THE INFLUENCE OF FOOD AND TEMPERATURE ON THE LIFE CYCLE CHARACTERISTICS OF TROPICAL CLADOCERAN SPECIES FROM KALAWEWA RESERVOIR, SRI LANKA

by

Yalagalage Nuwanrasie Amaramali JAYATUNGA

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Department of Zoology, Royal Holloway and Bedford New College

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DEDICATION

This thesis is dedicated to my husband, H. G. D. JAYATUNGA and my daughter, SEWWANDI, both of whom have lost two years of family life whilst I was in England working on this thesis.

ABSTRACT

This study analyses the effect of food concentration and temperature on growth, body size, development and reproduction on tropical cladoceran species: Diaphanosoma excisum, Moina micrura, Daphnia lumholzi and Ceriodaphnia cornuta from Kalawewa Reservoir in Sri Lanka. A review of relevant literature is given which deals with tropical plankton ecology, with ecological factors affecting growth, body size, development and reproduction and with the structure and functioning of cladoceran filtering limbs. The contents of this thesis falls into three parts of which the first contributes the major portion: experimental, field and the application of experimental findings to field data.

Long term growth experiments were carried out at three temperatures and six food concentrations. Animals were examined daily from neonate to fifth adult instar. Organic carbon content of different-sized animals which had been reared on different food concentrations were analysed. Computed length/carbon weight relationships were applied to measured lengths in order to obtain body size in terms of weight. Different growth curves were fitted to obtain the best fit at different food-temperature combinations. The growth rates for the juvenile, the primiparous female and fifth instar adult were computed for defined food-temperature combinations and compared. Significant relationships are given on embryonic and post-embryonic development with food quantity and temperature, both separately and in combination. The effect of food level and temperature on

reproduction were evaluated in relation to age and stage at maturity, fecundity and on the size of the young produced. The filtering structure was examined for a possible explanation of the observed differences in life-cycle characteristics of the four species.

The nutritional level of the reservoir was estimated four times in terms of total particulate carbon and chlorophyll "a" content. The field population was examined to determine the length/carbon weight relationships, the size of the primipara and the size of the neonates.

The field data was related with the experimental findings to see the possibility of applying an indirect method of predicting the field nutritional conditions.

The interpretations of the findings were discussed finally, particularly in relation to the comparisons between temperate and tropical species.

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CHAPTER 1

INTRODUCTION

1.1 AIM OF STUDY

Zooplankton production estimates for tropical freshwater bodies are still in their infancy, although information is available on species composition, distribution and seasonal variation. In order to estimate the species population it is necessary to know specific life-cycle parameters such as growth, duration of development and reproduction, as well as how these parameters are affected by a number of simultaneously occurring biotic and abiotic factors in the environment. According to Bottrell et.al. (1976) a knowledge of the accuracy and comparability of these basic parameters is essential if estimates of biomass and production are to be accurate and comparable. Due to the paucity of experimental work on tropical zooplankton species it is not yet possible to obtain good estimates of their biomass and production. Further, according to Krebs (1978), "In order to live in a given environment, an organism must be able to survive, grow and reproduce and consequently the physiological ecologist must try to measure the effect of environmental factors on survival growth and reproduction". Resource availability and temperature are major environmental factors which influence the adaptations of aquatic invertebrates. A number of researchers have evaluated the effects of either temperature or food concentration on life-history parameters of zooplankton species and these studies are discussed below. In these studies the

effects of temperature or food level have been examined either by looking at field data, or by experimental manipulation of one factor while holding the other factor constant. However, in nature a planktonic animal living in a body of freshwater experiences a wide range of food concentrations and temperatures both seasonally and diurnally, when migrating through a stratified water column and hence the sim ultaneous effects of both temperature and food concentration need to be studied. If temperature effects change according to resource availability, then commonly used mathematical functions, which only related to temperature, determined using well-fed animals, will not be accurate under food-limited conditions due to both low food quantity and poor food quality. Therefore, knowledge based on experimental investigation of the simultaneous effects of temperature and food concentration, and the mathematical relationships including both these factors, on life-cycle characteristics of freshwater zooplankters are important in understanding the events which take place in nature.

In order to contribute information to our, as yet, sparse knowledge of the effects of environmental factors on tropical zooplankters, experimental work was carried out in Sri-Lanka to evaluate the simultaneous effects of temperature and food concentration on four tropical cladoceran species under well defined food and temperature conditions. Further, an attempt was made to apply the experimental findings to a natural population in order to understand its nutritive status.

1.2 FRESHWATERS IN SRI LANKA AND INFORMATION ON THEIR ZOOPLANKTON

Sri Lanka is a country that lies within the tropical forest belt. In the past her economy has been based mainly on agriculture and even today it is understood that agricultural development has to remain one of the cornerstones of the island's economy. However, the climatic pattern of the island has required special approaches to water storage and supply.

