.

Table 41 shows that during the period of study, the population density of *M. triserialis* in the laboratory culture varied between 1124 and 16239 per litre of water. The growth of population is graphically represented in Fig. 17. It is evident from the figure that the population showed an increase in the initial period. After attaining its peak on 16^{th} day, the population declined associated with the appearance of males and ephippial females. A sudden increase in the population was further noted on 26^{th} day when the population comprised of only parthenogenetic females. The males and ephippial females however disappeared towards the end the period.

Life cycle of Male

Twenty male neonates were sorted out for the life history studies and cultured them individually in test tubes using the methodology mentioned in section 3. 2. 3.

The mean birth size (SaB) was 0.266 × 0.216 mm. The first moulting occurred in duration of 36.0 hrs and there was no moulting further. The first pre-adult neonates had a mean TL of 0.408 mm and CH of 0.248 mm. They attained maturity on 2^{nd} day after first moulting and attained mean TL

of 0.432 mm and CH of 0.256 mm (Table 32). The pre-adult duration was 36.0 hrs (1.5 days). The sexually mature individuals have yellow coloured testis.

The maximum percentage increment of TL was observed on 2nd day with 5.88%. The increment of TL showed variation subsequently (Table 32). Maximum size attained during the study was 0.518×0.318 mm on 9th day. The mean life span of male (Σlx) is calculated as 4.6 days while the maximum life span (Lmax) observed was 8.6 days.

Life cycle of parthenogenetic female

The majority of neonates produced were parthenogenetic females. The features characteristic of the reproduction and life cycle of parthenogenetic female is given as follows.

Moulting

M. triserialis underwent moulting towards the end of each instar. The release of whole part of the carapace as exuvium was observed in *M. triserialis* and the moulting was completed within duration of 50.0 minutes.

Pre-adult instar

The neonates produced from the parthenogenetic females had a mean birth size (SaB) of 0.318 × 0.207 mm. Both the first and second moulting occurred in a uniform duration of 29.0 hrs. The total pre-adult duration was

58.0 hrs and the mean pre-adult duration (PID) was 29.0 hrs. They started to reproduce after second moulting.

Attainment of maturity

The ovary of parthenogenetic female was clearly visible at 48±1 hrs of life; and appeared as green coloured elongated structures on either side of alimentary canal. They started to bear eggs after completion of 2nd moult at 58.0 hrs; and hence the AFR was 2.42 days. The SFR was 0.608×0.376 mm.

Egg production

Eggs were deposited into the brood pouch in a mean duration of 50.0 minutes after completion of the second moult. The eggs attained spherical shape with yellow green colour and measured a mean size of 0.175× 0.133 mm. During this primiparous instar (3rd instar) egg production started with a mean of 5.5 eggs/brood. The primiparous instar was completed in mean duration of 30.5 hrs and the first generation time FGT is calculated as 88.5 hrs.

During the subsequent instars there was a steady increase in egg production to 20.4 eggs/ brood in 6th instar; followed by a slight decrease (Table 30). In the succeeding instars the egg production increased to attain a peak production of 24.2 eggs/ brood during 10th instar. This maximum clutch size (Emax) was attained in the 10th instar, followed by a slow decline till death (Fig. 16 c). The bimodal pattern of egg production is observed in *M. triserialis* with two peaks. They continued egg production throughout the life span.

Each clutch consisted of 1-2 rows of eggs deposited on either side of the brood pouch. The female underwent moulting towards the end of each adult instar. The adult instar durations varied from 30.5 and 36.5 hrs (Table 30); with a mean adult duration (AID) of 32.9 hrs. The relationship between instar number and instar duration is represented in Fig. 18 b.

Fecundity

The relationship of egg production with instar number is represented in Fig. 16 c. The range of egg production of a single female was from 4 to 26 with 13.24 eggs/day of adult life. The cumulative number of eggs produced (Σmx) during entire life span was 181.5 (Table 30). Ten broods were produced during the entire life with a mean of 18.15 eggs/ brood. The Σmx was linearly correlated with instar number Fig. 16 d. The rate of egg production REP of *M. triserialis* is calculated as 17.9329.

5. 7. 3 Growth

The first pre-adult neonates had a mean TL of 0.410 mm and the second instar had TL of 0.464 mm. Primiparous stage was attained during the 3rd instar when the mean TL was 0.608 mm. The maximum mean TL of 0.966 mm was attained at the end of 12th instar (Table 31).

The mean CH during the first pre-adult instar was 0.248 mm and attained CH of 0.366 mm at second instar. A mean CH of 0.376 mm was attained during the primiparous condition. Maximum mean CH of 0.608 mm was attained in 12th instar (Table 31). During the life span each individual has undergone two pre-adult and ten adult moults. The relationship between TL,

CH and instar number of *M. triserialis* has been represented in Fig. 16 a. The correlation coefficients of life history characters are given in Table 33, which shows a positive correlation between TL and CH (r= 0.971).

The increment of TL and CH during each instar is given in Table 31. Maximum growth increment recorded during the life cycle was with TL of 31.03% in 3rd instar and with CH of 47.58% was observed in 2nd instar.

5. 7. 4 Embryonic Development

The stages of embryonic development of *M. triserialis* are represented in Plate 28. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of oval-shaped egg with dark granular yolk. Mean duration: 1.0 hrs. Mean size: 0.178 mm.

Stage II: The embryo has two distinct areas. The outer area of the embryo appears transparent while the yolk granules are concentrated at the inner granular zone. Mean duration: 1.5 hrs. Mean size: 0.180 mm.

Stage III: The embryo has elongated in anterio-posterior axis and shows cellular divisions. Mean duration: 4.0 hrs. Mean size: 0.182 mm.

Stage IV: The head lobe and antennary bud made their first appearance during this stage. Mean duration: 1.5 hrs. Mean size: 0.183 mm

Stage V: During this stage the head lobe and the rudiment of antennae become more distinct. The cellular divisions can be clearly seen. Mean duration: 3.0 hrs. Mean size: 0. 214 mm.

Stage VI: This stage can be recognized by the development of rudiments of head, antennae, antennules and postabdomen. Mean duration: 5.5 hrs. Mean size: 0.218 mm

Stage VII: This stage can be recognized by the presence of pink eye. Head become more distinct. Mean duration: 3.8 hrs. Mean size: 0.236 mm

Stage VIII: The eye become dark, ocellus appears. Setae appear on antennae, antennules and thoracic legs and postabdomen which become more distinct. Mean duration: 7.4 hrs. Mean size: 0.266 mm

Stage IX: The development of antennae, antennules, thoracic limbs and postabdomen is completed Mean duration: 2.0 hrs. Mean size: 0. 0.298 mm.

Release of neonates

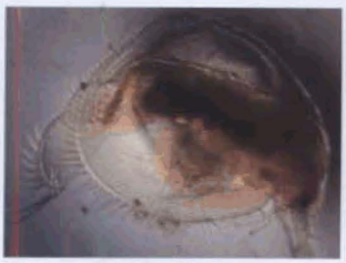
Embryonic development of primiparous instar of *M. triserialis* was completed in a mean duration of 29.7 hrs and the neonates were released by the movement of postabdomen of the female.

5. 7. 5 Life Span and Survivorship

The survivorship curve (Fig. 16 e) indicates the relationship of age (days) and percentage survival of *M. triserialis.* The mean life span (Σlx) of female is calculated as 7.55 days, while the maximum life span (Lmax) of was 16.1 days. As evident from the data survival was higher near the age of maturity and declined further a few days after maturity.

Plate 23

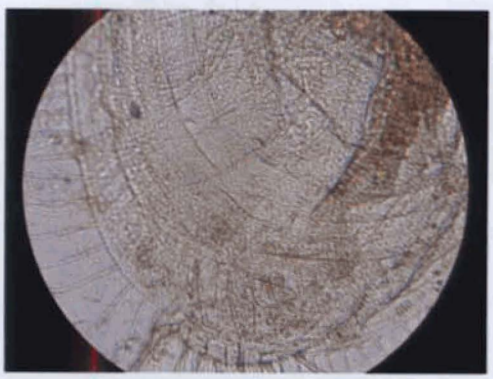
A



B

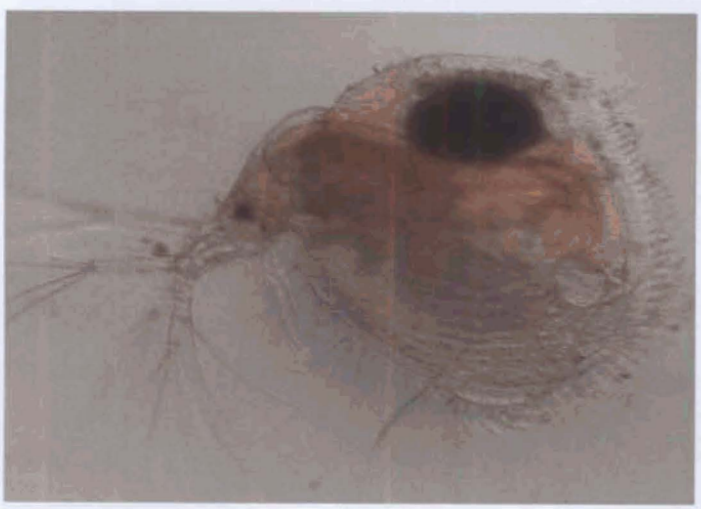
C

D



E

F



G

H

Ilyocryptus spinifer

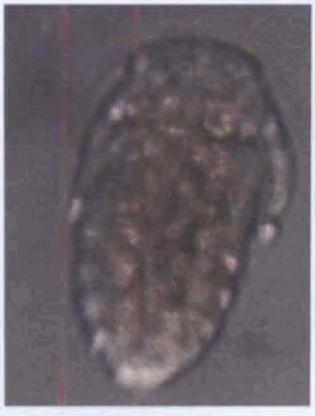
Fig. **A**. Pre-adult (0.346 mm), **B**. Female with ovary (0.502 mm), **C**. Parthenogenetic female with egg (0.596 mm), **D**. Head enlarged, **E**. Part of shell enlarged to show growth lines, **F**. Postabdomen enlarged, **G**. Ephippial female (0.571 mm), **H**. Ephippium (0.506 mm).

Plate 24

Stage-I (0.187 mm)

Stage-II (0.194 mm)

Stage-III (0.204 mm)



Stage-IV (0.219 mm)

Stage-V (0.224 mm)

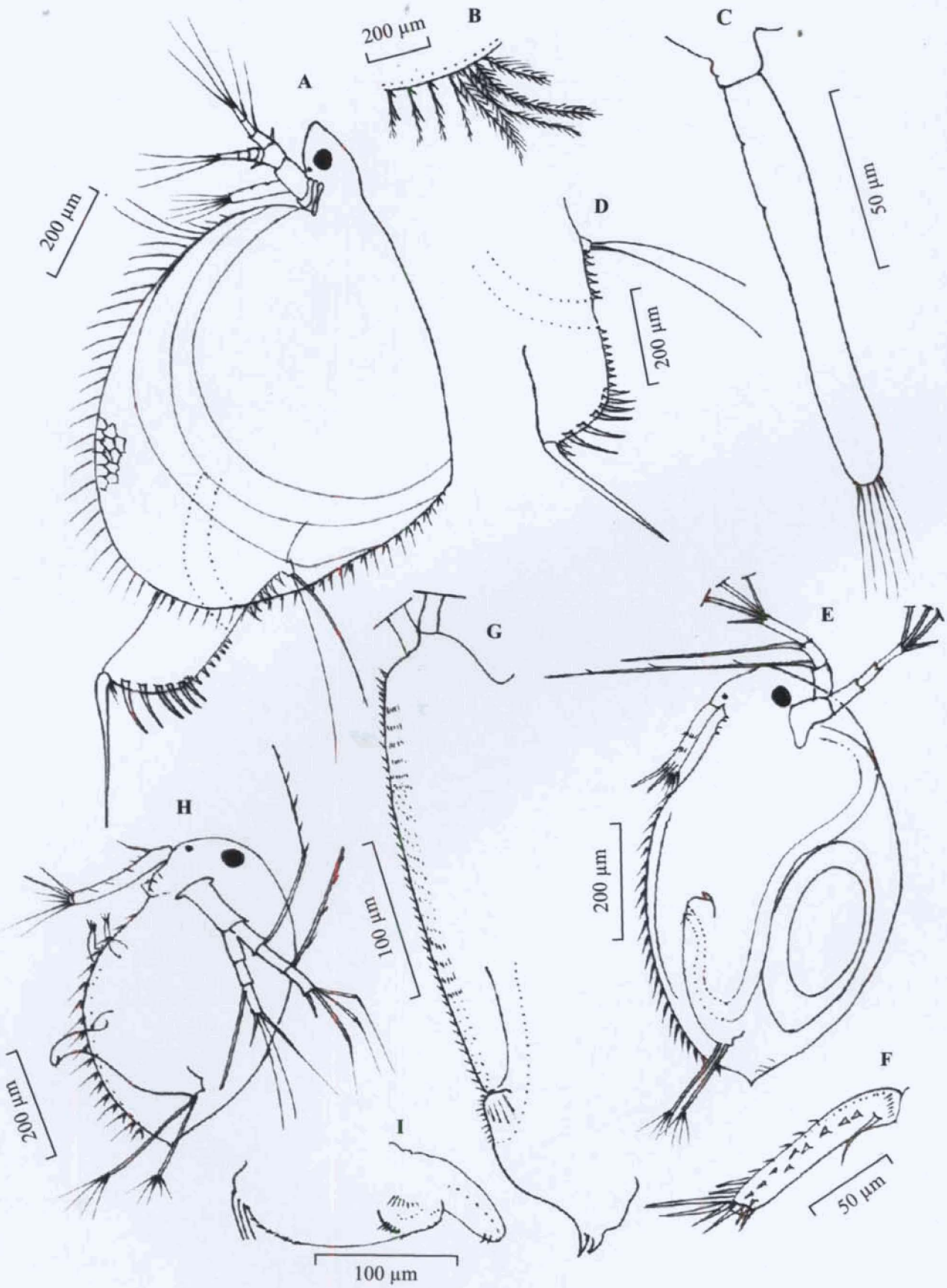
Stage-VI (0.259 mm)

Stage-VII (0.286 mm)

Stage-VIII (0.302 mm).

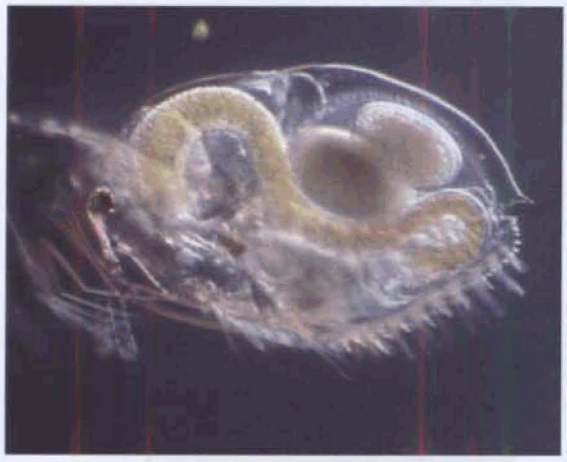
Ilyocryptus spinifer Embryonic development

Plate 25

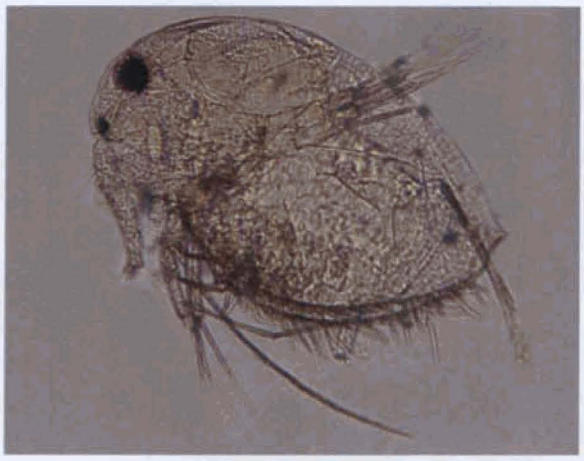


Ilyocryptus spinifer Herrick Fig. **A**. Female, **B**. Anteroventral shell margin, **C**. Antennule of female, **D**. Postabdomen of female.
Macrothrix triserialis (Brady) **E**. Female, **F**. Antennule of female. **G**. Postabdomen of female. **H**. Male, **I**. Postabdomen of male.

