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Parthenogenetic reproduction of *Diaphanosoma celebensis* (Crustacea: Cladocera). Effect of algae and algal density on survival, growth, life span and neonate production

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Abstract The cladoceran *Diaphanosoma celebensis* Stingelin is reported on for the first time from Indian waters (Mandovi estuary, Goa). Amictic females were maintained in the laboratory (temperature 24 ± 1 °C and salinity 17 psu) for three successive generations in order to follow the parthenogenetic reproductive behaviour, growth, survival and neonate production. The mean life span and body length of adult females in the three generations showed some variations and ranged from 9 to 12.5 d and 842 to 932 µm, respectively. The mean length of the neonates produced also varied (283 to 446 µm) in the three generations. Cladoceran preference for three phytoplankton food sources, i.e. *Isochrysis galbana* (Parke), *Chaetoceros calcitrans* (Paulsen) and *Tetraselmis gracilis* (Kylin), was determined. Growth was faster in the initial stage with all three diets but slowed down in later life. Increased food concentrations resulted in higher neonate production but reduced the life span of females. However, long-term feeding experiments revealed that the percentage survival was high with *I. galbana* and low with *C. calcitrans*.

Introduction

Diaphanosoma celebensis (Crustacea:Cladocera:Sididae) is a low to medium saline estuarine cladoceran showing wide but patchy distribution in tropical Asia (Korovchinsky 1993a). This species has been redescribed by Korovchinsky (1989) from the south of Vietnam. On the Indian subcontinent, 130 species of Cladocera have been recorded (Fernando and Kanduru 1984); in the present paper the occurrence of *D. celebensis* is reported on for the first time from Indian waters (Mandovi estuary, Goa; 150°N; 74°E). The southeastern Asian species *D. aspinosum*, the parthenogenetic biology of which was studied by Segawa and Yang (1987, 1988), has been considered synonymous with *D. celebensis* (Korovchinsky 1993a).

The reproductive biology of marine cladocerans was recently reviewed by Egloff et al. (1997). It is apparent from the review that very little is known about the morphology, biology, gamogenic and parthenogenic reproductive behaviour of *Diaphanosoma celebensis* (Inoue and Aoki 1971; Segawa and Yang 1987, 1988, 1990). Although some information is available on the reproductive behaviour of freshwater cladocerans from Indian waters (Pandian 1994), very little is known on this aspect of marine cladocerans (Vijayalakshmi and Venugopal 1972; Shirgur and Naik 1977); reproduction, feeding behaviour, growth and survival of *D. celebensis* from Indian waters have not yet been studied. Further, it is most likely that earlier reports (Menon et al. 1971; Madhupratap and Haridas 1975; Madhupratap 1981; Haridas et al. 1980) on the occurrence of *Penilia avirostris* (a high saline species) in the estuarine and coastal waters of the west coast of India during the low saline period may be due to mistaken identification of the species, as *P. avirostris* morphologically closely resembles *D. celebensis*. Therefore, it appears that *D. celebensis* has a wider distribution in Indian waters during the low saline period.

In this communication, various aspects of parthenogenetic neonate production in three successive generations and the influence of microalgal diets on neonate production by amictic females of *Diaphanosoma celebensis* under laboratory conditions are dealt with in detail. In view of the short regeneration time and easy maintainability in the laboratory, the results of the studies provide

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guidelines for the mass culture of this species, for use as natural live feed in shrimp and fish hatcheries.

Materials and methods

Zooplankton samples were collected from the Mandovi estuary (Goa) during August 1997 by towing a square zooplankton net (mouth area of 0.25 m^2, mesh size of 300 μm) for 10 min. They were immediately transferred into clean glass beakers (2 litres) containing aerated ambient seawater and transported to the laboratory. Adult parthenogenetic females of *Diaphanosoma celebensis* Stingelin were carefully removed with a fine capillary tube under a stereo zoom microscope. Cells of *Isochrysis galbana* (Chrysophyta), *Tetraselmis gracilis* (Chlorophyta) and *Chaetoceros calcitrans* (Bacillariophyta), isolated from the wild and cultured in the laboratory in *F*/2 media (Guillard and Ryther 1962) under a 10 h light:14 h dark period (Omori and Ikeda 1984), were used as food for experimental animals.