The pattern of rainfall over the island is not uniform. A large part of the country is exposed to drought conditions for much of the year. On the basis of its rainfall the island is divided into dry and wet zones (Fig 2.1; Domras, 1974). The dry zone receives high rainfall only during the north-east monsoon (October - January) (Dobesch, 1983).

During the time of the Sinhala kingdom which extended back to about 4,000 years BP (Brohier, 1934) a large number of reservoirs were constructed in the dry zone (Fig 2.1) to collect water during the rains and make it available for irrigation during the drought period. However, over the years the reservoirs fell into disrepair and the paddy lands gradually returned to forest. During the past few decades agricultural practice has been changed once more. There has been a planned programme of reconstructing and returning to use the very numerous reservoirs and irrigation systems of the past. While continuing to exploit and expand the plantation economy . . of the past century, a great effort has been mounted in order to make the country self-sufficient in cereals and in other food crops. In addition to restoring old reservoirs, many new irrigation works have been

undertaken. One of these and certainly the biggest of them is the Mahaweli Diversion Scheme. Under this scheme the Mahaweli River (the longest river in the island - 335 km in length) is to be diverted and a network of reservoirs (new and old), rivers and channels brought together into a massive system serving for irrigation and power generation.

This new irrigation scheme has changed the hydrological regimes of the reservoirs. In addition, increasing numbers of settlements and intensification of land cultivation will increase the accumulation of nutrients, pesticides and silt through land erosion in the reservoir catchments and these will affect the aquatic biota. On the other hand, the larger number of water bodies increases habitat for fish production. As Schiemer (1983) states "In order to master the forthcoming problems of eutrophication and pollution and to allow a successful integration of the multiple uses of the reservoirs (irrigation, fisheries, recreation, washing facilities, pastures for live-stock etc.), a profound insight into how they are functioning is indispensable". The only whole-ecosystem study that has been carried out on Sri-Lankan reservoirs was that on Parakramas Samudra by a research party which included scientists from Austria, England, West Germany and the Netherlands in collaboration with scientists from Sri-Lanka. The results of this study are given as 21 papers in the book "Limnology of Parakrama Samudra" (Schiemer, ed., 1983). The study includes detailed water and energy budgets (Dobesch, 1983), phosphorous dynamics (Gunatilaka, 1983), primary productivity (Dokulil

et al., 1983), composition density and distribution of zooplankton (Fernando & Rajapaksa, 1983; Duncan, 1983; Duncan & Gulati, 1983), as well as experimental work on their feeding and duration of development (Duncan, 1983; Duncan & Gulati, 1983), the sediment and benthic community (Newrkla, 1983), feeding ecology, feeding apparatus, body composition and reproductive strategies of fish (Schiemer & Hofer, 1983; Hofer & Schiemer, 1983; Dokulil, 1983; Adamicka, 1983; De Silva et al. 1983) and finally the ecology of cormorants (Winkler, 1983).

There are no natural lakes in the island and at the present approximately 3,500 reservoirs are in existence (Schiemer, 1983) covering an area of 1,250 km^2 in an island which covers 66,000 km^2. Despite this large number of reservoirs in relation to its small area the biology of these water bodies, with the exception of Parakrama Samudra, are very poorly known. The species composition and distribution of the zooplankton fauna is better known for Sri Lanka than in many other tropical countries. The earliest work by Brady (1886) and the past literature is reviewed in Fernando (1980a). Other than this only a handful of information is available on the zooplankton ecology of Sri Lankan reservoirs. There have been three investigations of seasonal variation in zooplankton: that on Beira Lake (Costa & DeSilva, 1978), which is in the wet zone of the country, only considered the seasonal variation major groups, Cladocera, Copepoda, Rotifera and Nauplii; that on Kalawewa reservoir (Jayatunga, 1982), which is part of the irrigation network of the Mahaweli diversion scheme and for which an attempt was made to examine the

effect of environmental factors on seasonal variation and percentage composition of the zooplanton at a species level; that on Parakrama Samudra (Fernando and Rajapakse, 1983), which also belongs to the Mahaweli diversion scheme. In this last study, the pattern of seasonal variation in major groups (Rotifera and Crustacea) as well as total zooplankton were studied for a period of three years and variations in the species composition over a period of 25 years were indicated. There have been only two investigations of diurnal variation in zooplankton, one on Kalawewa reservoir (Jayatunga, 1982) and one on Parakrama Samudra reservoir (Duncan & Gulati, 1983). In addition to these the only information available on the zooplankton of Sri Lankan water bodies is that from the joint research programme on Parakrama Samudra mentioned above. It is thus clear that there are a large number of water bodies in the island that require investigations of their secondary production, particularly if they are to provide an efficient harvest of freshwater fish.