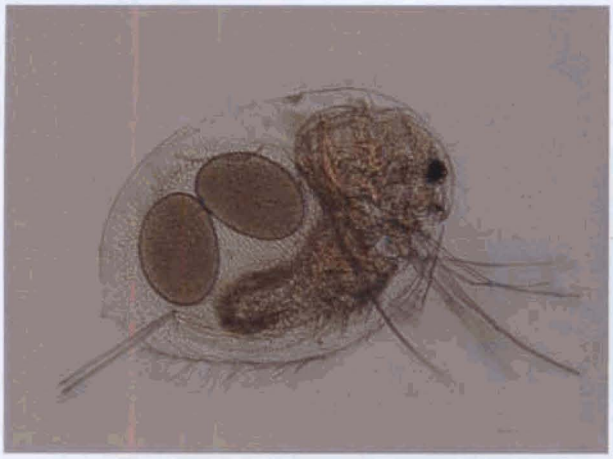
Plate 26



A

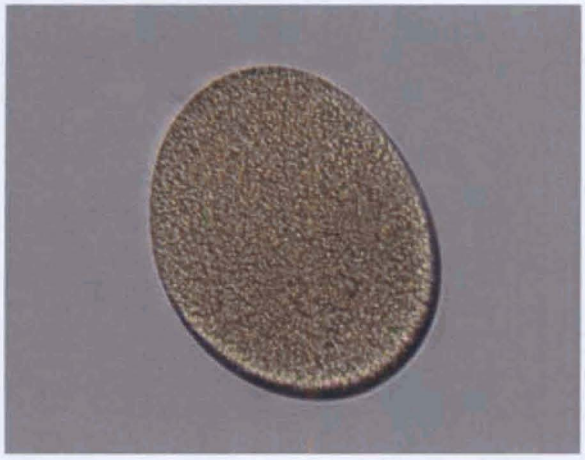


B



C

D

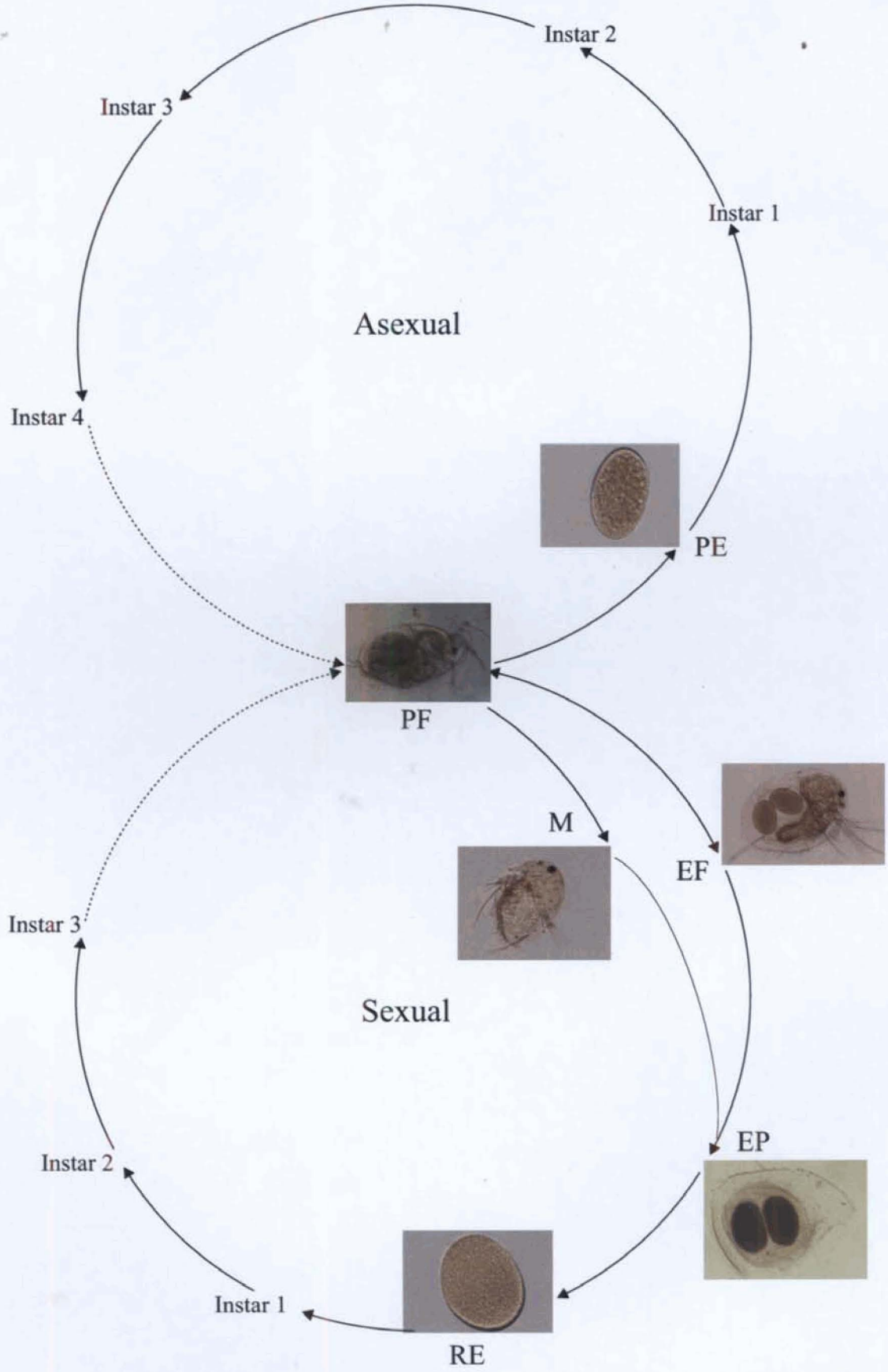


E

Macrothrix triserialis

Fig. **A**.Parthenogenetic female (0.884 mm), **B**. Male (0.521 mm), **C**. Ephippial female (0.770 mm), **D**. Ephippium (0.452 mm) **E**. Ephippial egg enlarged (0.216 mm).

Plate 27

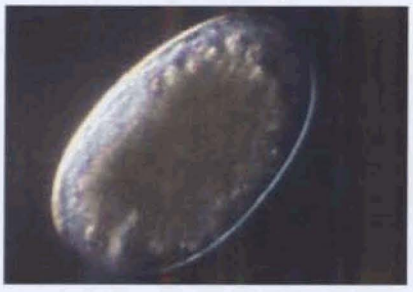


Macrothrix triserialis Life cycle

EP-Ephippium **EF**-Ephippial female **M**-Male **PE**-Parthenogenetic egg, **PF**-Parthenogenetic female **RE**- Resting egg.

Plate 28

Stage I (0.176 mm)



Stage II (0.180 mm)

Stage III (0.182 mm)

Stage IV (0.184 mm)

Stage-V (0.216 mm)

Stage-VI (0.220 mm)

Stage-VII (0.238 mm)

Stage-VIII (0.262 mm)

Stage-IX (0.296 mm)

Macrothrix triserialis Embryonic development

Ilyocryptus spinifer

Fig. 15 a Relationship between Total length (TL), Carapace height (CH) and instar number

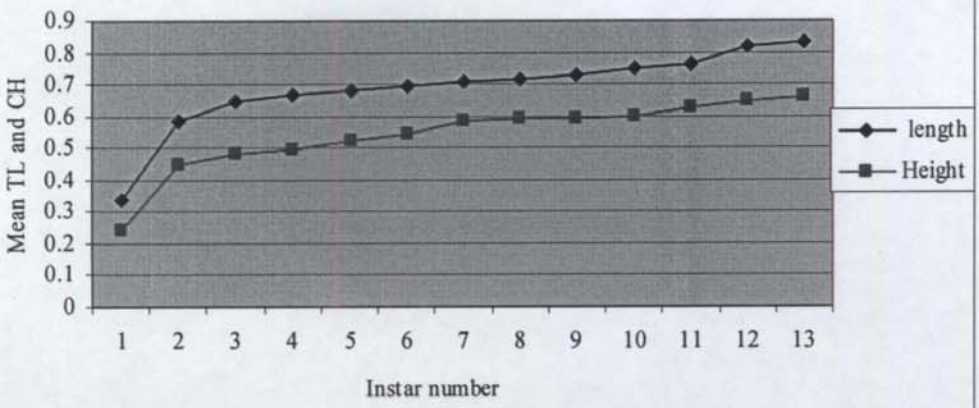


Fig. 15 b Egg production in relation to instar number

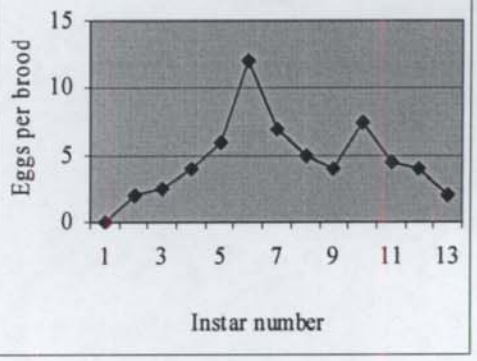


Fig. 15 c Cumulative egg production related to adult instar number

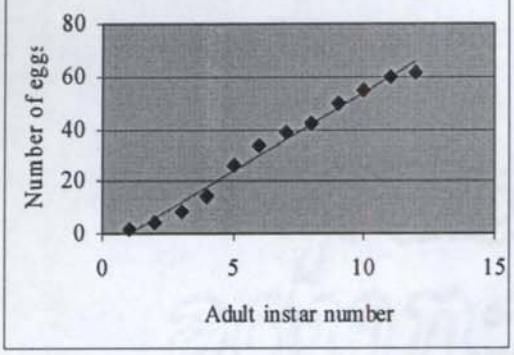
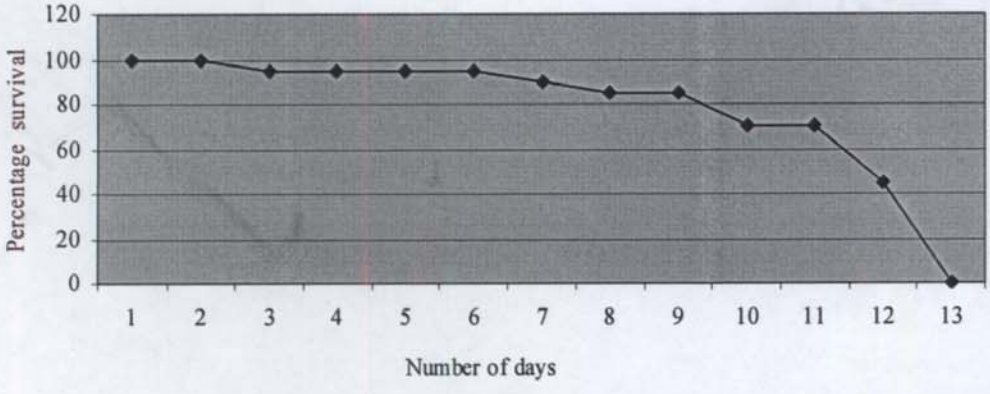


Fig. 15 d Survivorship curve



Macrothrix triserialis

Fig.16 a Relationship between TL, CH and instar number in female

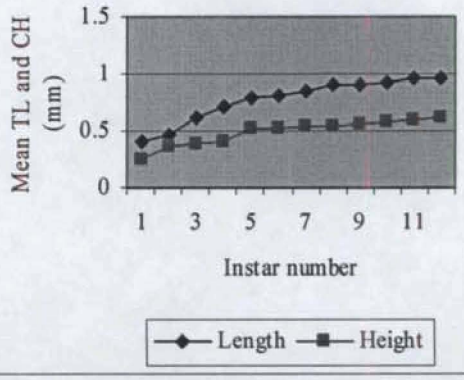


Fig. 16 b Relationship between TL, CH and age in male

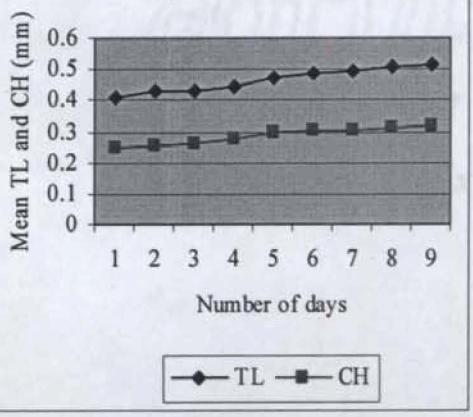


Fig. 16 c Egg production in relation to instar number

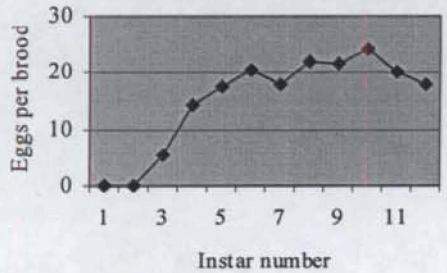


Fig. 16. d. Cumulative egg production related to adult instar number

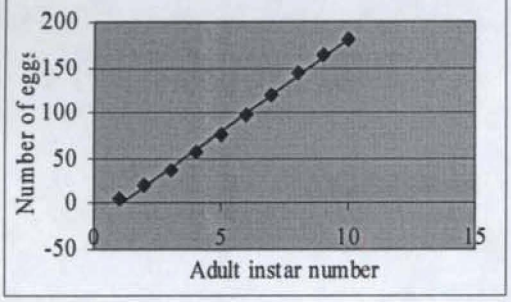


Fig. 16 e Survivorship curve

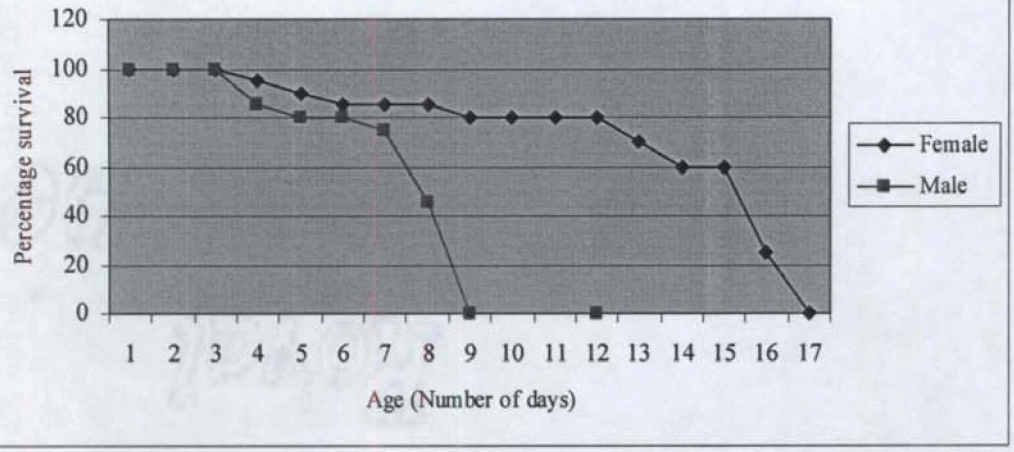


Fig. 17 Population growth in *M. triserialis*

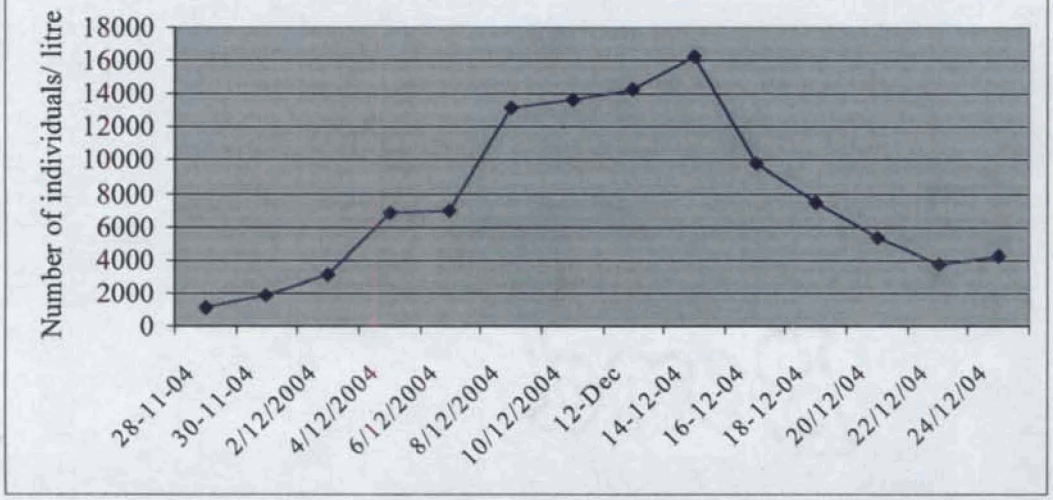


Fig. 18 a Growth increment in Macrothricidae

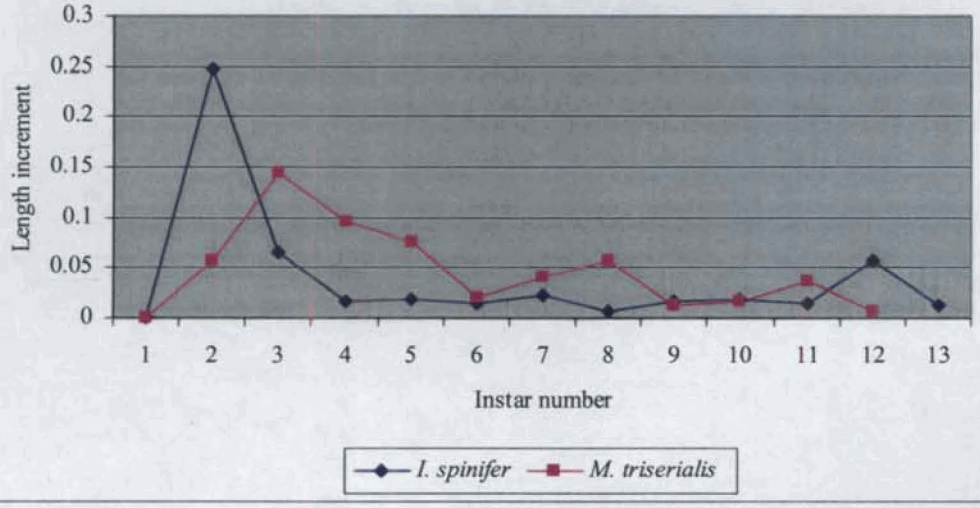
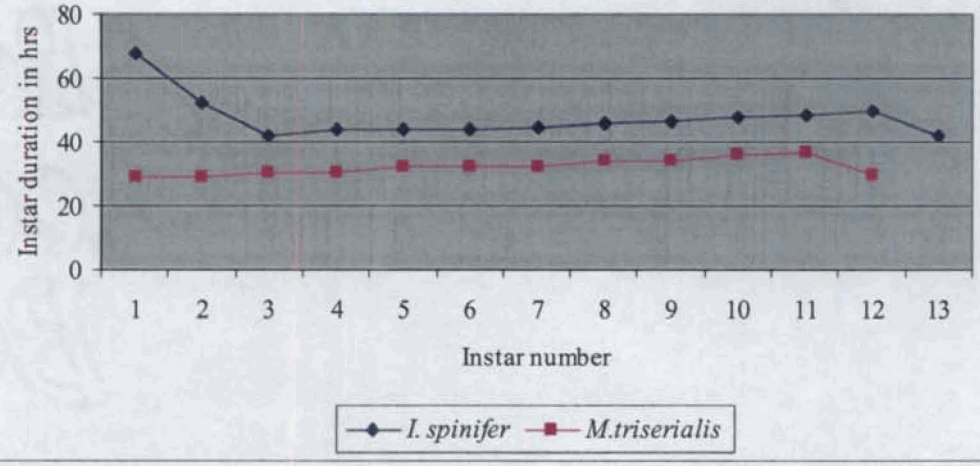