Adult females were transferred into cell plates of 24 cell wells, one in each cell well containing about 4.5 ml of Nucleopore-filtered seawater (FSW) of ambient salinity (17 psu). Monoculture of *Isochrysis galbana* in growth phase (100×10^4cells ml^{-1}) was used as the diet for maintaining the experimental females. For experiments on growth and neonate production, the newly released neonates (0 d old) were transferred to cell plates, one in each cell well containing 4.5 ml of FSW (17 psu). These were considered the first generation population. Before transferring them into the cell wells, gross body length (μm) of each neonate was measured to the nearest division (Onbé 1983) under a Leica inverted microscope (40×) with the help of an ocular scale. For measuring the length, the cladocerans were not narcotized, as even the slightest handling resulted in their death. Therefore, the cell plate was mounted on the inverted microscope, and the measurements were taken when the cladocerans remained in a dorsal position. Food and water in each cell well were changed every day. Daily record was maintained on body length, moulting, neonate production and the length of neonates. The neonates produced by the first generation were treated as the second generation. They were transferred, one each to new cell wells. Similarly, the neonates produced by the second generation females were considered the third generation population. The second and third generation females were subjected to all the observations and measurements as detailed above. The experiments were conducted in the laboratory (temperature 24 ± 1 °C and salinity 17 psu) and lasted till the death of all individuals.

In order to determine the influence of various phytoplankton species on reproduction, two sets, each consisting of three cell plates containing 0-d-old neonates (one neonate per cell well), were maintained at the same temperature and salinity mentioned above. In each set, one cell plate was used for each diet, i.e. monocultures of *Isochryis galbana*, *Tetraselmis gracilis* and *Chaetoceros calcitrans*. Cell densities were determined using a Neubauer haemocytometer under a Leica inverted microscope (100×). For the first set, all diets were maintained at concentrations of 10×10^4 cells ml^{-1} in each plate. This concentration was obtained by diluting the culture with the required quantity of autoclaved FSW. The volumes of water and phytoplankton were maintained at a constant ratio of 4.3 ml (water) to 500 μl (phytoplankton). Food and water were changed every day.

For the second set, dense cultures of three phytoplankton species (growth phase) were used for feeding. The densities of the cultures varied with species due to the differences in size and growth rates. In the case of *Isochrysis galbana* the cell density ranged from 100 to 104×10^4 cell ml^{-1}, whereas counts of *Chaetoceros calcitrans* and *Tetraselmis gracilis* varied from 85 to 92×10^4 and 26 to 32×10^4 cells ml^{-1}, respectively. Due to high density of cells, the volume of the three diets used for this experiment was 100 μl per cell well. In both experiments, growth, survival and neonate production in each cell well were monitored daily till the death of all individuals.

Kruskal–Wallis's *H*-test (Kruskal and Wallis 1952), Mann–Whitney's *U*-test (Downie and Heath 1970) and one-way ANOVA (Sokal and Rohlf 1981) were used whenever necessary to test significance of the treatments.

Results

Growth and life cycle in relation to generations

The mean life spans showed a gradual decrease from the first (12.5 d) to the third generation (9.0 d), but the longest life span (33 d) was observed in the second generation (Table 1).

Survival was better in the first generation, where no mortality was observed until Day 8. It dropped to 50% on Day 9 and decreased thereafter (Fig. 1). On the other hand, in the second and third generations, mortality was sometimes observed on Day 1 itself, and after Day 15 survival was extremely poor. A gradual increase in the mean body length of females was observed from the first to third generation (842 to 932 μm). Length increased at a faster rate in the initial phase of growth and gradually decreased towards the end, irrespective of the generation (Fig. 2). This was also clear from the intermoult periods, which were of short duration (1 d) in the initial stages of growth and increased (up to 3 d) towards the end of the life cycle. Kruskal–Wallis' *H*-test revealed that growth, neonate production and survival differed significantly ($p < 0.05$) between the three generations.

