DISCUSSION

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Chapter 6

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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.