Fig. 18 b Instar duration in Macrothricidae



Family Chydoridae Stebbing, 1902

5. 8 *Alona pulchella* King, 1853

Alona pulchella, a common littoral species which occurs in ponds, paddy fields, streams and reservoirs (Idris, 1983). This species was reported first in India by Petkovski (1966) from Gujarat. Subsequent reports are from Rajasthan (Nayar, 1971); West Bengal (Sharma, 1978); Tamil Nadu (Michael and Sharma, 1988) and Andhra Pradesh, (Durga Prasad and Padmavathy, 2003a).

5. 8. 1. External Morphology

Parthenogenetic female (Plate 29. Fig. A)

Body oval. The valves punctuate, without longitudinal striations, posterodorsal and posteroventral corner rounded; ventral margin with series of setae (Plate 30. Fig. H). Eye distinct; ocellus slightly smaller than the eye, situated half way between eye and tip of the rostrum. Antennules small, not reaching the tip of the rostrum (Plate 30. Fig. F). Labrum with convex anterior margin (Plate 30. Fig. G). Postabdomen with parallel dorsal and ventral edges; with 9 anal teeth, lateral setae arranged in 7 fascicles, distalmost seta being the longest; claw with basal spine, about 1/3rd the length of claw (Plate 30. Fig. I). Mean size: 0.438 × 0.258 mm.

Male (Plate 29. Fig. B)

Smaller than female. Valves with almost parallel dorsal and ventral margins, posterodorsal and posteroventral corner rounded. Antennules stout and broad extending beyond the apex of rostrum with sensory setae (Plate 30. Fig. J), First thoracic leg with a copulatory hook. Postabdomen slightly narrowing distally, with groups of lateral setae; claw with long basal spine. Mean size: 0.350 × 0.225 mm

Ephippial female (Plate 29. Fig. C)

Ephippial female similar to parthenogenetic female; but with distinct black pigmentation. Ephippium single, attached to carapace with single egg placed in its upper half and shed along with a major portion of the valve (Plate 29. Fig. D). Egg single, oval and brown coloured. Mean size of ephippial female: 0.475 × 0.291 mm. Mean size of ephippium: 0.421 mm.

5. 8. 2 Reproduction

Males and Ephippial females were produced in the laboratory culture throughout the period of study. However the parthenogenetic females dominated the culture. The events in the life cycle of parthenogenetic female are described below.

Life cycle of parthenogenetic female

The population obtained in the collection from Site 6 comprised of a good number of adult parthenogenetic females and ephippial females. The

adult females were found to bear only 2 eggs. The egg bearing females were sorted out for the laboratory culture.

The population developed in the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. A good number of males and ephippial females appeared in the culture throughout the period of study. This indicates the frequent occurrence of sexual reproduction in this species. However, the parthenogenetic females dominated the culture. The life cycle of *A. pulchella* is described below.

Pre-adult instar

The neonates produced from parthenogenetic females had a mean birth size (SaB) of 0.291×0.175 mm. Both the first and second moulting occurred in a uniform duration of 23.5 hrs. The total pre-adult instar duration was 47.0 hrs and mean PID was 23.5 hrs.

Attainment of maturity

The ovary was clearly seen at 26±1 hrs during the 2nd instar. They started to bear eggs after completion of 2nd moult at 47.0 hrs; and hence the AFR was 1.96 days. The SFR was 0.425× 0.250 mm.

Egg production

The eggs were deposited into brood pouch after completion of two moults. The eggs measured a mean size of 0.166×0.096 mm. During this primiparous instar (3rd instar) egg production started with an average of

2.0 eggs/ brood. The primiparous instar was completed in 26.5 hrs and the FGT is calculated as 73.5 hrs.

During the subsequent instars there was a steady production of 2.0 eggs/ brood. This trend of egg production continued throughout the life span upto the 12th instar (Table 34). The female underwent moulting towards the end of each instar.

Fecundity

The relationship of egg production with instar number is represented in Fig. 19 b. A constant clutch size of 2 eggs/ brood was produced throughout the early adult instars with 1.45 eggs/ day of adult life. The cumulative number of eggs produced (Σmx) during the entire life span was 20.0 (Table 34). Ten broods were produced during the entire life.

The Σmx is linearly correlated with instar number Fig. 19 c. The rate of egg production (REP) of *A. pulchella* is calculated as 1.923.

5. 8. 3 Growth

The first pre-adult neonates had a mean TL of 0.325 mm and second instar had TL of 0.350 mm. Primiparous stage was attained during the 3rd instar when the mean TL was 0.425 mm. The maximum mean TL of 0.525 mm was attained at the end of 12th instar (Table 35).

The mean CH during the first pre-adult instar was 0.183 mm and attained CH of 0.204 mm at second instar. They attained a mean CH of 0.250 mm during the primiparous condition. Maximum CH was attained in 12th

instar with CH of 0.328 mm (Table 35). During the life span each individual has undergone two pre-adult and ten adult moults.

The relationship between TL, CH and instar number of *A. pulchella* has been represented in Fig. 19 a. The correlation coefficients of the life history characters are given in Table 36, which shows positive correlation between TL and CH ($r = 0.996$).

The increment of TL and CH during each instar is given in Table 35. Maximum growth increment recorded during the life cycle was in 3rd instar with TL of 21.42% and CH of 22.54% respectively. The most significant decrease in growth increment occurred after 7th instar.

5. 8. 4 Life Span and Survivorship

The survivorship curve (Fig. 19 d) indicates the relationship of age (days) and percentage survival of *A. pulchella.*

The rate of survival was higher near the age of maturity and declined further a few days after maturity. The maximum life span (Lmax) observed during the present study was 15.75 days.

5.9 *Oxyurella singalensis* (Daday, 1898)

Oxyurella singalensis is a littoral cladoceran inhabiting in all types of freshwater habitats especially ponds, paddy fields and marshes. The name *Oxyurella* was coined by Dybowski and Grochowski (1894). The first description of this species is by Daday (1898).

In India, this species is reported first by Sharma (1978) from West Bengal and by Michael and Sharma (1988) from Kerala. A study on the biology of this littoral species is lacking, and hence the present study of the life history of male and parthenogenetic female has been made.

5. 9. 1 External Morphology

Parthenogenetic female (Plate 31. Fig. B)

Body evenly rounded, posterior margin convex, maximum height in the middle. Head shield broad in the middle, narrowly rounded anteriorly and broadly rounded posteriorly. Rostrum blunt. Ocellus smaller than eye, situated closer to the eye than to the apex of rostrum. Plate of labrum rounded with slightly acute apex (Plate 30. Fig. A). Valves with almost straight ventral margins, with a series of setae and setules along the posteroventral corner. Antennules short, not reaching the apex of rostrum. Antennae with 3-segmented dorsal and ventral ramus. Antennary setation: (0-0-3)/(1-1-3). Postabdomen slightly tapering distally, with 12 sharply pointed anal denticles which decrease in size proximally; distinct preanal and postanal corners,

distal corner rounded; claw long, curved dorsally, setules on the concave surface; basal spine long about ½ the length of claw and an additional small spine at the base of the claw (Plate 30. Fig. B). Mean size: 0.733 × 0.475 mm.

Male (Plate 31. Fig. C)

Valves with rounded posterodorsal and posteroventral corner; ventral margin with convexity in the middle; short marginal setae before convexity and longer setae behind it (Plate 30. Fig C). The first thoracic leg bears a curved blunt hook (Plate 30. Fig. D). Postabdomen almost uniformly wide, but slightly narrowed distally; anal spines confined mostly to rounded dorsal end (Plate 30. Fig. E). Mean size: 0.583 ×0.341 mm.

Ephippial female (Plate 31. Fig. D)

The ephippial female in external appearance resemble parthenogenetic females. The shell does not bulge outward. The ephippium is transparent, light yellow coloured. The egg single, dark brown in colour, and occupied almost at the centre of ephippium surrounded by a foamy mass. Ephippium cast of along with a major portion of the carapace (Plate 31. Fig. E). Some of the ephippium was cast off without eggs within it (Plate 31. Fig. F). Mean size of female: 0.670 mm. Size of ephippium: 0.570 mm.

5. 9. 2 Reproduction

Males were present throughout the period, while the ephippial females made their appearance only occasionally. However, the parthenogenetic females dominated the culture. The important events in the life cycle of male and female are given below.

Life cycle of Male

The males of *O. singalensis* were reported first from India by Michael and Sharma (1988). The males were produced in the laboratory throughout the period of present study. Twenty neonates of less than 12 hrs of age were sorted out and reared individually for life cycle studies following the methodology cited in section 3. 2. 3.

The male neonates had a mean birth size (SaB) of 0.437×0.269 mm. The first moulting was at 96.0 hrs after birth. There was no moulting further and they attained maturity. The sexually mature individuals could be recognized by their yellow coloured testis. After the first moulting they attained mean TL of 0.516 mm and CH of 0.320 mm (Table 40). The maximum percentage increment of CH was observed on 4th day with 6.67%. The size increments decreased subsequently (Table 40).

The relationship between TL and CH are represented in Fig. 20 b. Maximum size attained during the study was 0.560×0.344 mm on 23rd day. The mean life span of male is calculated as 9.37 days while the maximum life span observed during the present study was 22.79 days.

Life cycle of parthenogenetic female

The population developed during the laboratory culture comprised asexually reproducing females, ephippia bearing females and males. The parthenogenetic reproduction occurred throughout the period. The features characteristic of the life cycle is given as follows.

Pre-adult instar

The neonates produced from the parthenogenetic females had a birth size (SaB) of 0.492×0.318 mm. The first moulting occurred after duration of 36.0 hrs; while the second moulting occurred at an interval of 38.5 hrs. The total pre-adult instar duration of *O. singalensis* was 74.5 hrs. The mean duration (PID) is 37.25 hours.

Attainment of maturity

The ovary was conspicuous at 40±2 hrs towards the beginning of 2nd instar (Plate 31. Fig. A). However, they attained maturity after the second moult and started to bear eggs at 74.5 hrs (Table 37), and hence the AFR was 3.10 days. They attained a mean SFR of 0.544×0.400 mm during 3rd instar.

Egg production

The eggs were deposited into the brood pouch within 15.0 minutes after the second moult. During this primiparous instar (3rd instar) egg production started with 2.0 eggs/ brood. Soon after deposition into brood pouch the eggs attained a triangular shape. The primiparous instar was completed in a mean duration of 40.2 hrs and the first generation time (FGT) is calculated as 114.7 hrs.

During the succeeding instars each clutch comprised of 2.0 eggs/ brood. This steady egg production continued until 15th instar without any peak (Fig. 20 c). They continued moulting and egg production until the penultimate instar (16th). However, there was no egg production in the last two instars. The relationship of instar duration and instar number is represented in Fig. 22.

Fecundity

The relationship of egg production with instar number is represented in (Fig. 20 c). The females produced a constant number of eggs throughout the adult instars with 0.70 eggs/day of adult life.

The cumulative number of eggs produced (Σmx) during the entire life span was 26.0 (Table 37). Thirteen broods were produced during the entire life with an average of 2.0 eggs /brood. The cumulative number of eggs produced Σmx is linearly correlated with instar number (Fig. 20 d). The rate of egg production (REP) of *O. singalensis* is 1.848.

5. 9. 3 Growth

The first pre-adult neonates had a mean TL of 0.504 mm and 2nd instar had TL of 0.520 mm. Primiparous stage was attained during the 3rd instar when the mean TL was 0.544 mm. The maximum mean TL of 0.824 mm was attained at 14th instar, and no increase further upto 17th instar (Table 38).

The mean CH during the first pre-adult instar was 0.328 mm and attained CH of 0.344 mm at 2nd instar. A mean CH of 0.400 mm was attained during the primiparous condition. The maximum CH was attained in 14th instar with a mean CH of 0.536 mm and no increase further (Table 38). During the life span each individual has undergone two pre-adult and fifteen adult moults.

The size increments during each instar are represented in Table 38. Maximum growth increment recorded during the life cycle was with TL (23.52%) and CH (16.27%). The relationship between TL, CH and instar

number of *O. singalensis* female is represented in Fig. 20 a. The correlation coefficients of the life history characters are given in Table 39.

5. 9. 4 Embryonic Development

The stages of embryonic development of *O. singalensis* are represented in Plate 32. The most conspicuous features of the developmental stages are given below.

Stage I: This stage is recognized by the presence of oval egg with yellow colour. Mean duration: 1.5 hrs. Mean size: 0.223 mm

Stage II: This stage is recognized by the presence of triangular-shaped egg with dark-brown colour. Mean duration: 3.0 hrs. Mean size: 0.20 mm.

Stage III: The embryo shows two distinct zones. The outer area becomes transparent while the inner area is granular with a yellow coloured fat globule. Mean duration: 8.4 hrs. Mean size: 0.234 mm.

Stage IV: The head lobe starts to develop. The yolk and fat globules get concentrated in the middle. Mean duration: 6.0 hrs. Mean size: 0.256 mm.

Stage V: During this stage the head lobe become more distinct. The cellular divisions are clearly visible. Mean duration: 5.6 hrs. Mean size: 0.268 mm.

Stage VI: The cellular divisions of anterior region are completed with the development of rudiments of head and antennae. Mean duration: 3.5 hrs. Mean size: 0.286 mm.

Stage VII: This stage could be recognized by the presence of eye, antennules and antennae. Mean duration: 9.5 hrs. Mean size: 0.294 mm

Stage VIII: During this stage the development of head and postabdomen is completed. The eye and ocellus become more conspicuous, thoracic legs are developed and the embryo resembled the adult in external morphology. Mean duration: 2.5 hr. Mean size: 0.326 mm.

Release of neonates

Embryonic development of primiparous instar of *O. singalensis* was completed in a mean duration of 40.0 hrs. At the end of each adult instar two neonates were released from the brood pouch of the mother by the movement of of female postabdomen followed by moulting.

Moulting

The moulting was completed in two steps. First, the carapace is separated from the body at the region of head shield. This is followed by the separation of the exoskeleton of the head shield and postabdomen. The old carapace is shed as exuvium which gets detached from the animal and sinks to the bottom.

5. 9. 5 Life span and Survivorship

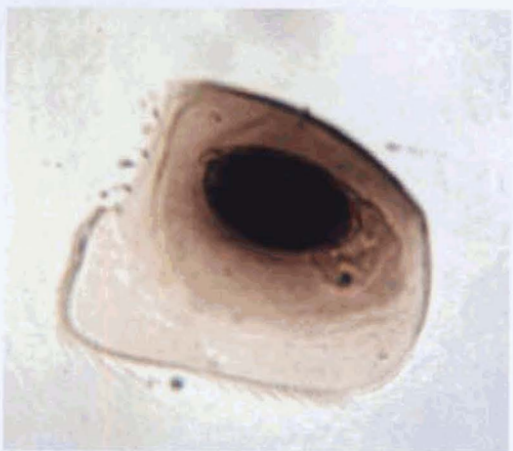
Fig. 20. e represents the survivorship curve of *O. singalensis*. The mean life span (Σlx) of female is 17.14 days, while the maximum life span (Lmax) observed in the present study was 40.0 days (Table 38).