1.3 EXPERIMENTAL WORK ON OTHER TROPICAL ZOOPLANKTON

From tropical Africa there is experimental information on the copepods and cladocerans of Lake Chad in relation to the effects of temperature on both their embryonic and post-embryonic duration (Gras, 1970; Gras & Saint-Jean, 1969; 1976; 1978; 1981; Leveque & Saint-Jean, 1983) and there is information on the effect of temperature on only the egg development time of Thermocyclops hyalinus from Lake George, Uganda (Burgis, 1971). This latter study was carried out over a temperature range of

15°-40°C in which an increase in egg development rate was demonstrated up to an optimal temperature of 32.5°C. The rate then fell with further increases in temperature and 100% mortality was observed at 40°C. The effect of temperature on the post-embryonic duration of the very similar copepod, Thermocyclops neglectus was studied by Magadza and Mukwena (1979) from Lake Mcllwaine, Rhodesia. In this study the optimum temperature for development was found to be 26.5°C for nauplii, 25°C for the copepodite stages and 25.5°C for the duration of complete development from nauplius to adult. These are lower temperatures than the optimum found for embryonic duration of the similar species from Lake George and Magazda and Mukwena (1979) suggested that this may be due to the acclimatization of growth rates to local climates with equatorial forms showing higher developmental temperature. Magazda (1977) also investigated the embryonic and post-embryonic duration in relation to temperature of Moina dubia from a stabilization pond at Lusaka Manchinchi Sewage Works in Zambia, but the food quality and quantity he used was not defined.

There is a series of life-cycle studies of tropical cladoceran species from Indian waters which evaluate instar duration, juvenile period, egg production and growth in terms of length with relation to instar number. These studies were done on Ceriodaphnia cornuta (Michael, 1962; Murugan, 1975a), Moina micrura (Murugan, 1975b), Simocephalus acutirostratus (Murugan & Sivaramakrishnan, 1976), Daphnia carinata (Navaneethakrishnan & Michael, 1971) and Scapholeberis kingi (Murugan &

Sivaramakrishnan, 1976). All these studies were conducted using filtered natural pond water in which neither quality nor quantity was defined and with temperature fluctuations of 2-3°C. Due to these two drawbacks the comparisions made by the above authors both interspecifically and intraspecifically are invalid. Further, the suggestion by Murugan (1975a) that the differences in life-cycle characteristics of Ceriodaphnia cornuta were due to latitudinal differences can no longer be accepted because these studies were not conducted under comparable conditions. Venkataraman & Job (1980) made an attempt to evaluate the effect of temperature on life-cycle characteristics of Daphnia carinata from a seasonal pond in Puliangutam, India, by conducting the experiments at constant temperatures of 10° and 35°C, feeding with same food (50µm filtered pond water) though not quantified. Therefore there is a possibility of comparing the results within the experiments but the findings cannot be generalized as food is not quantified and hence there is a possibility that these experimental animals were food limited. In two recent papers on the growth and development of Moina micrura (Jana & Pal, 1985a) and Daphnia carinata (Jana & Pal, 1985b) under various circumstances, the authors report results obtained using various organic wastes (cow dung, rice bran) as culture media. Although these media were defined in terms of their nitrogen and carbon contents in earlier papers (Jana & Pal, 1983a,b) their results were not directly related to the supply of these resources.

For tropical rotifers, there is experimental information on the influence of temperature on the duration of egg development of Brachionus species and on feeding studies with natural food particles (Duncan and Gulati, 1983b). Both these studies used animals from Parakrama Samudra reservoir in Sri Lanka.

Piyasiri (1984) provided valuable comparable information on the ecology of tropical zooplankton by evaluating the simultaneous effect of food concentration and temperature on the growth and development of Phyllodiaptomus annae under well defined conditions.

From examination of the available literature it is evident that our experimental knowledge of tropical zooplankton species is far behind that for species of temperate regions. Limnological information from the temperate zones is not directly applicable to tropical aquatic systems because the structure and annual cycles of tropical aquatic animals, as well as conditions of temperature and light, rainfall patterns and nutrient situations are often very different. Therefore more information obtained under controlled experimental conditions is required for tropical zooplankton species in order to understand secondary production in tropical freshwaters.

1.4 THE EFFECTS OF ENVIRONMENTAL FACTORS ON LIFE-CYCLE CHARACTERISTICS

Water temperature and food supply are the two most influential environmental factors affecting the characteristics of cladoceran life-cycles. A great deal of work has concerned the effects of temperature but there has been much less work done on the effects of food supply. It is now known that both quantity and quality of the food supply are important. In experimental conditions it is possible to provide the animals with food that is known to be of good quality (ie. will enable

them to achieve maximal rates of growth and reproduction providing other conditions are favourable), but in natural conditions the animals have available a wide variety of potential food organisms which may or may not be "good food" and it is difficult for the investigator to be sure whether or not the animals can utilise the food available. In the last fifteen years a number of studies have been published which address this problem.