The mean length of the neonates produced was greatest in the third generation (446 μm). The mean neonate production was also high (8.7 neonates, female^{-1}) in the third generation.

In all generations, only parthenogenetic females were produced. Resting egg production did not occur during the experimental period. However, three sexual males were encountered during the later part of the study under mass rearing conditions (Achuthankutty et al. in preparation) when females were fed exclusively with *Isochrysis galbana*. Males were identified by their slender

Table 1 *Diaphanosoma celebensis*. Mean age and size of primiparous females, and size, life span and neonate production of adult females in three generations. Range in parentheses

Generation	Primiparous female		Adult female		Neonate	
	Age (d)	Length (μm)	Life span (d)	Body length (μm)	Total neonates female^{-1}	Length (μm)
First	6.3 (5–10)	729 (575–950)	12.5 (8–15)	842 (350–1075)	4.2 (1–12)	433 (300–525)
Second	9.0 (6–14)	872 (750–1000)	11.7 (1–33)	896 (300–1050)	3.8 (2–30)	283 (300–500)
Third	6.31 (3–10)	853 (750–1000)	9.0 (1–26)	932 (300–1050)	8.7 (2–24)	446 (325–550)

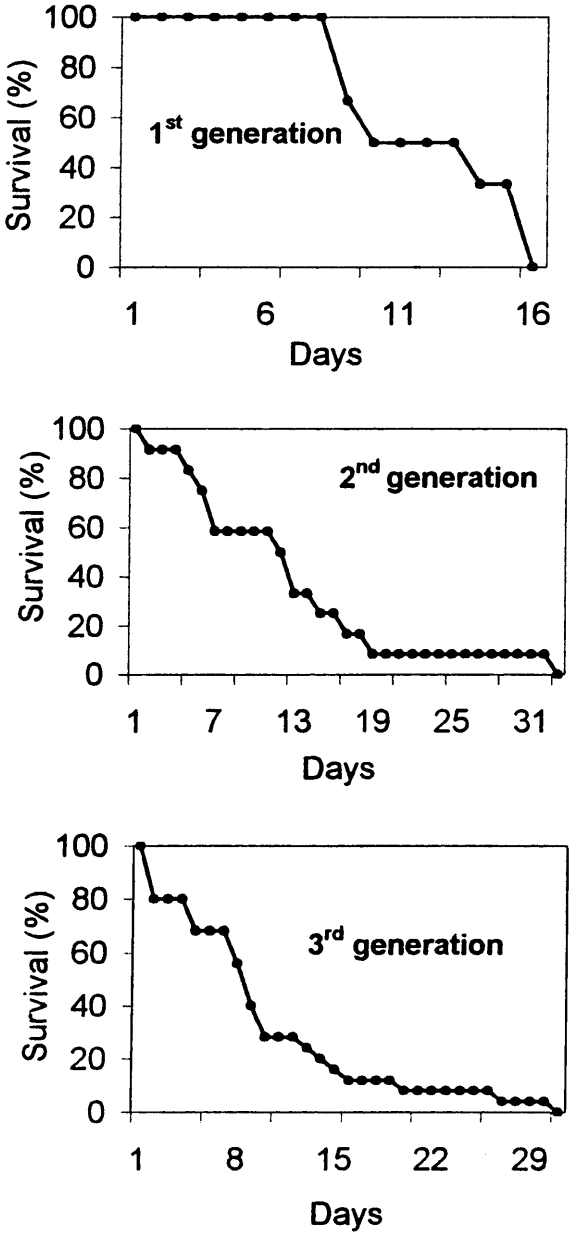

Fig. 1 *Diaphanosoma celebensis*. Percentage survival in the three generations

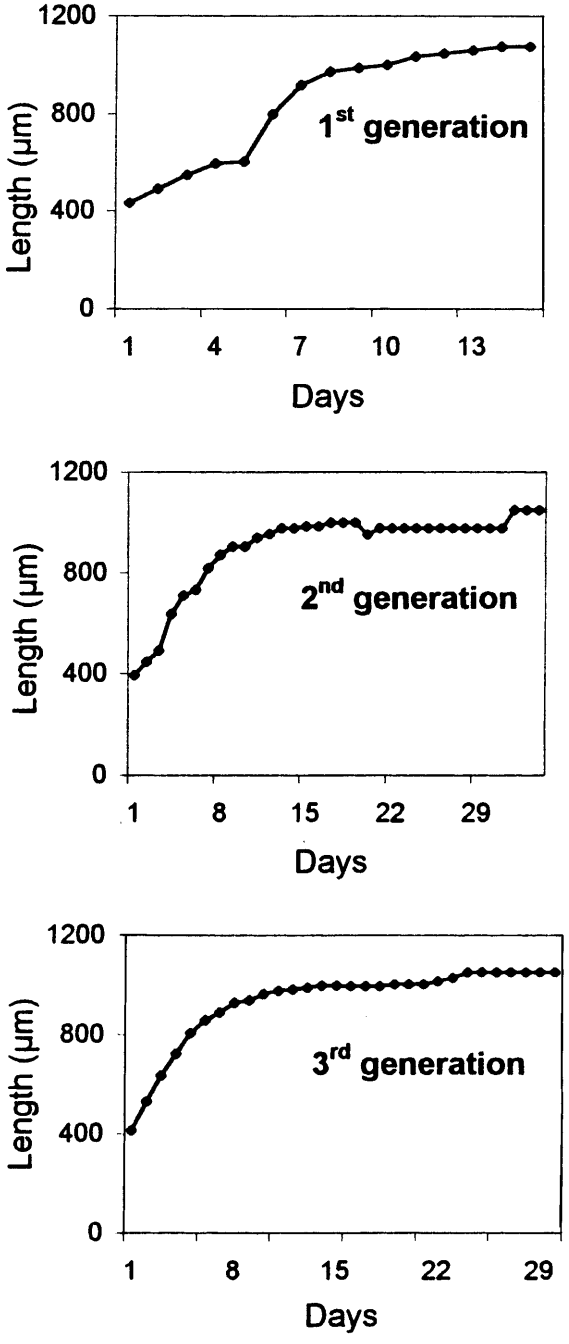

Fig. 2 *Diaphanosoma celebensis*. Daily growth in the three generations

shape and a pair of elongate copulatory organs (antennules) originating from the sides of the thorax (Korovchinsky 1989, 1993b). The antennules were much longer than those encountered in females and extended nearly the entire length of the carapace.

Feeding and neonate production

Feeding experiments with the three different phytoplankton species in the two concentrations showed that mean life span of the females increased at high concentrations of *Isochrysis galbana* (11 d) and *Tetraselmis gracilis* (12 d), while little effect was seen with *Chaetoceros calcitrans* (Table 2).

The pattern of daily growth remained more or less the same with all diets, irrespective of cell concentration, as faster growth was recorded initially and slowed down in the later stages (Fig. 3). However, cell concentration within a specific algal diet was found to exert some effect on the survival (Fig. 4). For instance, at low concentrations of *Isochrysis galbana* a sharp decline in the population occurred from Day 3 onwards, leading to total mortality on Day 13. At high concentrations, the survival rate was quite high for the first 5 to 6 d, and total mortality did not occur until Day 27. In the case of