Plate 29

A

B

C

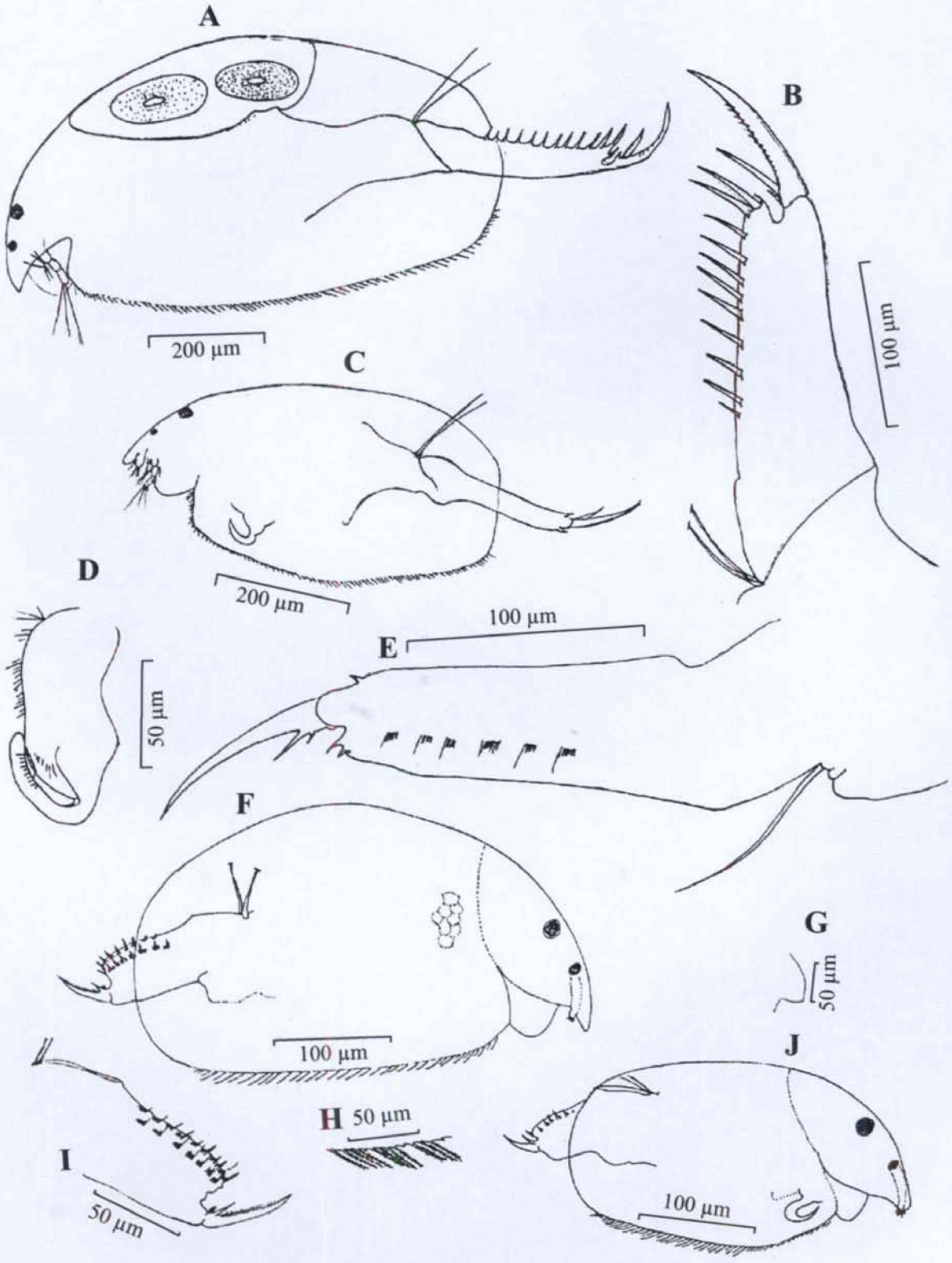


D

Alona pulchella

Fig. **A**. Parthenogenetic female (0.443 mm), **B**. Male (0.348 mm), **C**. Ephippial female (0.468 mm), **D**. Ephippium (0.426 mm).

Plate 30



Oxyurella singalensis (Daday) Fig.**A**. Female. **B**. Postabdomen of female, **C**. Male, **D**. 1st Thoracic leg, **E**. Postabdomen of male.
Alona pulchella King **F**. Female. **G**. Plate of labrum. **H**. Posteroventral margin of shell, **I**. Postabdomen of female, **J**. Male.

Plate 31

A

B

C

D

E

F

Oxyurella singalensis

Fig. **A**. Pre-adult (0.524 mm), **B**. Parthenogenetic female (0.735 mm), **C**. Male (0.580 mm), **D**. Ephippial female (0.677 mm), **E**. Ephippium with egg (0.539 mm) **F**. Ephippium without egg (0.548 mm).

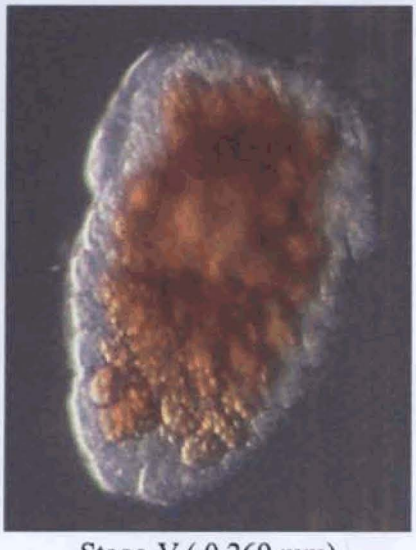
Plate 32

Stage-I (0.226 mm)

Stage-II (0.230 mm)

Stage-III (0.232 mm)

Stage-IV (0.258 mm)



Stage-V (0.269 mm)

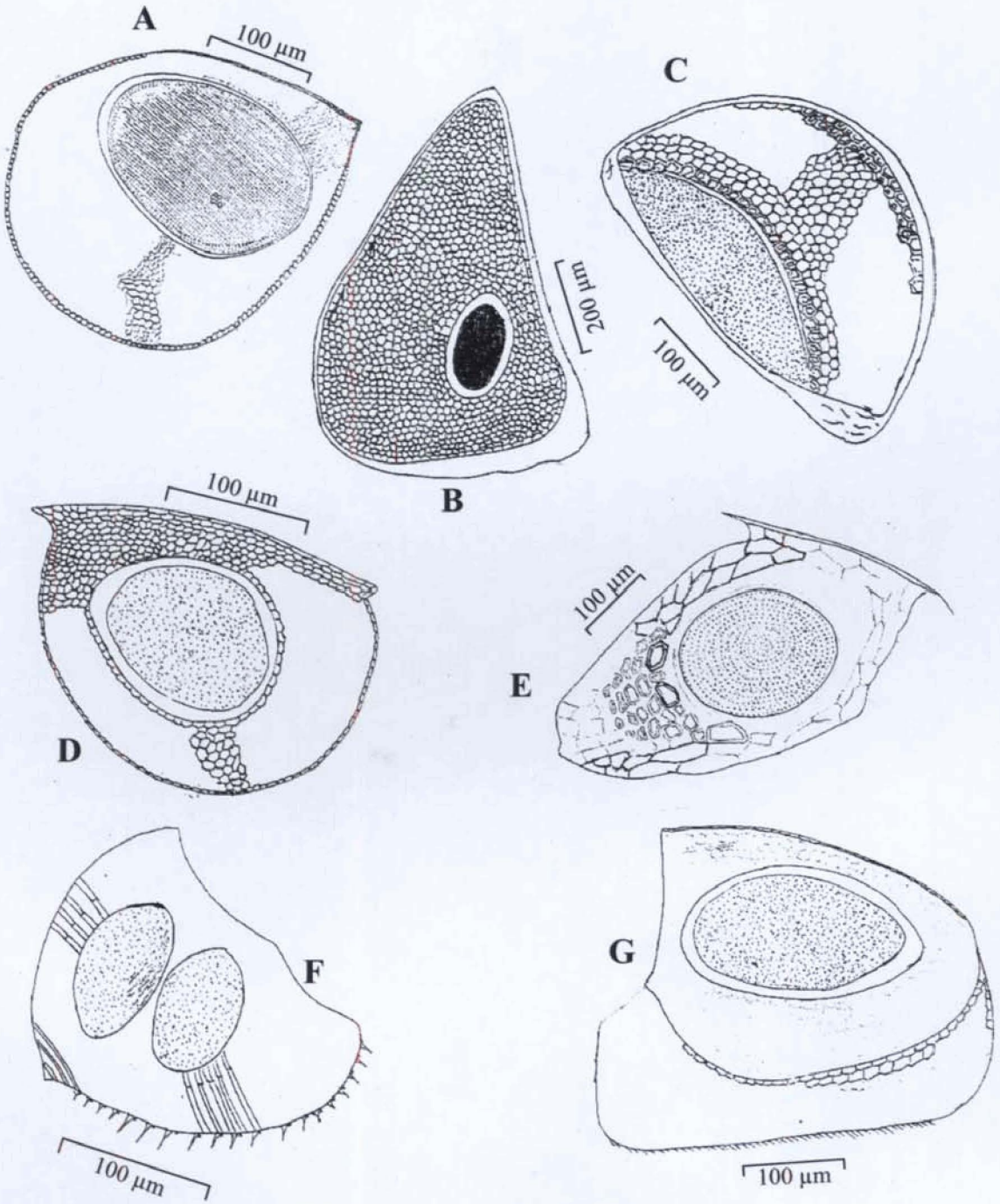
Stage-VI (0.285 mm)

Stage-VII (0.298 mm)

Stage VIII (0.340 mm)

Oxyurella singalensis Embryonic development

Plate 33



Ephippial Morphology

Fig. **A**. *Ceriodaphnia cornuta*, **B**. *Simocephalus serrulatus*, **C**. *Scapholeberis kingi*, **D**. *Moina brachiata*, **E**. *Moinodaphnia macleayi*, **F**. *Macrothrix triserialis*, **G**. *Alona pulchella* (In Figs. A, C, D, F and G ornamentation is shown only partly)

Alona pulchella

Fig. 19 a Relationship between Total length (TL), Carapace height (CH) and instar number

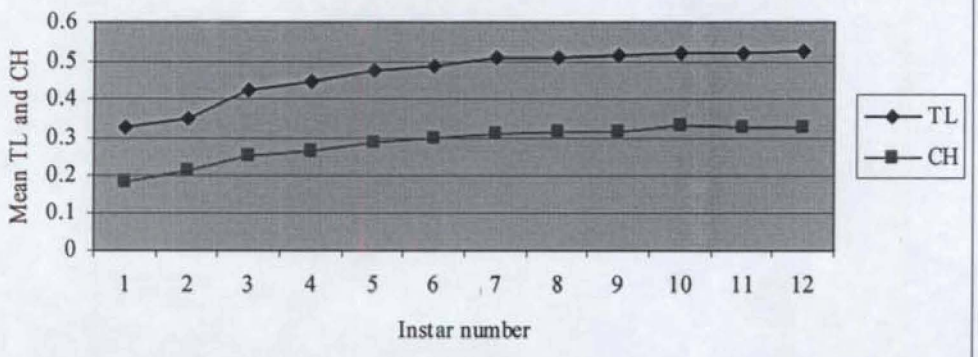


Fig. 19 b Egg production in relation to instar number

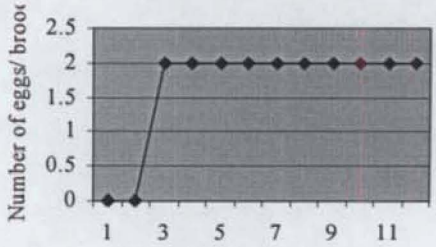


Fig. 19 c Cumulative egg production related to adult instar number

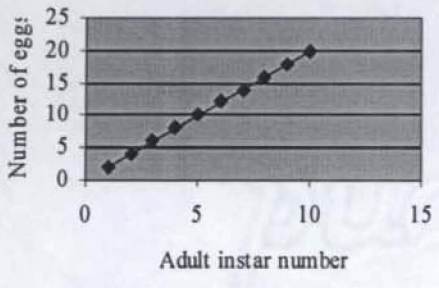
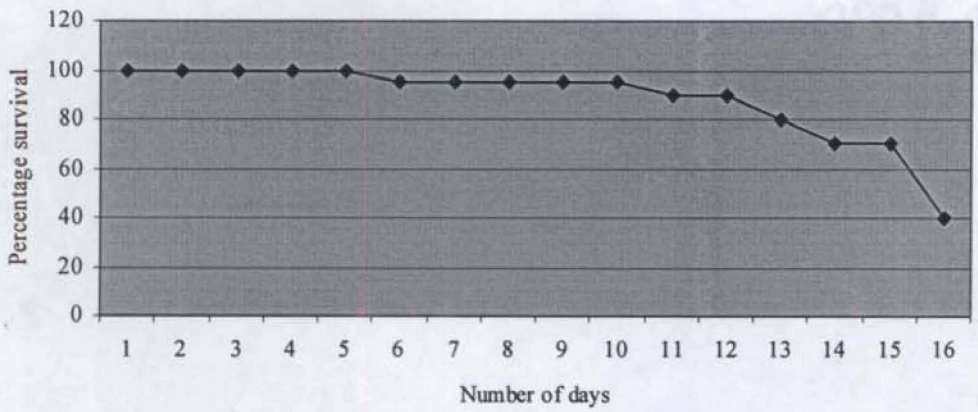
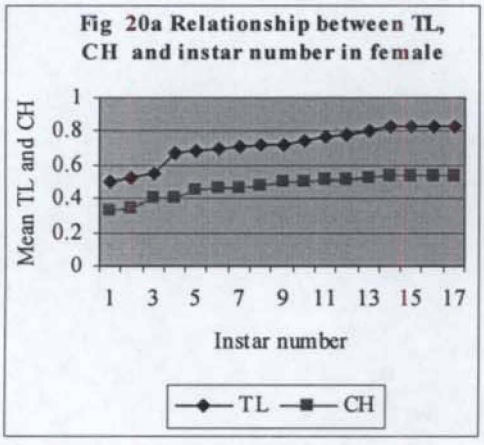
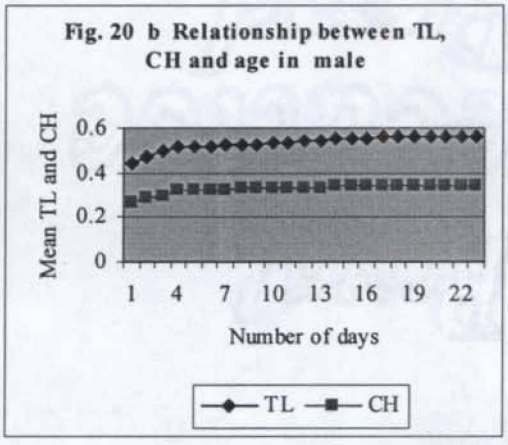