1.4.1. FOOD QUALITY

The quality of particulate food for filter-feeding planktonic herbivores is ultimately determined by the presence of essential chemical substances, such as carbo hydrates, proteins and lipids together with various micro-elements necessary for body maintenance, growth and reproduction. However, before these essential chemicals are available for internal digestion and assimilation, the food particle has to be captured by a specific feeding mechanism which has evolved to operate efficiently for dealing with particles of particular characteristics and which can be interfered with in various ways by particles not conforming with these properties. There is survival advantage for living food particles such as planktonic algae to evolve shapes and sizes that reduce the grazing pressure upon their populations by herbivores.

The whole question of the lower and upper limits of the size of ingestible particles in relation to the filtering structures of cladoceran species has been reviewed in section 1.5 and will not be considered here. It is necessary to know these limits for

particular herbivores in order to be able to assess realistically the available food quantity in a water body. It is also necessary to know whether there are algal species which by their presence can interfere with the capture of ingestible particles since this also can affect available food quantity.

There is the possibility that food cell size within the ingestible range can affect feeding rates. McMahon & Rigler (1967) determined what they called the incipient limiting food concentration for adult Daphnia magna fed on a variety of monospecific food of different cell size. The incipient limiting concentration represents that food level at which the changeover from concentration dependent to independent feeding occurred. They found that as the food cell size increased, the incipient limiting concentration declined, when expressed as numbers of cells per ml. Chalk (1981), also working with adult Daphnia magna, found a similar effect when she tested thirteen different food species of a wide range of size. The incipient limiting concentration, expressed numerically, differed for each food species. This was also true when both the limiting food concentration and the cell size was expressed in other units, such as volume or carbon weight, although the differences in the limiting concentration for various cell sizes were less. The smallest differences of all were obtained when cell size and concentration were measured in 'projected area'. Chalk interprets this in the light of a hypothesis that feeding in daphnids is a function of the filter area offered by the animals so that the incipient limiting 'areal' concentration is likely to be the same, irrespective of ingestible food size.

Filamentous shape seems to be a property that can cause such an interference by reducing filtering rates, as shown by Burns (1968) for Daphnia rosea in the presence of high densities of Anabaena filaments. The need to reject these filaments frequently resulted in an interruption of the pumping action of the thoracic appendages and so lowered the filtering rates of the animals. Other authors have recorded similar observations with filamentous algae (Webster & Peters, 1978; Porter & Orcutt, 1980). The consequence of such an interference in the feeding mechanism can be to reduce reproductive rates (Porter et.al., 1982). Infante & Abella (1985) recorded reduced growth and reproductive rates in Daphnia pulicaria and Daphnia thorata in the presence of up to 400 filaments per ml of Oscillatoria agaardi, despite the presence of an adequate quantity of good food in the form of Cryptomonas sp. Some cladocerans can avoid such interference by narrowing the gape between the two edges of the carapace, thus denying larger algae and colonies entry to the filter chamber. Gliwicz (1980) discovered this possibility when he recorded narrower carapace gapes in the field populations of Daphnia cucculata coinciding with a summer phytoplankton dominated by blue-green filaments. In laboratory experiments, when subjected to a continuously flowing suspension of Anabaena filaments ranging in concentration from 1-100 mg fresh weight per litre, adult Daphnia magna were capable of reducing their carapace gape from 300 μm to 100 μm (Gliwicz, 1980).

Another form of interference in the uptake of ingestible food particles is the presence of algae with toxic properties (Collins, 1978). Blue-green algae such as Anabaena flos-aquae and Microcystis aeruginosa have been identified with possessing such toxic properties. A toxin has been isolated from Microcystis and identified as a cyclic polypeptide which is normally not released into the water but has to be ingested in particulate form. As with non-ingestible algal shapes, the production of toxic products which can suppress populations of grazers is clearly advantageous to the algal species. Several authors have confirmed the toxic effect of monospecific suspensions of Microcystis and Anabaena on daphnids (Sirenko et al., 1976; Porter & Orcutt, 1980) but not on Ceriodaphnia reticulata or chydorid cladocerans (O'Brien & de Noyelles, 1974; Gentile & Maloney, 1969). When tested in the presence of a good food alga rather than as a monospecific suspension, single or double celled Microcystis was toxic to Daphnia magna whereas other common planktonic blue-greens (Aphanizomenon gracile or Synechococcus elongatus) were not (Lampert, 1981a,b). In the pure suspension of Microcystis, daphnids did not survive longer than 48 hours. Even very low quantities (50 $\mu gC.L^{-1}$) added to an adequate amount of a good food alga reduced growth and growth ceased in concentrations of 250 $\mu gC.L^{-1}$. The toxic effect involves ingestion of the cells since Lampert found that both the Microcystis cells and the Scenedesmus cells were filtered with equal efficiency and were not rejected. He concludes that toxicity of Microcystis is an effective defence mechanism against grazing pressure. From the point of view of the herbivore, its presence prevents an effective exploitation of ingestible food algal species.