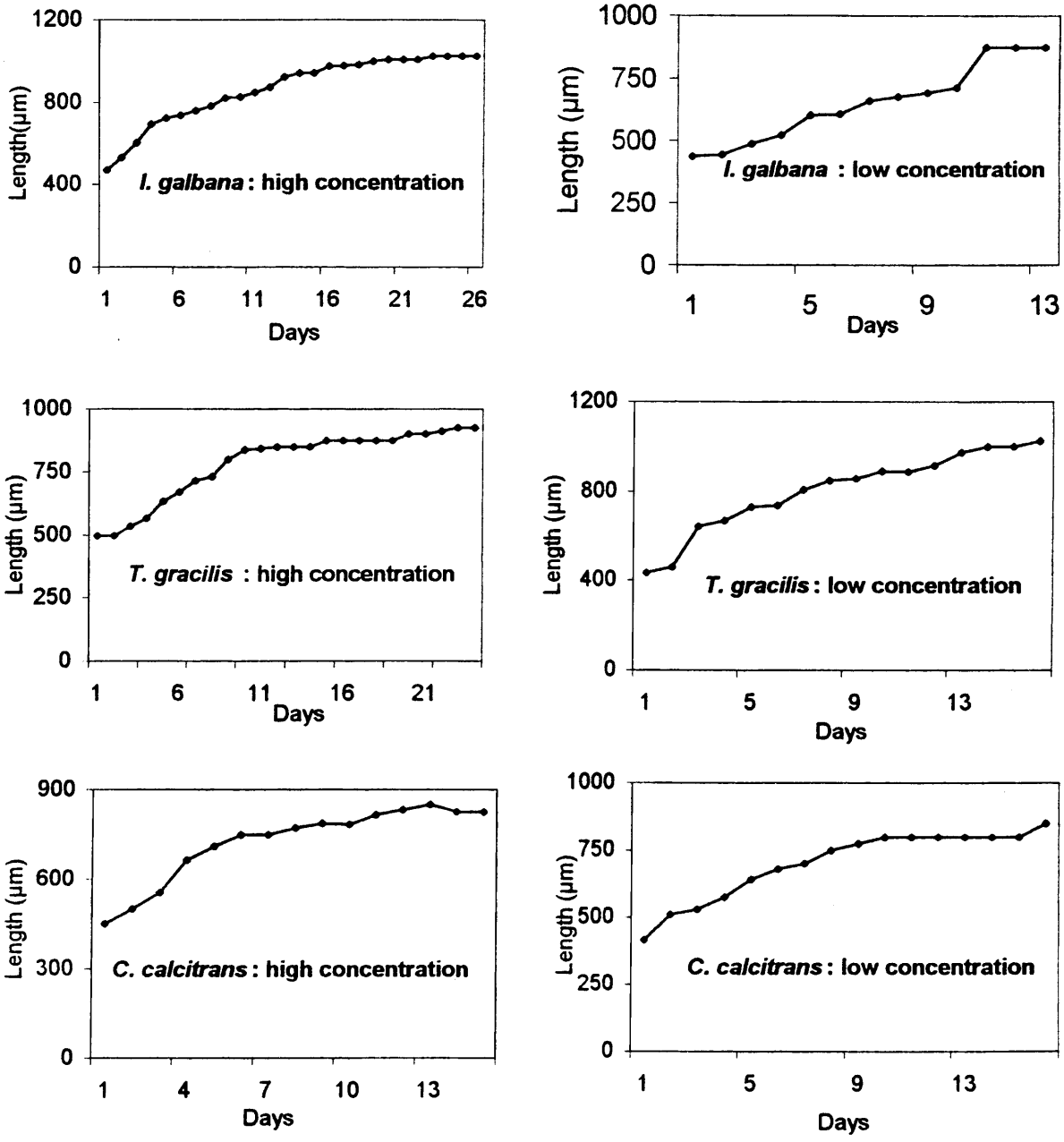

Table 2 *Diaphanosoma celebensis*. Mean age and size of primiparous females and size, life span and neonate production of adult females fed on three species of microalgae. Range in parentheses. Concentrations see "Materials and methods"

Diet	Primiparous female		Adult female		Neonate	
	Age (d)	Length (µm)	Life span (d)	Body length (µm)	Total neonates female^{-1}	Length (µm)
Isochrysis galbana						
Low conc.	5.0 (5)	605 (600–625)	6.2 (2–13)	595 (425–875)	1.5 (1–3)	416.6 (375–450)
High conc.	5.4 (4–8)	726 (675–800)	11.1 (1–26)	826 (425–1025)	10.9 (1–25)	426.8 (350–550)
Tetraselmis gracilis						
Low conc.	5.0 (5)	731 (650–750)	10.6 (2–16)	872 (400–1025)	8.5 (1–13)	436.3 (325–525)
High conc.	9.6 (8–11)	803 (700–875)	12.9 (2–24)	797 (450–925)	2.1 (1–4)	410.0 (325–475)
Chaetoceros calcitrans						
Low conc.	7.6 (7–9)	730 (675–775)	8 (4–16)	745 (400–850)	1.7 (1–3)	416.6 (400–450)
High conc.	7.1 (5–9)	750 (700–800)	8 (1–15)	733 (450–825)	2.8 (1–7)	440.0 (375–525)