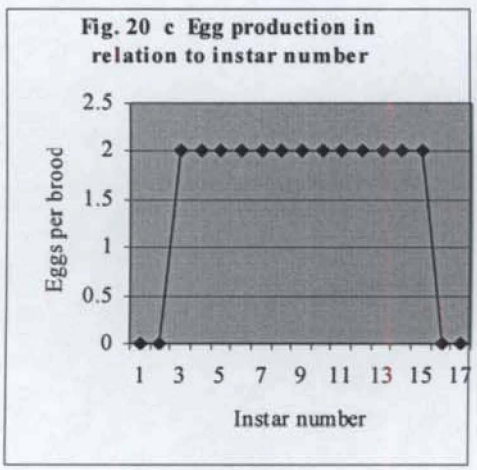
Fig. 19 d Survivorship curve

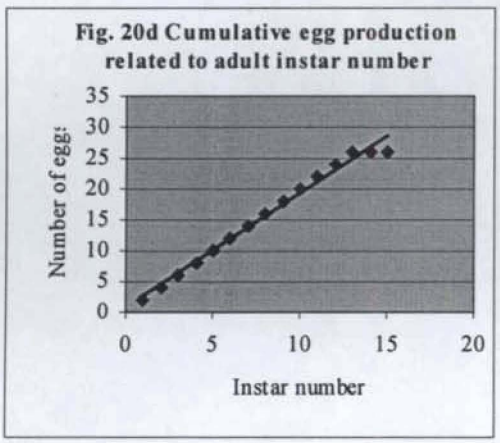


Oxyurella singalensis









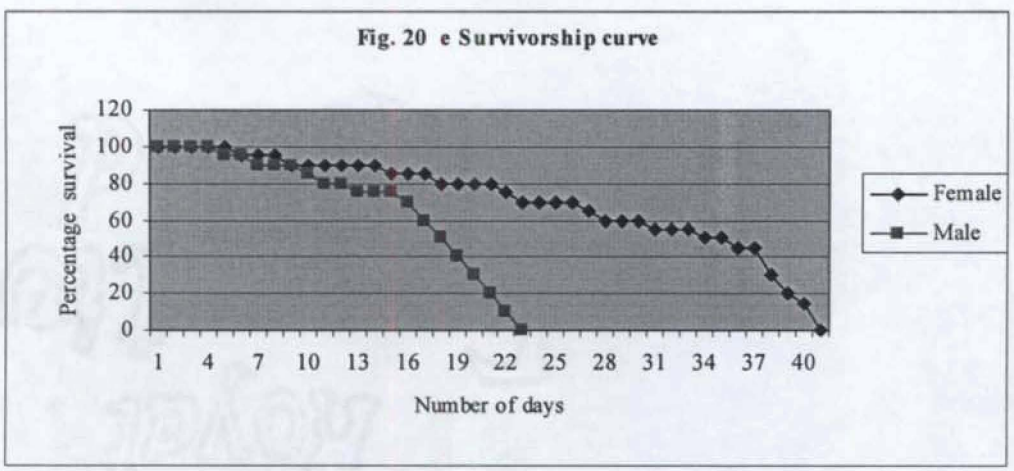


Fig. 21 Growth increment in Chydoridae

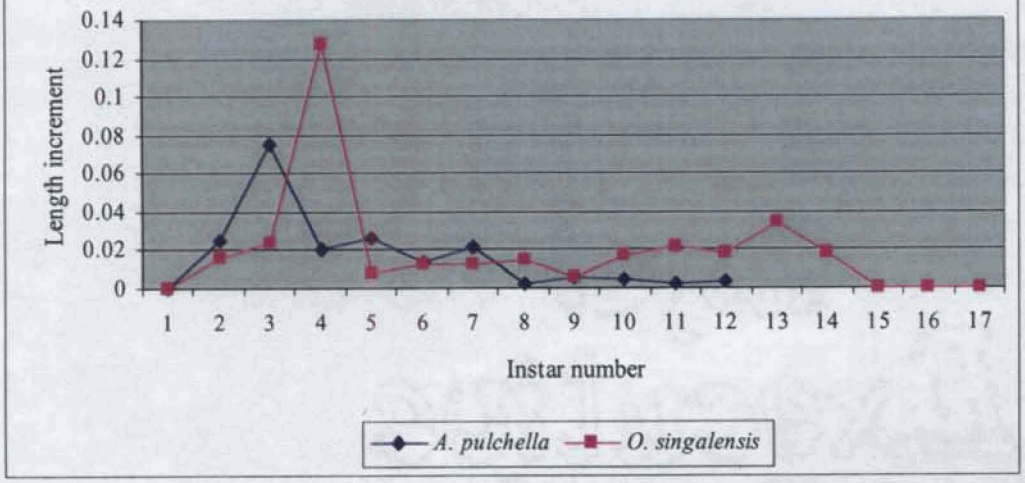
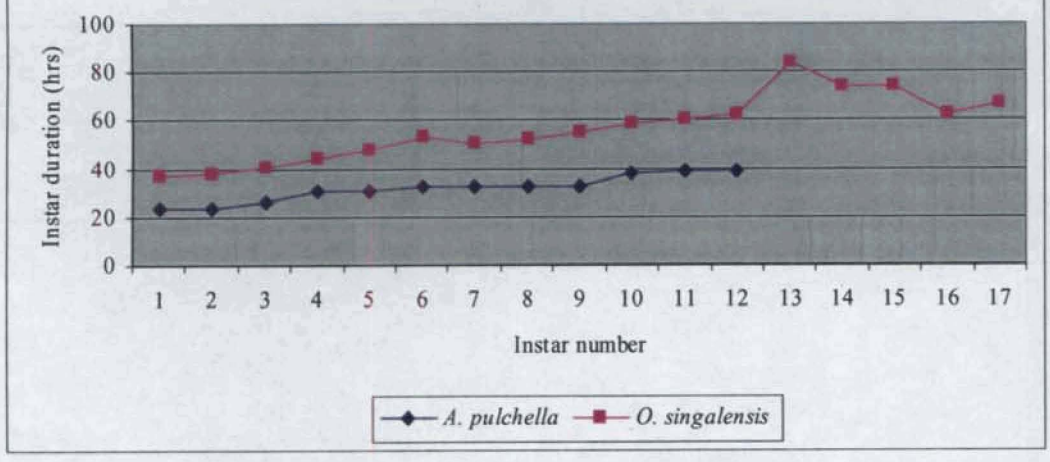


Fig. 22 Instar duration in Chydoridae



DISCUSSION

Britto Joseph. K “Studies on the biology of freshwater Cladocera: Crustacea ” Thesis. Department of Zoology, St. Thomas College Thrissur, University of Calicut, 2007

Chapter 6

DISCUSSION

DISCUSSION

Although, India is potentially rich in cladoceran fauna, both numerically as well as in diversity, information on biology of Indian Cladocera is meager. The early researchers of our country chiefly made systematic studies. The monograph on "Indian Cladocera" by Michael and Sharma (1988) is an important contribution in this field. Information on the biology of Indian Cladocera is limited to the studies of a few species, and a good number of which are restricted to species from Tamil Nadu. But, there are only a few published reports of investigations on biology from Kerala (Thresiamma *et al.* 1991; Babu and Nayar, 1993, 1997).

Based on the observations from field study made in different habitats of Thrissur district, Kerala; it is evident that cladocerans form an important group in the temporary as well as permanent water bodies. The total number of cladoceran species so far reported from Kerala is 35 (Babu and Nayar, 2004; Babu and John, 2007). In the present study 19 freshwater cladoceran species were collected from different freshwater bodies of this locality, of which 12 species belonging to five families viz. Family Sididae, Daphniidae, Moinidae, Macrothricidae and Chydoridae have been selected for biological studies by rearing them in the laboratory.

Out of the 12 species investigated, the biology of 9 species: *Diaphanosoma sarsi* Richard, *Pseudosida bidentata* var. *szalayi* (Daday), *Latonopsis australis* Sars, *Moina brachiata* (Jurine), *Moinodaphnia macleayi* (King), *Ilyocryptus spinifer* Herrick, *Macrothrix triserialis* (Brady), *Alona pulchella* King and *Oxyurella singalensis* (Daday) has been studied for the first time from our country. The biology of *Ceriodaphnia cornuta* Sars, *Scapholeberis kingi* Sars and *Simocephalus serrulatus* (Koch) has also been investigated to compare with earlier reports. The studies made on the life cycle of males of 4 cladoceran species: *Pseudosida bidentata, Moinodaphnia macleayi, Macrothrix triserialis,* and *Oxyurella singalensis* is a new contribution to the cladoceran biology.

In the present study the samples collected from the field were dominated by parthenogenetic females while the ephippial females and males were scarcely represented. Out of the 19 species collected for the present study, the males and ephippial females were obtained from the natural habitat only in *D. lumholtzi* and *D. sarsi*. However, ephippial females were produced under laboratory conditions in all the 12 species studied. In the laboratory culture males were found to be produced in 10 species.

The cladocerans studied herein were reared in laboratory simulating the natural condition of 12 hrs light: 12 hrs dark photoperiod. Stock culture of each species was developed and maintained by providing *Chlorella* as food. All the studies were made in the laboratory where the water temperature

varied from 26 to 30°C and pH from 6.2-6.8. The life history studies were done after isolating the animals from this stock culture; and their neonates were individually reared in separate glass vessels providing similar culture conditions.

The cladocerans as any other crustaceans also grow by moulting. During moulting the inner part of carapace is reabsorbed and a new one is developed below the old one which is shed as exuvium (Dodson and Frey, 1991). In the present study cladocerans viz. *D. sarsi*, *P. bidentata*, *L. australis*, *C. cornuta*, *S. kingi*, *S. serrulatus*, *M. brachiata*, *M. macleayi*, *M. triserialis*, *A. pulchella*, and *O. singalensis* underwent moulting towards the end of each instar while moulting was not observed in *Ilyocryptus spinifer*. The specimens of *I. spinifer* collected from the field were always found to be encrusted with detritus and sand particles. In the laboratory, however the carapace was found profusely covered with algae (*Chlorella*). The presence of encrustations both in the field and in the laboratory probably indicates camouflage as a protective mechanism. Absence of true moulting enables these animals to retain its old carapace without renewing the encrustation. Fryer (1974) has also observed encrustation of detritus in the body of *Ilyocryptus sordidus*. Both these species are bottom dwelling detritus feeders and the camouflage is possibly an adaptation to escape from enemies.

The number of pre-adult instars of parthenogenetic females recorded during the present study is given in Table 45. In *C. cornuta* 2 pre-adult instars are recorded presently which is in conformity with that of earlier investigators like Murugan (1975b) and Babu and Nayar (1993). In *S. serrulatus* 3 pre-adult instars recorded during the present study is also in conformity with Babu and Nayar (1997). The presence of 2 pre-adult moults in *S. kingi* as observed in the present study is also in agreement with the observations made by Murugan and Sivaramakrishnan (1976). The present study indicates that the number of pre-adult instars is constant for a species, hence can be of taxonomic value in confirmation of identification. However, the number of adult instars is found to vary from species to species based on culture conditions. For example, in *C. cornuta* the number of adult instars recorded is 9 by Michael (1962), 18 by Murugan (1975b), 25 by Kanaujia (1982), 12 by Babu and Nayar (1993) and 15 in the present study.

Kanaujia (1982) suggested the possibility of the influence of temperature and food in determining the number of adult instars. Since the present study as well as the studies made by Murugan and Sivaramakrishnan (1976), Murugan and Job (1982) and Babu and Nayar (1993, 1997) were conducted in similar tropical conditions, the temperature can not be taken as a single factor in influencing the number of adult instars. However, the quantity and quality of the available food can be a possible factor in influencing the growth and moulting.

The present investigations indicate that the primiparous instar is distinctly longer than any pre-adult instars in all the species studied except in *I. spinifer* (Table 42). The primiparous instar duration is almost double the pre-adult instar in *M. brachiata*. Longer primiparous instar duration has also been reported earlier in *D. carinata* by Navaneethakrishnan and Michael (1971), in *S. acutirostratus* by Murugan and Sivaramakrishnan (1973) and in *D. senegal* by Venkataraman and Krishnaswamy (1985). In the light of above observations longer primiparous instar duration could be considered a general feature of the cladoceran life cycle.

Another general feature of the cladoceran life cycle is the gradual increase in the duration of successive adult instars (Table 1, 5, 8, 11 and 17). Hutchinson (1967) also proposed a similar view that the time of brooding depends on the length of instar and increases progressively with age. In *Simocephalus acutirostratus,* Murugan and Sivaramakrishnan (1973) observed that the adult instars are of longer duration than the pre-adult. Sharma and Sharma (1989) also noticed an increasing trend during the successive adult instars of *Simocephalus exspinosus*.

Earlier investigators like Anderson *et al.* (1937), Ingle *et al.* 1937, Anderson and Jenkins (1942) have considered temperature a probable factor in determining the duration of instars. Kanaujia (1982) suggested that in *C. cornuta* increase in instar duration at low water temperature could be one of the factors for producing more number of eggs per brood where females

get more time to produce and accumulate yolk with required quantity of food. Vijverberg and Richter (1982) have also reported that instar durations of cladocerans are mainly affected by temperature and to a much lesser degree by food conditions.

In the members of the Family Daphniidae, Sididae, Moinidae and Macrothricidae the egg production is found to increase gradually from the primiparous instar and attained a peak during the adult instars and then declined till the end of life span. However, in *A. pulchella* and *O. singalensis* (Chydoridae) the number of eggs produced in each clutch was always found to be two (Tables 34 & 37). Similar pattern of egg production has also been reported earlier in another chydorid, *Leydigia acanthocercoides* by Murugan and Job (1982) where also the number of eggs produced was two. The constancy in the number of eggs produced can be considered a characteristic feature of chydorids.

Bottrell (1975) and Wetzel (1975) have suggested that the life of chydorids in a stable littoral environment may be the reason for the production of a constant clutch size. They pointed out that chydorids usually feed on the organic detritus present in the bottom of the littoral region throughout the seasons, so that there is no scarcity of food material at any time. They attributed this as the reason for the uniformity of clutch size in chydorids, unlike other species whose food supply is seasonal. The production of constant clutch size in chydorids observed in the present study

can also be attributed due to availability of sufficient food in the laboratory culture.

The total number of eggs produced by a cladoceran species is variable depending on culture conditions. For example, the cumulative number of eggs produced in C. *cornuta* is 63.0 (Table 11); while Babu and Nayar (1993) observed 67.3, Michael (1962) 42.0 and Murugan (1975b) 194.0. Similar observation is also made in *S. serrulatus* where the number of eggs was 151.3 (Table 17), while 384.5 eggs were recorded by Babu and Nayar (1997). This indicates that the total number of eggs produced during the life span is a variable character depending on culture conditions.

The positive correlation between the Total Length (TL) and Carapace Height (CH) as observed in the twelve species studied point out allometric growth as another general feature of cladoceran life cycle. In *C. cornuta, S. kingi* and *S. serrulatus* (Daphniidae) highest growth increment is recorded during the pre-adult instar followed by a decline in growth rate after the attainment of sexual maturity (Fig. 9). Maximum growth has also been recorded in the pre-adult instars by Green (1956) in temperate species of *Daphnia*. Early investigators like Weglensca (1971), Murugan (1975b), Murugan and Sivaramakrishnan (1976), Venkataraman (1981), Kanaujia (1988a; 1988b), Sharma and Sharma (1989) have also observed a higher growth rate during the pre-adult instars in Daphniidae. A similar trend is also observed in *P. bidentata, L. australis,* and *D. sarsi* (Sididae) (Fig. 4);

M. triserialis and *I. spinifer* (Macrothricidae) (Fig. 18 a) and in *A. pulchella, O. singalensis* (Chydoridae) (Fig. 21). Kryutchkova and Sladeck (1969) suggested that the fall in growth rate after commencement of egg production may be attributed to the energy requirement for reproductive activity.

One of the notable observations made in the present study regarding the embryonic development is the delayed deposition (13.0 to 14.0 hrs) of eggs into the brood pouch in *I. spinifer* compared with the time duration required in other species for egg transfer. The longer duration for egg development and its subsequent deposition into the brood pouch in *I. spinifer* could be attributed due to the absence of moulting which provides the animal more time for egg production. However, the deposition of eggs within a very short duration as observed in all other species is due to the presence of moulting. Green (1956) has reported that in mature *Daphnia* the parthenogenetic eggs are deposited into the brood pouch about half an hour after moulting.

The present study as well as earlier studies made by Green (1956) in *D. magna*, Murugan and Sivaramakrishnan (1973) in *S. acutirostratus*, Lie and Clifford (1974) in *Daphnia schodleri,* Murugan (1975a) in *M. micrura*, Murugan and Venkataraman (1977) in *D. carinata* and Sureshkumar *et al.* (1999) in *Pleuroxus aduncus* indicate that the stages of embryonic development follows a general pattern in Cladocera. However, the total

duration of embryonic development is relatively lower in tropical species (Murugan and Sivaramakrishnan, 1976).

A comparison of the life span of the parthenogenetic female shows a general phenomenon of lower duration (10.77 days) in *M. brachiata* (Table 45). Murugan (1975a) has also observed a shorter life span (13.0 days) in *M. micrura*. The shorter life span exhibited by *M. brachiata* and *M. micrura* can be attributed to their sporadic occurrence and swarming behaviour characteristic of the species occurring in temporary water bodies. This may be a survival strategy to build up population before adverse environmental conditions set in.

Edmondson (1955) has pointed out that certain species of Cladocera resorts to asexual reproduction alone. Byars (1960) and Michael (1962) have reported the absence of males in the natural population of *C. cornuta.* Kanaujia (1988a) and Babu and Nayar (1993) based on their laboratory studies have also suggested the absence of sexual reproduction in the life cycle of this species. However, Babu and Nayar (2004) reported the occurrence of males in the collection made from Periyar Lake. The appearance of males and ephippial females in the present laboratory culture of *C. cornuta* confirms the existence of sexual reproduction in this species. Therefore, *C. cornuta* is not an exception to the typical cladoceran life cycle.

Rarity of males in the natural population of Cladocera is a general phenomenon. In the present study also the males were scarcely represented in the collection made from natural habitats. Similar observations were also made by Chengalath (1982) and Frey (1987). However, the presence of males in the present laboratory culture can be attributed to the influence certain environmental factors. The appearance of males in the dense population of *M. triserialis* indicates the possibility of crowding as an environmental factor in inducing male production. Banta and Brown (1929) and Hutchinson (1967) have also suggested the effect of crowding on male production. Babu and Nayar (1997) while studying the life cycle of *S. serrulatus* observed the appearance of males when the population was at its peak.

Dodson and Frey (1991) has pointed out that the asexual formation of male is induced by some deterioration of the environment such as change in food concentration, crowding and decrease in photoperiod. Zhang and Baer (2000) observed male production in *D. magna* under reduced photoperiod (8 hrs dark and 16 hrs light) and lower feeding rates. Nayar and Babu (2002) also observed the appearance of males associated with the decline in the population of *S. serrulatus* indicating the possibility of scarcity of food as an environmental factor to induce male production. These studies point out the possible role of different environmental factors such as crowding, reduced photoperiod and change in food availability in inducing male production. This principle can be applied to produce males in the laboratory culture by

environmental manipulation. Further investigations are needed to identify the role of environmental factors in influencing male production.