Once eaten, the ingestible algal species may differ in their nutritional quality or may possess various defences against digestion. There is evidence that nitrogen-deficient algae and detritus lower the feeding and assimilation rate (Schindler, 1968). Yesipova (1969) found shortened developmental duration, higher growth rates and increased fecundity in Daphnia magna and Daphnia longispina when they were fed with fresh algal detritus compared with when they were fed on old detritus from the bottom of a pond. She concluded that calorific value rather than bacterial content was a major factor determining the difference in the quality of these two foods. Arnold (1971) and Lampert (1977b) found that in spite of high assimilation rates, some foods do not result in good growth and reproduction. From amongst two green algae (Scendesmus acutus, Stichococcus minutissimum), two desmids (Closterium sp., Staurastrum cf. planctonium), two diatoms (Nitzschia actinastroides, Asterionella formosa) and three blue-green species without gelatinous sheaths (Synechococcus elongatus, Microcystis cf. aeruginosus and Anabaerna cf. plantonica), Lampert found the highest assimilation rates in Daphnia pulex fed with Synechococcus but this blue-green species did not support good growth when offered on its own. Porter (1980) and Lampert (1981) suggested that such results may be due to a nutritional inadequacy or lack of some essential compounds in the food offered.

There is some recent evidence to show that the best quality of food for a species is not solely dependent on high nutritional status of the food itself but that its size and shape also

matters (Infante & Litt, 1985). These two authors found Stephanodiscus niagarae and Tabellaria fenestrata to have the highest carbon and nitrogen contents out of ten algal species they investigated from Lake Washington but Daphnia pulicaria and Daphnia thorata showed their highest egg production and increase in biomass with Cryptomonas erosa and Stephanosdiscus hantzschii. The authors suggest that this could be due to their single-cell form and their size, despite their low content of carbon and nitrogen.

In the experiments reported here, the food has been the same throughout. Scenedesmus acutus has been shown to be a good quality food for daphnids compared with the other algal species tested (Lefevre, 1942; Lampert, 1977b) and only bettered by species of Cryptomonas (Infante & Litt, 1985; Duncan, personal communication). It is a small, green alga of simple shape and without mucilage or a thick cell wall(10.34 x 4.68 μm; 102.39 μm^3; Rocha, 1983). Chemically and in picograms, Rocha (1983), working with the same laboratory culture, records 19.74 ash free dry weight, 0.49 chlorophyll "a", 11.78 total organic carbon, 2.70 total organic nitrogen, 4.83 Biuret protein and 16.88 protein calculated as 6.25N. Thus the experimental variables were confined to food quantity and temperature both of which were accurately controlled and kept constant throughout each experiment (Chapter 3). There is now a large body of literature concerning the effects of both these factors on the life cycle characteristics of temperate cladocerans. Relatively few of these previous studies consider the simultaneous effects of food quantity and temperature (Rocha, 1983; Piyasiri, 1983; Orcutt & Porter, 1984) and none of them concern tropical species of Cladocera.

1.4.2 FOOD QUANTITY

The effects of quantity of food, assumed to be ingestible, digestible and nutritionally adequate, on the feeding rates of cladocerans has been well worked by a large number of authors. There is also more known about this in daphids than in any other cladoceran family. Rigler and his co-workers introduced the concept of 'incipient limiting food concentration' (McMahon & Rigler, 1965) to define the food level at which dependency on food ceases and the feeding rate remains maximal. As has been mentioned earlier, this level varies with food cell size when measured as numbers per ml, but less so when estimated in a more functionally relevant form (Chalk's (1981) "projected area"). It has proved more difficult to define the lowest food concentration at which feeding occurs, despite its obvious ecological importance, particularly in oligotrophic waters. In marine planktonic crustaceans, several authors have been able to demonstrate a feeding threshold concentration below which several species of calanoid copepods stop filtering (Adam & Steele, 1966; Parsons et al., 1967; Gamble, 1978) but in the two freshwater forms studied, Daphnia longispina and Eudiaptomus gracilis, such a cessation does not occur (Lampert, 1980) and these two species carried on filtering in very low food concentrations until exhausted.