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Fig. 3 *Diaphanosoma celebensis*. Daily growth in two concentrations of three microalgal diets (*Isochrysis galbana*, *Tetraselmis gracilis*, *Chaetoceros calcitrans*)

Tetraselmis gracilis, survival was better initially at both concentrations, but longevity was enhanced in the high concentration treatment (24 d). With *Chaetoceros calcitrans*, cladoceran survival was better in low density treatments in the initial stages of the experiment, but the surviving population was very small after Day 9. At high concentrations, gradual diminishing of the population resulted in a longer life span (up to 15 d) for a small percentage (20%) of the population. The results thus indicate that higher concentrations of algae had a positive influence on the life span of parthenogenetic females. Mann–Whitney's *U*-test indicated that growth, life span and survival differed significantly ($p < 0.05$) between the two concentrations of each diet.

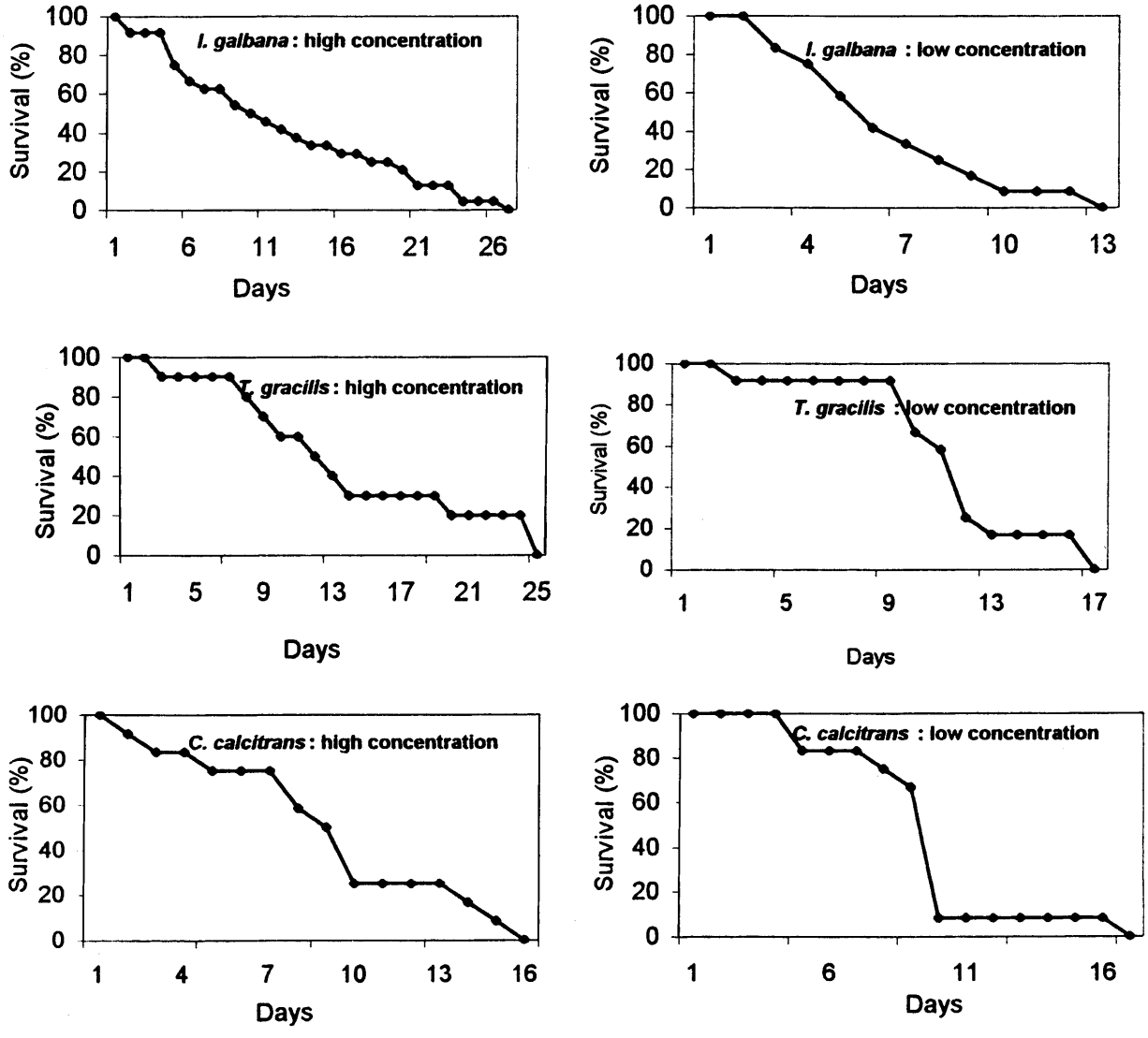
The rate of neonate production also appears to be influenced by the algal species and cell concentrations, with a direct bearing on their survival and longevity (Table 2). Longer females generally produced more neonates. With *Isochrysis galbana*, the maximum number of neonates (up to 25 neonates, mean 10.9) was produced by the females fed with a high cell density, while the reverse was true with *Tetraselmis gracilis*. However, no significant difference was noted with *Chaetoceros calcitrans*. One-way ANOVA showed that growth, life span, survival and neonate production differed significantly ($p < 0.05$) with diet.