Asexual formation of males from parthenogenetic females is a general feature of cladocerans. However, Dodson and Frey (1991) reported the appearance males in *Moina* from sexually derived resting eggs. In all the species studied including *M. brachiata* males are found to be produced asexually from parthenogenetic females and no males appeared when the resting eggs collected from the soil sample was hatched. It is likely that males are produced in *Moina* species from parthenogenetic females as well as from resting eggs. Further studies are required to confirm this.

Absence of moulting in the adult male is a general feature in Cladocera. The present study also shows absence of adult moulting as observed in *P. bidentata, M. macleayi, M. triserialis* and *O. singalensis*. Babu and Nayar, (1997) also observed absence of moulting during the adult instars in *S. serrulatus* males. From this it could be assumed that cladoceran males undergo moulting only during the pre-adult instars.

The appearance of ephippial females in all the species studied indicates the obligatory nature of ephippia in the cladoceran life cycle. The role of environmental factors in inducing male production and consequent ephippia formation has been studied by several workers. Banta and Brown (1939) have pointed out that ephippial production takes place when the food supply is low.

Banta and Wood (1939) and D'Abramo (1980) have pointed out the role of scarcity of food in inducing ephippial production. Michael (1962) and Kanaujia (1982; 1984) have reported the influence of crowding in the production of ephippial females in *C. cornuta* and *D. lumholtzi* respectively. Thresiamma *et al.* (1991) indicated that ephippial females made their appearance when the population attained its peak as a mechanism to minimize population explosion. Babu and Nayar (1997) and Nayar and Babu (2002) have observed the presence of large number of ephippial females in an over-crowded population of *S. serrulatus*. Berner *et al.* (1991) suggested that sexual reproduction in *Scapholeberis armata* could be induced with respect to seasonal changes particularly in short photoperiod and cool water.

The presence of several ephippia containing no eggs observed in the present laboratory culture indicates that this may be a general phenomenon in the cladoceran life cycle. The production of ephippium without egg as observed in six species viz. *P. bidentata, L. australis* (Plate 5. Fig. H), *C. cornuta*, *S. serrulatus* (Plate 16. Fig. F), *S. kingi* and *O. singalensis* (Plate 31. Fig. F) suggest the possibility of the occurrence of this phenomenon in most of the cladoceran species. This can be attributed to the failure of synchronization of the stimuli involved in the formation of egg and the ephippium. Goulden (1968) has observed in *Moina,* that the eggs and ephippia develop simultaneously and the ephippial formation is not depended on the fertilization of the eggs.

A comparative study of the morphological features of the ephippia points out their taxonomic importance and evolutionary trends. In the members of the Order Ctenopoda (*P. bidentata, L. australis* and *D. sarsi*) are with more than one ephippium and are shed without any attached part of the carapace (Plate 3. Fig. E; Plate 5. Fig. F). The members of the Order Anomopoda (*C. cornuta, S. kingi, S. serrulatus, M. brachiata, M. macleayi, M. triserialis*, *I. spinifer*, *O. singalensis* and *A. pulchella*) are with single ephippium which is cast off along with a part of the carapace (Plate 33. Figs. A-G). Among these the ephippia of *C. cornuta*, *S. serrulatus, S. kingi* (Daphniidae) and *M. brachiata*, *M. macleayi* (Moinidae) are cast off along with a part of posterodorsal carapace. In *M. triserialis*, *I. spinifer* (Macrothricidae), *O. singalensis* and *A. pulchella* (Chydoridae) a major part of the carapace including the posteroventral part remain attached with ephippia. The increased surface area due to the presence of carapace may help in buoyancy. Dodson and Frey (1991) have suggested that the ephippia float on the surface of water due to their specific gravity and hydrophobic nature of the carapace.

The ephippia are specialized structures produced by cladocerans to enable them survive in adverse environmental conditions and to assist dispersal. The different mechanisms involved in the dispersal of ephippia comprise presence of hooks and spines, air vacuoles, oil globules, light weight, sticky substances etc. The presence of spines on the ephippia of

M. triserialis and *I. spinifer*, the villi-like outgrowths over the surface of *D. sarsi* and the marginal spinules below the air vacuole in *L. australis* and *P. bidentata* are structures by which ephippia can adhere to any substratum especially aquatic plants and moving objects to enable dispersal. Fryer (1972) suggested that ephippia are dispersed by sticking to the feathers of birds. The sticky envelope present on the ephippial surface enables adherence of ephippia to plants which allows rapid dispersal through animals that feed on macrophytes (Korovchinsky, 1993, Vandekerkhove *et al.* 2005 b).

Goulden (1968) has also observed distinct reticulations in members of Family Moinidae. The present study as well as the studies made by other investigators indicates that the morphological features of ephippium especially the pattern of ornamentation may be diagnostic in species level. The ornamentation in the ephippium of *S. serrulatus, S. kingi, C. cornuta, M. brachiata, M. macleayi, M. triserialis* and *A. pulchella* are different (Plate 33. Figs. A-G). The ephippia of *M. macleayi* (Plate 21. Fig. G) and *I. spinifer* (Plate 23. Fig. H) have polygonal reticulations. Distinct ornamentations are observed in the ephippia of *P. bidentata* (Plate 3. Fig. F), *L. australis* (Plate 5. Fig. G) and *D. sarsi* (Plate 7. Fig. E). Korovchinsky (1995) have also observed distinct reticulations among different species of *Diaphanosoma.* These observations point out the importance of the morphological features of ephippia in taxonomy particularly in species identification.

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* Original article not seen

TABLES

Table 1
Mean length(TL), carapace height (CH) , eggs per brood, total eggs produced and instar duration in *Pseudosida bidentata* female

Instar No.	MeanTL (mm±SE)	Mean CH (mm± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.721±0.032	0.250±0.014	0		21.5	21.5
2	0.808±0.076	0.285±0.020	0		21.5	43.0
3	1.100±0.062	0.391±0.015	0		29.0	72.0
4	1.300±0.030	0.533±0.008	4.37	4.37	38.0	110.0
5	1.416±0.034	0.550±0.010	7.2	11.50	36.0	146.0
6	1.526±0.023	0.604±0.009	10.33	21.83	36.0	182.0
7	1.664±0.015	0.686±0.009	10.4	32.23	37.0	219.0
8	1.760±0.007	0.756±0.002	12.2	44.43	37.0	256.0
9	1.796±0.014	0.762±0.009	12.6	57.03	37.0	293.0
10	1.852±0.012	0.772±0.006	14.0	71.03	37.0	330.0
11	1.864±0.008	0.788±0.002	15.2	86.23	38.0	368.0
12	1.912±0.007	0.806±0.004	12.4	98.63	36.0	404.0
13	1.940±0.012	0.834±0.002	4.0	102.63	38.0	442.0
14	1.986±0.013	0.860±0.006	3.5	106.13	38.0	480.0
15	2.030±0.008	0.892±0.003	2.0	108.13	43.0	523.0
16	2.042±0.007	0.906±0.004	0	108.13	42.0	565.0
17	2.046±0.003	0.906±0.001	0	108.13	45.0	610.0

Table 2

Size increment in *Pseudosida bidentata* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.721			0.250		
2	0.808	0.087	12.06	0.285	0.035	14.00
3	1.100	0.292	36.14	0.391	0.106	37.19
4	1.300	0.200	18.18	0.533	0.142	36.32
5	1.416	0.116	8.92	0.550	0.017	3.19
6	1.526	0.110	7.77	0.604	0.054	9.82
7	1.664	0.118	7.73	0.686	0.082	13.58
8	1.760	0.116	6.97	0.756	0.07	10.20
9	1.796	0.036	2.05	0.762	0.006	0.79
10	1.852	0.056	3.12	0.772	0.01	1.31
11	1.864	0.012	0.65	0.788	0.016	2.07
12	1.912	0.048	2.58	0.806	0.018	2.28
13	1.940	0.028	1.46	0.834	0.028	3.47
14	1.986	0.046	2.37	0.860	0.026	3.12
15	2.030	0.044	2.22	0.892	0.032	3.72
16	2.042	0.012	0.59	0.906	0.014	1.57
17	2.046	0.004	0.20	0.906	0	0.00

Table 3

Correlation coefficients for 8 life history characters in *P. bidentata* female

	1	2	3	4	5	6	7	8
1.Total length (TL)	----	0.997	-----		* 0.337	0.966	0.906	0.930
2.Carapace height (CH)					* 0.294	0.962	0.907	0.945
3.Increment of TL				0.863	* -0.081	-0.882	* -0.233	* -0.574
4. Increment of CH					* -0.060	-0.646	* -0.067	* -0.442
5 Mean clutch size						* -0.357	* 0.223	* 0.027
6. Total egg production							0.590	0.968
7.Instar duration								0.823
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence.

Table 4

The mean length (TL), carapace height (CH) and percentage of growth increment/day in *Pseudosida bidentata* male.

Days	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		(mm)	%		(mm)	%
1	0.683			0.241		
*2	0.975	0.292	42.75	0.341	0.100	41.49
*3	1.120	0.225	23.08	0.430	0.089	26.10
4	1.142	0.022	1.96	0.440	0.010	2.33
*5	1.148	0.006	0.53	0.448	0.008	1.82
6	1.152	0.004	0.35	0.460	0.012	2.68
7	1.160	0.008	0.69	0.472	0.012	2.61
8	1.172	0.012	1.03	0.504	0.032	6.78
9	1.186	0.014	1.19	0.520	0.016	3.17
10	1.190	0.004	0.34	0.542	0.022	4.23
11	1.190	0	0.00	0.546	0.004	0.74
12	1.196	0.006	0.50	0.562	0.016	2.93
13	1.198	0.002	0.17	0.576	0.002	0.36
14	1.198	0	0.00	0.580	0.004	0.69
15	1.198	0	0.00	0.588	0.008	1.38
16	1.198	0	0.00	0.602	0.014	2.38
17	1.200	0.002	0.17	0.620	0.018	2.99
18	1.200	0	0.00	0.620	0	0.00
19	1.202	0.002	0.17	0.624	0.004	0.65
20	1.202	0	0.00	0.636	0.012	1.92
21	1.202	0	0.00	0.640	0.004	0.63
22	1.208	0.006	0.50	0.640	0	0.00
23	1.208	0	0.00	0.640	0	0.00

* indicates moulting

Table 5

Mean length (TL), carapace height (CH), eggs per brood, total eggs produced; and instar duration in *Latonopsis australis* female

Instar No.	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean instar duration in hrs	Cumulative duration of instars in hrs
1	0.608±0.013	0.256±0.009	0	0	26.0	26.0
2	0.728±0.018	0.312±0.014	0	0	28.0	54.0
3	0.888±0.023	0.356±0.010	0	0	28.0	82.0
4	1.104±0.036	0.472±0.023	4.2	4.2	31.5	113.5
5	1.240±0.028	0.496±0.016	7.4	11.6	32.1	145.6
6	1.661±0.042	0.714±0.020	11.3	22.9	38.0	183.6
7	1.721±0.018	0.738±0.014	12.3	35.2	42.0	225.6
8	1.836±0.013	0.754±0.008	13.4	48.6	48.0	273.6
9	1.952±0.003	0.772±0.006	8.0	56.6	48.0	321.6
10	1.986±0.006	0.804±0.002	7.5	64.1	48.0	369.6
11	2.002±0.002	0.826±0.004	5.0	69.1	48.0	417.6
12	2.016±0.005	0.840±0.002	4.0	73.1	50.0	467.6

Table 6

Size increment in *Latonopsis australis* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.608			0.256		
2	0.728	0.120	19.74	0.312	0.056	21.88
3	0.888	0.160	21.98	0.356	0.044	14.10
4	1.104	0.216	24.32	0.472	0.116	32.58
5	1.240	0.136	12.32	0.496	0.024	5.08
6	1.661	0.421	33.95	0.714	0.218	43.95
7	1.721	0.060	3.61	0.738	0.024	3.36
8	1.836	0.115	6.68	0.754	0.016	2.17
9	1.952	0.116	6.32	0.772	0.018	2.39
10	1.986	0.034	1.74	0.804	0.032	4.15
11	2.002	0.016	0.81	0.826	0.022	2.74
12	2.016	0.014	0.70	0.840	0.014	1.69

Table 7
Correlation coefficients for 8 life history characters in *L. australis* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.996			0.678	0.939	0.975	0.962
2.Carapace height (CH)					0.695	0.927	0.967	0.954
3.Increment of TL				0.925	* 0.279	* -0.389	* -0.262	* -0.280
4. Increment of CH					* 0.198	* -0.294	*-0.209	* -0.195
5 Mean clutch size						* 0.442	0.602	* 0.478
6. Total egg production							0.975	0.978
7.Instar duration								0.964
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 8
Mean length (TL), carapace height (CH), eggs per brood, total eggs produced and instar duration in *Diaphanosoma sarsi* female

Instar No.	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean instar duration in hrs	Cumulative duration of instar in hrs
1	0.552±0.023	0.184±0.016	0	0	24.0	24.0
2	0.732±0.035	0.248±0.012	0	0	24.5	48.5
3	0.809±0.027	0.302±0.013	4.2	4.2	32.0	80.5
4	0.840±0.018	0.330±0.006	5.4	9.6	30.0	110.5
5	0.904±0.020	0.364±0.012	8.5	18.1	30.6	141.1
6	0.990±0.016	0.372±0.007	10.0	28.1	32.0	173.1
7	1.022±0.008	0.404±0.004	11.2	39.3	32.5	205.6
8	1.060±0.023	0.412±0.004	12.0	51.3	34.0	239.6
9	1.096±0.008	0.428±0.012	13.0	64.3	36.0	275.6
10	1.120±0.006	0.436±0.007	8.5	72.8	36.0	311.6
11	1.152±0.004	0.448±0.003	9.0	81.8	37.5	349.1
12	1.212±0.007	0.462±0.004	9.0	90.8	39.0	388.1
13	1.280±0.002	0.472±0.001	9.5	100.3	38.0	426.1
14	1.300±0.006	0.482±0.002	9.0	110.3	39.0	465.1
15	1.320±0.004	0.486±0.003	7.5	117.3	39.0	504.1

Table 9

Size increment in *Diaphanosoma sarsi* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.552			0.184		
2	0.732	0.180	32.61	0.248	0.064	34.78
3	0.809	0.077	10.52	0.302	0.054	21.77
4	0.840	0.031	3.83	0.330	0.028	9.27
5	0.904	0.064	7.62	0.364	0.034	10.30
6	0.990	0.086	9.51	0.372	0.008	2.20
7	1.022	0.032	3.23	0.404	0.032	8.60
8	1.060	0.038	3.72	0.412	0.008	1.98
9	1.096	0.036	3.40	0.428	0.016	3.88
10	1.120	0.024	2.19	0.436	0.008	1.87
11	1.152	0.032	2.86	0.448	0.012	2.75
12	1.212	0.060	5.21	0.462	0.014	3.13
13	1.280	0.068	5.61	0.472	0.010	2.16
14	1.300	0.020	1.56	0.482	0.010	2.12
15	1.320	0.020	1.54	0.486	0.004	0.83

Table 10
Correlation coefficients for 8 life history characters in *D. sarsi* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.985			0.714	0.953	0.961	0.979
2.Carapace height (CH)					0.797	0.910	0.963	0.946
3.Increment of TL				0.731	* -0.341	* -0.377	* -0.379	* -0.359
4. Increment of CH					* -0.419	* -0.588	* -0.482	* -0.558
5 Mean clutch size						* 0.556	0.716	0.609
6. Total egg production							0.922	0.993
7.Instar duration								0.999
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 11

Mean length (TL), carapace height (CH), eggs per brood, total eggs produced and instar duration in *Ceriodaphnia cornuta* female

Instar No.	Mean TL (mm±SE)	Mean CH (mm±SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.232±0.013	0.120±0.006	0	0	23.1	23.1
2	0.352±0.033	0.248±0.021	0	0	23.0	46.1
3	0.426±0.023	0.288±0.008	2	2	24.2	70.3
4	0.462±0.008	0.352±0.012	3.3	5.3	24.7	95.0
5	0.520±0.026	0.408±0.016	6.3	11.6	26.5	121.5
6	0.576±0.004	0.424±0.006	6.6	18.2	26.7	148.2
7	0.606±0.012	0.440±0.008	8.6	26.8	27.3	175.5
8	0.616±0.002	0.446±0.004	9.3	36.1	30.3	205.8
9	0.624±0.006	0.449±0.002	6.6	45.4	30.0	235.8
10	0.636±0.010	0.452±0.006	6.0	51.4	29.3	265.1
11	0.650±0.006	0.464±0.002	4.6	56	33.0	298.1
12	0.672±0.012	0.476±0.008	2.0	58	33.3	331.4
13	0.672±0.002	0.496±0.002	0.70	58.7	33.3	364.7
14	0.678±0.006	0.496±0.002	1.30	60.0	33.4	398.1
15	0.682±0.004	0.496±0.002	2.0	62.0	34.6	432.7
16	0.682±0.002	0.496±0.004	1.0	63.0	33.3	466.0
17	0.696±0.002	0.498±0.002	0	63.0	30.0	496.0