Another approach to threshold food concentrations was introduced by Lampert & Schober (1980) when they defined this as the concentration at which an individual animal can just balance its metabolic losses so that it cannot and does not grow but yet

does not lose body weight. Both facets of production, body growth and reproduction, are zero. Such threshold food concentration for growth or for reproduction can be extrapolated from laboratory experiments at different food concentrations, hopefully ranging from food-limiting to non food-limiting levels. The linear relationship between growth rate or reproductive rate and food concentration in the limiting food concentration range can be used to determine the threshold food concentration for growth or reproduction. Lampert & Schober (1980) provide an example of such a calculation for experimental adult Daphnia pulex at 20°C. With Scenesdemus acutus as food, the threshold concentration for growth ranged from 0.04 to 0.12 $mgC.L^{-1}$ but was higher though still low when the less assimilable diatom, Nitzschia, was provided as food. These results were derived from short-term radio-tracer experiments reported by Lampert in his 1977 paper. A second experimental example is given by Lampert & Schober (1980), again for Daphnia pulex fed on Scenesdemus acutus at 20°C, but using egg production rates obtained during long-term growth experiments under constant food conditions in a flow-through apparatus (Lampert, 1976). The extrapolated threshold food concentration was very similar to those given for the first example. Using the only comparable data available at the time, Lampert & Schober have fitted on the same graph Peter's (1972) egg production rates for the much smaller Daphnia rosea, fed at 20°C on yeast. The maximal egg production rate in this species is lower than in Daphnia pulex and the threshold food concentration is lower. This kind of comparison of threshold food concentrations is clearly important in determining the outcome of competition between co-existing species for a scarce resource such as food supply.

The determination of the level of threshold food concentrations by the technique mentioned in Lampert's paper only works if a large number of food levels are investigated. The Michaelis-Monod Function, introduced into cladoceran literature by Hrbacek (1978), is more amenable to linear regression analysis when applied in its modified form and requires fewer food concentrations provided that these span the full range from limiting to non-limiting food levels. The function provides two ecological parameters: μ_{max} or the maximal rate at non-limiting by food quantity and k_s or the food concentration at which half μ_{max} is attained.

Rocha (1983) compared the primipara female absolute growth rates for three species of Daphnia using this techn ique and was able to demonstrate ecologically important differences which co-exist as populations in a local reservoir. As with the Daphnia rosea-Daphnia pulex example given above, the smallest species (Daphnia hyalina) has the lowest μ_{max} and the largest species (Daphnia magna) had the highest μ_{max}; the intermediate sized Daphnia pulicaria showed a μ_{max} that fell between these two. The values for k_s were also different in the three species. Daphnia pulicaria had the highest value for k_s (0.23-0.24 $mgC.L^{-1}$); for Daphnia magna, k_s had half of this value (0.12 $mgC.L^{-1}$) and it was lowest in Daphnia hyalina (0.02 $mgC.L^{-1}$). These are the values obtained from the 20°C experiments; the comparative pattern of response changed at the lower temperatures. Such specific differences in the growth rates in relation to food and temperature are clearly relevant to the outcome of inter-specific competition between the three co-existing species living in a temperate habitat where both food quantity and temperature varies seasonally.

Threshold food concentrations are virtually unknown for tropical species of cladocerans. Do they require higher food thresholds than temperate species to cover temperature-induced high respiratory rates or do they have thresholds similar to temperate species because of a metabolic adaptation to high temperature? There appears to be a species-body size effect in the pattern of threshold response in the temperate Daphnia studied by Rocha (1983), small species requiring lower food thresholds than larger ones under equivalent conditions. This gives small species a survival advantage in low food conditions. Is this one reason why many tropical planktonic forms are small in size? One aim of the present study is to provide some information in order to be able to answer some of these questions.

1.4.3. THE EFFECTS OF FOOD CONCENTRATION AND TEMPERATURE ON GROWTH

Until recently, the growth of cladocerans was expressed in terms of increase in length in relation to instar stage or age (Anderson, 1932; Ingle et al., 1937; Anderson & Jenkins, 1942; Green, 1956; Hrbackova-Esslova, 1966; Michael, 1962; Navaneethankrishnan & Michael, 1971; Murugan & Sivaramakrishnan, 1973; Murugan, 1975; Taylor, 1985; amongst others). In all of these studies, the pattern of growth was similar, showing a steeper increase in length during the early instars which became markedly slower after maturity. These growth patterns cannot be compared precisely, either interspecifically or intraspecifically, to evaluate the effect of environmental

factors because length is not a good comparable measure. Body shape varies interspecifically and length measurements do not account for food reserves stored in the body. Body weight instead of length is a more comparable measurement and this is discussed in detail in Chapter 4 as are the various mathematical models which can be used to analyse growth.

Rocha (1983) expressed growth in terms of increase in dry weight in relation to age and fitted her results to the Chapman-Richards growth function (Richards, 1959; Chapman, 1961). These growth curves give visual evidence of how temperature and food concentration affect the pattern of growth. Additionally, using the parameters of the Chapman-Richards function for each growth curve, it is possible to predict weight at a particular age and this allows one to evaluate growth rates more precisely. The same function has been used by Vidal (1978) to describe the growth of the marine calanoid copepods, Calanus pacificus and Pseudocalanus sp., and, by Piyasiri (1984) for the freshwater calanoid copepods, Phyllodiaptomus annae (tropical) and Arctiodiaptomus spinosus (temperate). Taylor (1985) compared the pattern of growth of Daphnia pulex and Daphnia pulicaria at 1.0 and 0.1 $mgC.L^{-1}$ by fitting the increase in length with age to von Bertalanffy's equation. She found that the growth curves for the two species were similar at each food level but it should be noted that, as lengths were used instead of weight, there may still be a tendency for body growth to differ in two species.