Discussion

Growth and neonate production in relation to generations

Diaphanosoma celebensis, like *Penilia avirostris*, belongs to the family Sididae and is likewise a filter feeder. Its

Fig. 4 *Diaphanosoma celebensis*. Survival in two concentrations of three microalgal diets (*Isochrysis galbana*, *Tetraselmis gracilis*, *Chaetoceros calcitrans*)



morphology is also similar to that of *P. avirostris*. In his description of the subclass Branchiopoda, Kaestner (1970) described the filter-feeding cladocerans as individuals with six pairs of leaf-like trunk limbs enclosed within a carapace. *D. celebensis* also has a carapace enclosing six pairs of appendages. In *P. avirostris* the vibration of the trunk limbs creates a water current, due to which the food particles are transferred to its food groove and are pushed into the mouth (Zhong et al. 1989). We observed that *D. celebensis* also produces a similar type of water current with the help of its trunk limbs while feeding.

In the three successive generations maintained in the laboratory, only apomixis by amictic females was observed. This agrees with the earlier observations of Segawa and Yang (1987, 1988, 1990) on the same species collected from regions of southeastern Asia. *Diaphanosoma excisum* has been reported (Hart 1992) to thrive in waters carrying suspended sediments, even at very low concentrations of food, suggesting that species belonging to this genus are adapted to live in turbid environments. This, coupled with the preference for low salinity, could be one of the reasons for the sudden appearance of this species in the study area immediately after the onset of the southwest monsoon, when the riverine discharge turns the estuary turbid.

Maturation of neonates into parthenogenetic females is probably an indication of the availability of an optimum quantity of food, because parthenogenetic reproduction in tropical cladocerans is normally triggered by the availability of food (Vijayalakshmi and Venugopal 1972). Sexual males of Cladocera have been reported to occur suddenly after a series of parthenogenetic generations (Zhong et al. 1989). In the present study also, three sexual males were encountered during the later part of the study while being mass reared. Studies have been initiated to examine the reasons for this phenomenon. However, no sexual females were encountered throughout the study.

Growth was found to be faster during the initial stages in all three generations, when moulting was also observed to be frequent. This supports the earlier observation of Kaestner (1970) that young cladocerans moult more frequently than adults. Increased mean body length and increased production of neonates over the generations may be attributable to the acclimatisation under laboratory conditions and the plentiful availability of food. The developmental cycle and the release of neonates in podonid cladocerans have been reported to follow a distinct diel rhythm (Bryan 1979; Egloff et al. 1997), and, in *Diaphanosoma celebensis*, neonate release and moulting occurred almost simultaneously in the early hours of the day.