Table 12

Size increment in *Ceriodaphnia cornuta* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.232			0.120		
2	0.352	0.120	51.72	0.248	0.128	106.67
3	0.426	0.074	21.02	0.288	0.040	16.13
4	0.462	0.036	8.45	0.352	0.064	22.22
5	0.520	0.058	12.55	0.408	0.056	15.91
6	0.576	0.056	10.77	0.424	0.016	3.92
7	0.606	0.03	5.21	0.440	0.016	3.77
8	0.616	0.01	1.65	0.446	0.006	1.36
9	0.624	0.008	1.30	0.449	0.003	0.67
10	0.636	0.012	1.92	0.452	0.003	0.67
11	0.650	0.014	2.20	0.464	0.012	2.65
12	0.672	0.022	3.38	0.476	0.012	2.59
13	0.672	0	0.00	0.496	0.020	4.20
14	0.678	0.006	0.89	0.496	0	0.00
15	0.682	0.004	0.59	0.496	0	0.00
16	0.682	0	0.00	0.496	0	0.00
17	0.696	0.014	2.05	0.498	0.002	0.40

Table 13

Correlation coefficients for 8 life history characters in *C.cornuta* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.990			* 0.203	0.902	0.886	0.891
2.Carapace height (CH)					* 0.241	0.852	0.851	0.854
3.Increment of TL				0.881	* -0.027	-0.693	-0.673	-0.628
4. Increment of CH					* -0.159	-0.648	-0.612	-0.592
5 Mean clutch size						* -0.097	* -0.042	* -0.200
6. Total egg production							0.952	0.965
7.Instar duration								0.906
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 14

Mean length(TL), carapace height (CH), eggs per brood, total eggs produced and instar duration in *Scapholeberis kingi* female

Instar No.	Mean TL (mm±SE)	Mean CH (mm±SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.290±0.040	0.187±0.012	0	0	32	32
2	0.376±0.052	0.226±0.024	0	0	32	64
3	0.483±0.010	0.283±0.016	1.5	1.5	34.5	98.5
4	0.504±0.015	0.328±0.004	2.5	4.0	35.0	133.5
5	0.536±0.016	0.344±0.008	4.0	8.0	36.0	169.5
6	0.568±0.030	0.368±0.002	5.0	13.0	36.0	205.5
7	0.640±0.022	0.424±0.001	7.5	20.5	36.0	241.5
8	0.684±0.003	0.440±0.001	9.0	29.5	38.0	279.5
9	0.694±0.002	0.440±0.001	11.0	40.5	38.0	317.5
10	0.704±0.001	0.456±0.003	14.0	54.5	39.5	357.0
11	0.704±0.001	0.460±0.002	10.0	64.5	36.5	393.5
12	0.724±0.004	0.486±0.006	8.0	72.5	36.0	429.5
13	0.731±0.002	0.496±0.002	9.0	81.5	38.0	467.5

Table 15

Size increment in *Scapholeberis kingi* female

Instar No.	Mean TL (mm)	Length increment (mm)	Length increment %	Mean CH (mm)	Height increment (mm)	Height increment %
1	0.290			0.187		
2	0.376	0.086	29.66	0.226	0.036	19.25
3	0.483	0.107	28.46	0.283	0.057	25.22
4	0.504	0.021	4.35	0.328	0.045	15.90
5	0.536	0.032	6.35	0.344	0.016	4.88
6	0.568	0.032	5.97	0.368	0.024	6.98
7	0.640	0.072	12.68	0.424	0.056	15.22
8	0.684	0.044	6.88	0.440	0.016	3.77
9	0.694	0.01	1.46	0.440	0	0.00
10	0.704	0.01	1.44	0.456	0.016	3.64
11	0.704	0	0.00	0.460	0.004	0.88
12	0.724	0.02	2.84	0.486	0.026	5.65
13	0.731	0.007	0.97	0.496	0.01	2.06

Table 16

Correlation coefficients for 8 life history measurements in *S.kingi* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.993			0.911	0.840	0.900	0.946
2.Carapace height (CH)					0.900	0.866	0.881	0.963
3.Increment of TL				0.806	* -0.466	* -0.539	* -0.382	* -0.487
4. Increment of CH					* -0.391	* -0.433	* -0.290	* -0.358
5 Mean clutch size						0.791	0.926	0.868
6. Total egg production							0.675	0.966
7.Instar duration								0.801
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 17

Mean length (TL), carapace height (CH), eggs per brood, total eggs produced and instar duration in *Simocephalus serrulatus* female

Instar No.	Mean TL (mm ± SE)	Mean CH (mm ± SE)	Mean number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean instar duration in hrs	Cumulative duration of instars in hrs
1	0.576 ± 0.016	0.296 ± 0.020	0	0	43.00	43.00
2	0.600 ± 0.008	0.344 ± 0.022	0	0	43.33	86.33
3	1.108 ± 0.040	0.664 ± 0.024	0	0	43.66	130.0
4	1.144 ± 0.028	0.880 ± 0.016	5.2	5.2	44.0	174.0
5	1.344 ± 0.032	0.988 ± 0.018	6.8	12	44.5	218.5
6	1.560 ± 0.026	1.106 ± 0.012	8.7	20.7	44.6	263.1
7	1.624 ± 0.016	1.136 ± 0.013	14.6	35.3	44.5	307.6
8	1.832 ± 0.008	1.150 ± 0.006	16.9	52.2	44.0	351.6
9	2.000 ± 0.012	1.280 ± 0.008	18.0	70.2	46.0	397.6
10	2.050 ± 0.016	1.360 ± 0.002	18.6	88.8	49.6	447.2
11	2.120 ± 0.006	1.56 0 ± 0.004	26.2	115	50.2	497.4
12	2.280 ± 0.002	1.560 ± 0.001	10.4	125.4	50.6	548.0
13	2.280 ± 0.003	1.580 ± 0.004	10.0	135.4	50.4	598.4
14	2.280 ± 0.006	1.586 ± 0.002	6.2	141.6	52.2	650.6
15	2.280 ± 0.002	1.586 ± 0.004	6.0	147.6	52.0	702.6
16	2.280 ± 0.002	1.586 ± 0.002	4.0	151.3	52.0	754.6

Table 18

Growth increment in *Simocephalus serrulatus* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		(mm)	%		(mm)	%
1	0.576			0.296		
2	0.600	0.024	4.17	0.344	0.048	16.22
3	1.108	0.508	84.67	0.664	0.320	93.02
4	1.144	0.036	3.25	0.880	0.216	32.53
5	1.344	0.200	17.48	0.988	0.108	12.27
6	1.560	0.216	16.07	1.106	0.188	19.03
7	1.624	0.064	4.10	1.136	0.030	2.71
8	1.832	0.208	12.81	1.150	0.014	1.23
9	2.000	0.168	9.17	1.280	0.130	11.30
10	2.050	0.050	2.50	1.360	0.080	6.25
11	2.120	0.070	3.41	1.560	0.200	14.71
12	2.280	0.160	7.55	1.560	0	0.00
13	2.280	0	0.00	1.580	0.020	1.28
14	2.280	0	0.00	1.586	0.006	0.38
15	2.280	0	0.00	1.586	0	0.00
16	2.280	0	0.00	1.586	0	0.00

Table 19

Correlation coefficients for 8 life history measurements in *Simocephalus serrulatus*

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.988			* 0.547	0.913	0.863	0.951
2.Carapace height (CH)					* 0.544	0.902	0.869	0.945
3.Increment of TL				N.A	N.A	N.A	N.A	N.A
4. Increment of CH					* 0.054	* -0.481	* -0.397	* -0.443
5 Mean clutch size						* 0.325	* 0.252	* 0.347
6. Total egg production							0.972	0.979
7.Instar duration								0.944
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 20

Mean length (TL), carapace height (CH), eggs per brood, total eggs produced; and instar duration in *Moina brachiata* female.

Instar No.	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.528 ± 0.016	0.272 ± 0.007	0	0	11.0	11
2	0.636 ± 0.019	0.332 ± 0.004	0	0	11.0	22
3	0.758 ± 0.016	0.508 ± 0.008	6.4	6.4	21.0	43
4	0.805 ± 0.041	0.528 ± 0.028	12.4	18.8	22.0	65
5	0.988 ± 0.024	0.684 ± 0.006	15.6	34.4	23.0	88
6	1.008 ± 0.010	0.698 ± 0. 002	14.7	51	23.5	111.5
7	1.096 ± 0.022	0.706 ± 0.003	7.7	58.7	24.5	136
8	1.152 ± 0. 016	0.712 ± 0.001	10.2	68.9	26.0	162
9	1.196 ± 0.012	0.720 ± 0.004	14.7	83.6	26.0	188
10	1.248 ±0. 008	0.732 ± 0. 006	15.7	99.3	25.5	213.5
11	1.292 ± 0.004	0.742 ± 0.002	12.0	111.3	32.0	245.5
12	1.302 ± 0. 002	0.752 ± 0.002	5.0	116.3	35.0	280.5

Table 21

Size increment in *Moina brachiata* female.

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.528			0.272		
2	0.636	0.108	20.45	0.332	0.06	22.06
3	0.758	0.122	19.18	0.508	0.176	53.01
4	0.805	0.047	6.20	0.528	0.02	3.94
5	0.988	0.183	22.73	0.684	0.156	29.55
6	1.008	0.020	2.02	0.698	0.014	2.05
7	1.096	0.088	8.73	0.706	0.008	1.15
8	1.152	0.056	5.11	0.712	0.006	0.85
9	1.196	0.044	3.82	0.720	0.008	1.12
10	1.248	0.052	4.35	0.732	0.012	1.67
11	1.292	0.044	3.53	0.742	0.01	1.37
12	1.302	0.01	0.77	0.752	0.01	1.35

Table 22

Correlation coefficients for 8 life history characters in *M. brachiata* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.952			0.585	0.963	0.924	0.977
2.Carapace height (CH)					0.733	0.848	0.906	0.876
3.Increment of TL				0.834	* 0.100	* -0.362	* -0.195	* -0.309
4. Increment of CH					* -0.003	* -0.466	* -0.206	* -0.415
5 Mean clutch size						* 0.445	* 0.512	* 0.459
6. Total egg production							0.891	0.994
7.Instar duration								0.923
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 23

Mean length(TL), carapace height (CH), eggs per brood, total eggs produced and instar duration in *Moinodaphnia macleayi* female

Instar No.	Mean TL (mm ± SE)	Mean CH (mm ± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.480±0.052	0.296±0.048	0	0	23.26	23.26
2	0.584±0.022	0.312±0.010	0	0	23.56	46.82
3	0.628±0.048	0.432±0.016	2.2	2.2	29.3	76.12
4	0.724±0.020	0.492±0.012	4.2	6.4	30.4	106.52
5	0.764±0.025	0.518±0.008	7.6	14.0	32.2	138.72
6	0.814±0.029	0.600±0.010	9.0	23.0	33.98	172.7
7	0.873±0.017	0.624±0.006	11.0	34.0	33.72	206.42
8	0.908±0.032	0.652±0.012	13.4	47.4	34.22	240.64
9	0.973±0.008	0.699±0.004	13.8	61.2	34.40	275.4
10	0.990±0.005	0.718±0.006	17.2	78.4	36.0	311.04
11	1.001±0.027	0.729±0.012	18.4	96.8	37.08	348.12
12	1.056±0.008	0.760±0.002	19.6	116.4	37.04	385.16
13	1.072±0.002	0.777±0.001	14.4	130.8	37.80	422.96
14	1.076±0.010	0.777±0.001	10.0	140.8	38.0	460.96
15	1.097±0.003	0.780±0.003	5.6	146.4	38.0	498.96
16	1.104±0.002	0.786±0.001	2.5	148.9	36.0	534.96
17	1.104±0.002	0.786±0.001	2.0	150.9	28.0	562.96

Table 24

Size increment in *Moinodaphnia macleayi* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		(mm)	(%)		(mm)	(%)
1	0.480			0.296		
2	0.584	0.104	21.67	0.312	0.016	5.40
3	0.628	0.044	7.53	0.432	0.120	38.46
4	0.724	0.096	15.29	0.492	0.150	34.72
5	0.764	0.040	5.52	0.518	0.026	5.28
6	0.814	0.050	6.54	0.600	0.082	15.83
7	0.873	0.059	7.25	0.624	0.024	4.00
8	0.908	0.035	4.01	0.652	0.028	4.49
9	0.973	0.065	7.16	0.699	0.047	7.21
10	0.990	0.017	1.75	0.718	0.019	2.72
11	1.001	0.011	1.11	0.729	0.011	1.53
12	1.056	0.055	5.49	0.760	0.031	4.25
13	1.072	0.016	1.52	0.777	0.017	2.24
14	1.076	0.004	0.37	0.777	0	0.00
15	1.097	0.021	1.95	0.780	0.003	0.39
16	1.104	0.007	0.64	0.786	0.006	0.77
17	1.104	0	0.00	0.786	0	0.00

Table 25

Mean length (TL), carapace height (CH) and percentage of growth increment in *Moinodaphnia macleayi* male

Day	Mean TL (mm ± SE)	Mean CH (mm ± SE)	Length increment		Height increment	
			TL increment	%TL	CH increment	%CH
1	0.380± 0.028	0.218±0.012				
2*	0.464 ±0.016	0.236±0.008	0.084	22.11	0.018	8.26
3	0.568 ±0.026	0.272±0.016	0.104	22.41	0.036	15.25
4*	0.688 ±0.012	0.320±0.005	0.12	21.13	0.048	17.65
5	0.706 ±0.002	0.332±0.002	0.018	2.62	0.012	3.75
6	0.708 ±0.001	0.346±0.006	0.002	0.28	0.014	4.22
7	0.712 ±0.001	0.360±0.001	0.004	0.56	0.014	4.05
8	0.712 ±0.006	0.360±0.002	0	0	0	0.00
9	0.724 ±0.002	0.370±0.002	0.012	1.69	0.01	2.78
10	0.736 ±0.004	0.376±0.002	0.012	1.66	0.006	1.62
11	0.748 ±0.004	0.376±0.003	0.012	1.63	0	0
12	0.762 ±0.002	0.376±0.002	0.014	1.87	0	0
13	0.762 ±0.001	0.376±0.001	0	0	0	0

*indicates moulting on Day 2nd and 4th.

Table 26

Correlation coefficients for 8 life history characters in *M. macleayi* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.992			* 0.521	0.922	0.810	0.961
2.Carapace height (CH)					* 0.568	0.893	0.845	0.938
3.Increment of TL				0.581	* -0.088	-0.607	* -0.303	* -0.571
4. Increment of CH					* -0.128	* -0.544	* -0.150	* -0.489
5. Mean clutch size						* 0.286	0.729	* 0.305
6. Total egg production							0.641	0.982
7.Instar duration								0.667
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 27
Mean length (TL), carapace height (CH), eggs per brood, total eggs produced; and instar duration in *I. spinifer* female.