Like all biological processes, growth rates increase up to an optimum temperature and then decrease with further increase in temperature. (Lei & Armitage, 1980; Sutcliffe et al., 1981). Further, increase in food supply promotes growth rate (Richman, 1958; Hrbackova-Esslova, 1962; King, 1967; Kryutchkova & Sladecek, 1963; Vijverberg, 1976; Porter & Orcutt, 1980). In most of these studies, the nature of the increase is not described. In addition to the effect of temperature and food, decrease in growth rate as animals age has been recorded for daphnids by McArthur & Baillie (1929), Anderson (1932), Ingle et al. (1937), Anderson & Jenkins (1942), Green (1956), Richman (1958), Hall (1964), Hrbackova-Esslova (1962), Kryutchkova & Sladecek (1969), Buikema (1973), Lei & Clifford (1974), Lei & Armitage (1980) and Herzig (1985).

Since both temperature and food resource availability affect the rate of growth, a study of the simultaneous action of both these factors will give a more accurate picture of what occurs in nature than when each of these factors is considered alone. Rocha (1983) evaluated the effect of food and temperature in combination on the instantaneous growth rates of Daphnia magna, Daphnia pulicaria and Daphnia hyalina by examining their Q_{10} values. As would be expected (see Section 1.4.4), the higher Q_{10} values were found in the lower temperature range (5-15°C) compared with the higher temperature range (10-20°C). But she also found that Q_{10} decreased from the highest food concentration of 5 $mgC.L^{-1}$ to the lowest food concentration of 0.1 $mgC.L^{-1}$ with which she fed her animals. She records very high Q_{10} values for

the instantaneous growth rates of animals grown in non-limiting food concentrations, especially in the lower temperature range: Q_{10}s between 3.82 - 6.34. This probably reflects the species' full exploitation of an abundant food supply for growth.

The final size (measured as dry weight, length or carbon weight by different authors) of individuals reared on different foods at a particular temperature have been recorded by a number of authors, including Lang (1967), Paffenhoffer (1970), Mullin & Brooks (1970), Weglenska (1971), Montie (1976) and Vidal (1979). In this study, experiments were terminated when the animals reached the fifth instars so that their final size was not recorded.

1.4.4. THE EFFECTS OF FOOD CONCENTRATION AND TEMPERATURE ON THE DURATION OF DEVELOPMENT

(a) A review of temperature functions

It has been shown many times in the literature that temperature is a major influence on the duration of egg development in cladocerans. Earlier work on this is reviewed in Bottrell et.al. (1976) and further evidence has been given by Magadza (1977), Lei & Armitage (1980), Vijverberg (1980) and Herzig (1983, 1985). In all cases, the relationship of duration of egg development with temperature is a curvilinear one, with duration decreasing as temperature increases, or in other words, duration is a function of reciprocal temperature

D is a function of 1/T (1)

A variety of mathematical equations have been used by biologists to express how temperature affects biological rate processes and, in particular, biologists have tried to find a simple linear relationship. Many authors adopted the recommendation of Edmondson & Winberg (1971) and Winberg (1971) to use the reciprocal of duration (1/D) as the rate of development in order to quantify the temperature effect on duration of egg development. However, as Bottrell (1975) points out, the reciprocal transformation of duration is only useful if it produces a linear relationship:

$$1/D = a + bT \qquad (2)$$

where a is the intercept and b the slope, thus allowing the application of linear regression analysis. In most cases, plots of 1/D against T looked curvilinear and Bottrell (1975) found that his data for cladocerans from the River Thames departed significantly from linearity. He advocated the use of a logarithmic rather than reciprocal transformation of duration to linearise the relationship:

$$\log D = \log a + b \log T \qquad (3)$$

This had the additional bonus of normalising the original data, which is a requirement for regression analysis and one not provided by the reciprocal transformation.

Bottrell (1975) also tested two other temperature functions commonly used in biology: Krogh's Curve and the vant' Hoff-Arrhenius Function. Krogh (1914) produced an empirically derived curve against which subsequent workers compared their experimentally determined results and Edmondson & Winberg (1971)

provide a table of multipliers for calculating the theoretical time of development, based on Krogh's Curve, for temperatures ranging from 5-30°C. When the data for Thames cladocerans was compared with the theoretical curve, they deviated more and more as the temperature became higher. Bottrell found that the sums of squares of the predicted from the observed values were as high for Krogh's Curve as for equations (2) and (3) from above.