Feeding and neonate production

Paffenhöfer and Orcutt (1986) have reported a decrease in feeding activity of *Penilia avirostris* at very high concentrations of *Isochrysis galbana* and also low survival, poor growth and reproductive failure at very low concentrations. In the present study individuals were reproductively active with all phytoplankton diets, regardless of cell concentration, as low concentration did not inhibit maturity attainment of the individuals. Similar behaviour was exhibited by *Diaphanosoma excisum* at very low concentrations of suspended particles (Hart 1992). However, the rate of neonate production was found to be influenced by the type of alga and the cell concentration, indicating that quality and quantity of food are important factors in controlling the reproductive biology of *D. celebensis*, as is generally observed in other crustaceans (Provasoli et al. 1970).

Of the three algae tested as food sources, neonate production and life span remained low in those fed with *Chaetoceros calcitrans*, irrespective of cell concentration. Grazing experiments using *C. calcitrans* (Achuthankutty et al. unpublished data) showed that although the hourly ingestion rate was quite high, the pigment concentration in the gut (estimated fluorometrically) was low, indicating that the ingested cells were probably egested without proper assimilation. This suggests that *C. calcitrans* may not be a favoured food of *Diaphanosoma celebensis*. One of the reasons could be the difficulty in digesting the siliceous cell wall of this diatom. Many herbivorous zooplankton do not prefer spiny, chain-forming diatoms (Raymont 1983) a group to which *C. calcitrans* belongs.

On the other hand, low cell concentrations of *Isochrysis galbana* and *Tetraselmis gracilis* induced primiparity earlier than high concentrations. This indicates that *Diaphanosoma celebensis* is able to digest and assimilate food better at low cell concentrations than at high concentrations. This behaviour of zooplankton, where by the ingestion rate exceeds the assimilation rate at high food concentrations is termed superfluous feeding (Petipa 1964; Marshall 1973). Decrease in the feeding rate of the neonates at higher concentrations of algae may also be the result of clogging of the feeding appendages.

Although somatic growth in the cladocerans did not vary much with diet, the age of parthenogenetic maturity and neonate production varied. The somatic growth was faster with all diets in the initial phases of the life cycle, as was evident from the short and frequent intermoult periods. Egg production in zooplankton is generally used as a criterion for evaluating the suitability of a particular species of phytoplankton as a food source for zooplankton (Raymont 1983). Considering the total number of neonate produced during this study, *Isochrysis galbana* can be considered the most suitable food for *Diaphanosoma celebensis*. Optimal temperature and nutritionally rich food favour high parthenogenetic fertility in cladocerans (Onbé 1977; Madhupratap et al. 1996). Therefore, the variations observed in primiparity and neonate production in the present experiments could have been influenced by the nutritional quality of the microalgal feed.

Penilia avirostris prefers to feed on small-sized particles (Gore 1980). The same appears to be true of

Diaphanosoma celebensis, because among the three algae tested, *Isochrysis galbana* cells (diameter 4 μm) were preferred over *Tetragelmis gracilis* (diameter 12.75 μm), whereas the least preference was shown for the larger, chain-forming siliceous cells of *Chaetoceros calcitrans* (chain size of 12.5 to 30 μm). The results therefore support the observation that sidid cladocerans are generally adapted to filter-feed on smaller phytoplankton.

Short regeneration time, successful rearing and maintenance of several generations of this species in the laboratory open up several potential applications, particularly in aquaculture, where *Diaphanosoma celebensis* could substitute or supplement presently used natural live feed in hatcheries. Pandian (1994) stated that since the cladocerans have the ability to allocate large fractions of assimilated energy to reproduction, all effort must be made to cultivate them as food organisms for aquaculture animals. However, candidates must be selected only after a proper evaluation of their biochemical and organic constituents. Besides the three test generations, the species has been maintained successfully for more than 20 months under laboratory conditions, and efforts are being made to standardise appropriate techniques for mass rearing. Studies have also been initiated to evaluate the role of salinity on growth and reproductive maturity and to identify the chemical stimulus (if any) influencing the preference for feeding on a particular alga.

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