Instar No.	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.336±0.018	0.238±0.014	0	0	68.0	68.0
2	0.584±0.028	0.448±0.018	2.0	2	52.0	120.0
3	0.648±0.012	0.480±0.006	2.5	4.5	42.0	162.0
4	0.664±0.014	0.496±0.004	4.0	8.5	44.0	206.0
5	0.682±0.008	0.520±0.008	6.0	14.5	44.0	250.0
6	0.696±0.004	0.544±0.009	12.0	26.5	44.0	294.0
7	0.708±0.002	0.584±0.001	7.0	33.5	44.5	338.5
8	0.714±0.005	0.588±0.002	5.0	38.5	46.0	384.5
9	0.730±0.002	0.592±0.004	4.0	42.5	46.5	431.0
10	0.748±0.006	0.600±0.001	7.5	50	48.0	479.0
11	0.762±0.008	0.624±0.010	4.5	54.5	48.5	527.5
12	0.818±0.004	0.648±0.002	4.0	59.5	50.0	577.5
13	0.830±0.006	0.662±0.003	2.0	61.5	42.0	619.5

Table 28

Size increment in *I. spinifer*

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.336			0.238		
2	0.584	0.248	73.81	0.448	0.210	88.24
3	0.648	0.064	10.96	0.480	0.032	7.14
4	0.664	0.016	2.47	0.496	0.016	3.33
5	0.682	0.018	2.71	0.520	0.024	4.84
6	0.696	0.014	2.05	0.544	0.024	4.62
7	0.708	0.022	3.16	0.584	0.040	7.35
8	0.714	0.006	0.85	0.588	0.004	0.68
9	0.730	0.016	2.24	0.592	0.004	0.68
10	0.748	0.018	2.47	0.600	0.008	1.35
11	0.762	0.014	1.87	0.624	0.024	4.00
12	0.818	0.056	7.35	0.648	0.024	3.85
13	0.830	0.012	1.47	0.662	0.014	2.16

Table 29

Correlation coefficients for 8 life history characters in *I. spinifer*

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.989			* 0.394	* 0.394	-0.774	0.852
2.Carapace height (CH)					* 0.407	0.854	N. A	0.885
3.Increment of TL				0.972	* -0.274	* -0.361	* 0.098	* -0.358
4. Increment of CH					* -0.185	* -0.376	* 0.081	* -0.377
5 Mean clutch size						* 0.229	* -0.467	N. A
6. Total egg production							* -0.303	0.992
7.Instar duration								* -0.380
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 30
The mean length (TL), carapace height CH), eggs per brood, total eggs produced and instar duration in *Macrothrix triserialis* female

Instar No.	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean instar duration in hrs	Cumulative duration of instars in hrs
1	0.410±0.016	0.248±0.008	0	0	29	29.0
2	0.464±0.034	0.366±0.012	0	0	29	58.0
3	0.608± 0.026	0.376±0.016	5.5	5.5	30.5	88.5
4	0.704±0.018	0.400± 0.032	14.2	19.7	30.5	119.0
5	0.780± 0.026	0.512± 0.010	17.5	37.2	32.5	151.5
6	0.800± 0.010	0.526± 0.006	20.4	57.6	32.5	184.0
7	0.840± 0.016	0.536± 0.004	18.0	75.6	32.5	216.5
8	0.896± 0.003	0.544± 0.002	22.0	97.6	34.0	250.5
9	0.908± 0.002	0.560± 0.006	21.5	119.1	34.0	284.5
10	0.924± 0.012	0.582± 0.008	24.2	143.3	36.0	320.5
11	0.960±0.003	0.600± 0.001	20.2	163.5	36.5	357.0
12	0.966± 0.002	0.608±0.003	18.0	181.5	30.0	387.0

Table 31

Size increment in *Macrothrix triserialis* female

Instar No.	Mean TL (mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.410			0.248		
2	0.464	0.054	13.73	0.366	0.118	47.58
3	0.608	0.144	31.03	0.376	0.01	2.73
4	0.704	0.096	15.79	0.400	0.024	6.38
5	0.780	0.076	10.80	0.512	0.112	28.00
6	0.800	0.02	2.56	0.526	0.014	2.73
7	0.840	0.04	5.00	0.536	0.01	1.90
8	0.896	0.056	6.67	0.544	0.008	1.49
9	0.908	0.012	1.34	0.560	0.016	2.94
10	0.924	0.016	1.76	0.582	0.022	3.93
11	0.960	0.036	3.90	0.600	0.018	3.09
12	0.966	0.006	0.63	0.608	0.008	1.33

Table 32

The mean length (TL), carapace height (CH) and percentage of growth increment in *M. triserialis* male

Days	Mean TL (mm±SE)	Length increment		Mean CH (mm±SE)	Height increment	
		mm	%		mm	%
1	0.408±0.014			0.248± 0.002		
*2	0.432± 0.002	0.024	5.88	0.256± 0.006	0.008	3.23
3	0.434± 0.008	0.002	0.46	0.260± 0.002	0.004	1.56
4	0.448± 0.012	0.014	3.23	0.272± 0.008	0.012	4.62
5	0.472± 0.010	0.024	5.36	0.294± 0.006	0.022	8.09
6	0.488±0.002	0.016	3.39	0.302± 0.004	0.008	2.72
7	0.496± 0.005	0.008	1.64	0.306± 0.002	0.004	1.32
8	0.506± 0.004	0.01	2.02	0.312± 0.002	0.006	1.96
9	0.518± 0.002	0.012	2.37	0.318± 0.004	0.006	1.92

* indicates moulting on 2nd day.

Table 33

Correlation coefficients for 8 life history characters in *M. triserialis*

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.971			0.946	0.889	0.758	0.947
2.Carapace height (CH)					0.909	0.880	0.739	0.936
3.Increment of TL				* 0.239	* -0.281	* -0.525	* -0.211	* -0.437
4. Increment of CH					* -0.282	* -0.363	* -0.201	* -0.339
5 Mean clutch size						0.754	0.807	0.827
6. Total egg production							0.658	0.987
7.Instar duration								0.691
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 34
Mean length (TL), carapace height (CH), eggs per brood, total eggs produced; and instar duration in *Alona pulchella* female

Instar No.	Body length (mm± SE)	Carapace height (mm± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean instar duration in hrs	Cumulative duration of instars in hrs
1	0.325±0.012	0.183±0.008	0	0	23.5	23.5
2	0.350±0.028	0.214±0.013	0	0	23.5	47.0
3	0.425±0.010	0.250±0.006	2	2	26.5	73.5
4	0.446±0.018	0.264±0.004	2	4	30.4	103.9
5	0.472±0.022	0.286±0.009	2	6	30.2	134.1
6	0.486±0.016	0.298±0.005	2	8	32.0	166.1
7	0.508±0.008	0.308±0.003	2	10	32.6	198.7
8	0.510±0.006	0.312±0.002	2	12	32.0	230.7
9	0.516±0.006	0.316±0.001	2	14	32.5	263.2
10	0.520±0.004	0.332±0.002	2	16	37.4	300.6
11	0.522±0.001	0.326±0.001	2	18	38.8	339.4
12	0.525±0.001	0.328±0.002	2	20	38.6	378.0

Table 35

Size increment in *Alona pulchella* female

Instar No.	Mean TL mm	Length increment		Mean CH mm	Height increment	
		mm	%		mm	%
1	0.325			0.183		
2	0.350	0.025	7.69	0.204	0.021	11.47
3	0.425	0.075	21.42	0.250	0.046	22.54
4	0.446	0.021	4.94	0.264	0.014	5.60
5	0.472	0.026	5.82	0.286	0.022	8.33
6	0.486	0.014	2.96	0.298	0.012	4.19
7	0.508	0.022	4.52	0.308	0.01	3.36
8	0.510	0.002	0.39	0.312	0.004	1.30
9	0.516	0.006	1.17	0.316	0.004	1.28
10	0.520	0.004	0.77	0.332	0.006	1.90
11	0.522	0.002	0.38	0.326	0.004	1.20
12	0.525	0.003	0.57	0.328	0.002	0.61

Table 36

Correlation coefficients for 8 life history characters in *A. pulchella*

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.996			0.884	0.881	0.907	0.902
2.Carapace height (CH)				0.978	0.857	0.905	0.929	0.924
3.Increment of TL					* 0.093	* -0.529	* -0.448	* -0.490
4. Increment of CH					* 0.058	* -0.551	* -0.463	* -0.507
5 Mean clutch size						0.616	0.719	0.648
6. Total egg production							0.958	0.997
7.Instar duration								0.962
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 37
Mean length (TL), carapace height (CH), eggs per brood, total eggs produced; and instar duration in *Oxyurella singalensis* female

Instar No	Mean TL (mm± SE)	Mean CH (mm± SE)	Mean Number of eggs per brood	Cumulative number of eggs (Σ mx)	Mean Instar duration in hrs	Cumulative duration of instars in hrs
1	0.504±0.008	0.328±0.012	0		36.0	36
2	0.520±0.010	0.344±0.006	0		38.5	74.5
3	0.544±0.023	0.400±0.016	2	2	40.2	114.7
4	0.672±0.034	0.408±0.012	2	4	44.5	159.2
5	0.680±0.016	0.456±0.010	2	6	48.0	207.2
6	0.692±0.006	0.464±0.004	2	8	53.4	260.6
7	0.704±0.008	0.468±0.002	2	10	50.5	311.1
8	0.719±0.006	0.472±0.002	2	12	52.0	363.1
9	0.725±0.012	0.506±0.001	2	14	55.2	418.3
10	0.742±0.002	0.506±0.001	2	16	58.6	476.9
11	0.764±0.006	0.508±0.002	2	18	60.0	536.9
12	0.782±0.003	0.512±0.001	2	20	62.0	598.9
13	0.806±0.008	0.524±0.004	2	22	84.0	682.9
14	0.824±0.002	0.536±0.002	2	24	74.0	756.9
15	0.824±0.001	0.536±0.001	2	26	74.0	830.9
16	0.824±0.002	0.536±0.001	0	26	62.0	892.9
17	0.824±0.001	0.536±0.001	0	26	67.0	959.9

Table 38

Size increment in *Oxyurella singalensis* female

Instar number	Mean TL(mm)	Length increment		Mean CH (mm)	Height increment	
		mm	%		mm	%
1	0.504			0.328		
2	0.520	0.016	3.17	0.344	0.016	4.88
3	0.544	0.024	4.62	0.400	0.056	16.28
4	0.672	0.128	23.53	0.408	0.008	2.00
5	0.680	0.008	1.19	0.456	0.048	11.76
6	0.692	0.012	1.76	0.464	0.008	1.75
7	0.704	0.012	1.73	0.468	0.004	0.86
8	0.719	0.015	2.13	0.472	0.004	0.85
9	0.725	0.006	0.83	0.506	0.034	7.20
10	0.742	0.017	2.34	0.506	0	0.00
11	0.764	0.022	2.96	0.508	0.002	0.40
12	0.782	0.018	2.36	0.512	0.006	1.18
13	0.806	0.034	4.35	0.524	0.012	2.34
14	0.824	0.018	2.23	0.536	0.012	2.29
15	0.824	0	0.00	0.536	0	0.00
16	0.824	0	0.00	0.536	0	0.00
17	0.824	0	0.00	0.536	0	0.00

Table 39

Correlation coefficients for 8 life history characters in *O. singalensis* female

	1	2	3	4	5	6	7	8
1.Total length (TL)		0.971			* 0.252	0.946	0.894	0.941
2.Carapace height (CH)					* 0.317	0.932	0.874	0.928
3.Increment of TL				N. A	* 0.298	* -0.281	* -0.160	* -0.277
4. Increment of CH					N. A	N.A	N. A	N. A
5 Mean clutch size						* 0.047	* 0.240	* 0.000
6. Total egg production							0.910	0.995
7.Instar duration								0.888
8. Instar number								

* indicates that there is no correlation between the variables at 5% level of confidence

Table 40

The mean length (TL), mean carapace height(CH), and percentage of growth increment of *Oxyurella singalensis* male.

Days	Mean TL	Length increment		Mean CH	Height increment	
		mm	%		mm	%
1	0.442			0.272		
2	0.474	0.032	7.24	0.286	0.014	5.15
3	0.502	0.028	5.91	0.300	0.014	4.90
*4	0.516	0.014	2.79	0.320	0.020	6.67
5	0.520	0.004	0.78	0.322	0.002	0.63
6	0.520	0.000	0.00	0.322	0.000	0.00
7	0.522	0.002	0.38	0.324	0.002	0.62
8	0.526	0.004	0.77	0.328	0.004	1.23
9	0.530	0.004	0.76	0.332	0.004	1.22
10	0.536	0.006	1.13	0.334	0.002	0.60
11	0.540	0.004	0.75	0.334	0.000	0.00
12	0.544	0.004	0.74	0.336	0.002	0.60
13	0.546	0.002	0.37	0.336	0.000	0.00
14	0.550	0.004	0.73	0.338	0.002	0.60
15	0.554	0.004	0.73	0.340	0.002	0.59
16	0.558	0.004	0.72	0.344	0.004	1.18
17	0.560	0.002	0.36	0.344	0	0.00
18	0.560	0	0.00	0.344	0	0.00
19	0.560	0	0.00	0.344	0	0.00
20	0.560	0	0.00	0.344	0	0.00
21	0.560	0	0.00	0.344	0	0.00
22	0.560	0	0.00	0.344	0	0.00
23	0.560	0	0.00	0.344	0	0.00

* indicates moulting on 4th day

Table 41 Population growth in *M. triserialis*

Date	Pre-adult	Parthenogenetic females	Males	Ephippial females	Total
28-11-04	846	270	0	0	1124
30-11-04	1592	294	0	0	1886
2/12/04	2416	640	18	0	3074
4/12/04	5614	1098	42	36	6790
6/12/04	4846	1912	120	98	6976
8/12/04	10116	1051	356	1612	13135
10/12/04	7546	1904	646	3546	13642
12/12/04	8306	1062	422	4836	14256
14-12-04	9407	3608	656	2568	16239
16-12-04	6728	1732	460	826	9746
18-12-04	3610	3674	18	120	7422
20/12/04	2496	2826	08	0	5330
22/12/04	974	2710	12	16	3712
24/12/04	720	3218	0	0	4216

Table 42

Mean duration of pre-adult, primiparous and adult of the cladocerans studied

Sl.No.	Name of species	Pre-adult (PID)	Primiparous	Adult (AID)
1	*P. bidentata*	24	38	38.42
2	*L. australis*	27.33	31.5	42.85
3	*D. sarsi*	24.25	32	35.04
4	*C. cornuta*	23	24.2	30
5	*S. kingi*	32	34.5	36.68
6	*S. serrulatus*	43.33	44	48.04
7	*M. brachiata*	11	21	25.85
8	*M. macleayi*	23.41	29.3	34.43
9	*I. spinifer*	68	52	45.96
10	*M. triserialis*	29	30.5	32.9
11	*A. pulchella*	23.5	26.5	33.1
12	*O. singalensis*	37.25	40.2	59.03

Table 43 Size at different stages of growth in the cladocerans

No.	Name of species	Size at birth (SaB)		Size at first reproduction (SFR)		Relative size		Maximum mean size	
		TL	CH	TL	CH	RL	RH	TL	CH
1	*P. bidentata*	0.697	0.246	1.3	0.533	0.42	0.346	2.046	0.906
2	*L. australis*	0.512	0.208	1.104	0.472	0.464	0.441	2.016	0.84
3	*D.sarsi*	0.522	0.184	0.809	0.302	0.552	0.609	1.32	0.486
4	*C. cornuta*	0.232	0.120	0.426	0.288	0.545	0.471	0.696	0.498
5	*S. kingi*	0.29	0.187	0.483	0.283	0.594	0.66	0.731	0.496
6	*S.serrulatus*	0.576	0.296	1.144	0.88	0.399	0.336	2.28	1.586
7	*M. brachiata*	0.496	0.256	0.758	0.508	0.496	0.504	1.302	0.752
8	*M.macleayi*	0.48	0.296	0.628	0.432	0.764	0.685	1.104	0.786
9	*I. spinifer*	0.304	0.208	0.584	0.448	0.521	0.464	0.83	0.662
10	*M.triserialis*	0.318	0.207	0.608	0.376	0.474	0.468	0.966	0.608
11	*A. pulchella*	0.291	0.175	0.425	0.25	0.685	0.7	0.525	0.328
12	*O. singalensis*	0.492	0.318	0.544	0.4	0.803	0.672	0.824	0.536

Table 44 Correlation coefficients for the different stages of growth in cladocerans

		1	2	3	4	5	6	7	8
Size at birth (SaB).	1. Total length (TL)		0.695	0.839	0.751	-0.248	-0.373	0.861	0.736
	2. Carapace height (CH)			0.274	0.648	0.232	0.064	0.367	0.594
Size at first reproduction (SFR)	3.Total length (TL)				0.716	-0.652	-0.712	0.975	0.739
	4. Carapace height (CH)					-0.509	-0.678	0.798	0.979
Relative Size	5. Relative length (RL)	------					0.895	-0.648	-0.539
	6. Relative height (RH)							-0.714	-0.685
Maximum size	7. Maximum TL								0.834
	8. Maximum CH								

Table 45 Life history parameters of the cladocerans studied

Sl. No.	Species studied	Maximum size (mm)		Number of instars			Life span		Egg production		
		Total length (TL)	Carapace height (CH)	Pre-adult Instars	Adult instars	Total instars	Maximum life span (L max) days	Mean life span (Σlx) days	Maximum clutch size (E max) Eggs/brood	Cumulative number of eggs produced (Σ mx)	Rate of egg production (REP)
1	*P. bidentata*	2.046	0.906	3	14	17	25.41	9.85	15.2	108.13	9.0919
2	*L. australis*	2.016	0.84	3	9	12	19.49	7.95	13.4	73.1	7.9021
3	*D. sarsi*	1.32	0.486	2	13	15	21	9.0	13.0	117.3	9.2632
4	*C. cornuta*	0.696	0.498	2	15	17	20.66	8.23	9.3	63.0	4.8039
5	*S. kingi*	0.731	0.496	2	11	13	19.47	8.14	14.0	81.5	7.3434
6	*S. serrulatus*	2.28	1.586	3	13	16	31.44	13.37	26.2	151.3	12.2752
7	*M. brachiata*	1.302	0.752	2	10	12	11.69	5.63	15.6	116.3	11.771
8	*M. macleayi*	1.104	0.786	2	15	17	23.45	9.23	19.6	150.9	11.4968
9	*I. spinifer*	0.83	0.662	1	12	13	25.81	11.43	12.0	61.5	5.7637
10	*M. triserialis*	0.966	0.608	2	10	12	16.1	7.55	24.2	181.5	17.9329
11	*A. pulchella*	0.525	0.328	2	10	12	15.75	-	2.0	20.0	1.923
12	*O. singalensis*	0.824	0.536	2	15	17	39.99	17.14	2.0	26.0	1.848