The vant' Hoff-Arrhenius Function originally came from two commonly used temperature functions developed by chemists to relate the rate of chemical reactions and temperature and taken over by biologists for the temperature relations for whole organisms. One of these was the vant' Hoff or Q_{10} Rule:

$$Q_{10} = \frac{V_1}{V_2}^{(10/T_1-T_2)} \qquad (4)$$

where V is the rate of development (=1/D); T is temperature (°C) and Q_{10} is a constant indicating the increase in rate over a 10°C rise in temperature. This equation can be re-arranged as follows:

$$V_2 = V_1 \cdot Q_{10}^{(T_1-T_2/10)} \qquad (4a)$$

generalised as

$$V = ab^T \qquad (4b)$$

and transformed to a linear equation as

$$\log V = \log a + T\log b \qquad (4c)$$

If Q_{10} is found to be constant in biological data, then a plot of the logarithm of duration against temperature (°C) will be linear. The other temperature function is the Arrhenius equation which can be stated as:

$$V_2 = V_1 \cdot e^{0.5\mu \left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \qquad (5)$$

where V is the rate of development (1/D); T is temperature (^{o}Kelvin); e is the base of natural logarithms and μ is a constant. This equation can be generalised as

$$V = a \cdot e^{b\left(\frac{1}{T}\right)} \qquad (5a)$$

and transformed to a linear equation as

$$\ln V = \ln a + b\left(\frac{1}{T}\right) \qquad (5b)$$

If μ is found to be constant in biological data, then a plot of the logarithm of duration against reciprocal temperature (^{o}K) will be linear. According to Bělehrádek (1935), temperature in degree Celsius is more or less linearly related to the reciprocal absolute temperature (^{o}K), so that Q_{10} and Arrhenius μ are not different, which is why these two temperature functions have been combined into one.

There is plenty of evidence in the literature to show that neither Q_{10} nor the Arrenhius μ appear to be constant over the biological range of temperatures, although only Bottrell (1975a) has attempted to test this by demonstrating that the relationship between the logarithm of development rate and temperature is curvilinear in eight of his nine Thames cladoceran species. Adding a quandratic term to equation 4c significantly reduced the residual mean square:

$$\log V = \log a + T\log b + T^2 \log b \qquad (6)$$

and demonstrated both that the linear form of the equation is less than adequate and that Q_{10} is not constant. In the light of these findings that the vant' Hoff-Arrhenius Function could not be applied to most of his data, Bottrell advocated the use of D rather than its reciprocal (1/D or V is the above equations) and that this should be transformed logarithmically to ensure the normality of data which is assumed in regression analysis. Two equations could be tested:

$$\log D = \log a + b \log T \qquad D = aT^{b} \qquad (3)$$

and $$\log D = \log a + T \log b \qquad D = ab^{T} \qquad (7)$$

He found that adding a quadratic term $(c(\log T)^2)$ to equation (3) gave the best fit to eight of his nine cladoceran species, although a quadratic term $(T^2 \log c)$ added to equation (7) also gave a significant curvilinear fit.

Another equation relating embryonic development time to temperature was proposed by Bělehrádek (1935):

$$D = a/(t-b)^{c} \qquad (8)$$

where D is the duration of embryonic development; t is temperature (°C) and a, b and c are constants. 'a' represents a 'scaling factor', 'b' the 'theoretical biological zero' or t_o and 'd' in other literature and c is an exponent defining the degree of curvilinearity.

This equation was mentioned but not dealt with in detail by Bottrell (1975a) but Herzig (1983) has tested its performance on copepod embryonic development times against five other equations. The data set produced by Herzig for these tests was new and very complete as he determined the embryonic development times for six freshwater species of planktonic copepods at constant temperatures ranging from 1.4°C to 27.3°C and at very short temperature intervals. Herzig found that the inverse curvilinear relationship between the duration of embryonic development and temperature in these copepods was best described by the Belehradek equation, judging by the percentage of total explained variance. However, like Bottrell, he found that quadratic equations using log-transformed D were almost equally good. He

advocates the use of Bělehrádek's equation because of the possibility of interpreting biologically the meaning of the constants a and b: 'a' scales the temperature-duration relationship and 'b' defines the 'theoretical biological zero' or the temperature at which development times approach infinity. In the context of the subject matter of this thesis, this could be important, if such biological interpretation is possible, to distinguish the temperature responses of tropical and temperate cladocerans. However, as Herzig has shown, such a comparison would require very complete data sets obtained at small temperature intervals over the whole tolerated range of temperature and this is not yet available.

(b) A literature review of food and temperature effects on the duration of development

Bottrell (1975a) found Krogh's curve and vant' Hoff-Arrhenius' function to be inadequate for expressing the relationship between duration of embryonic development to temperature in cladocerans from the River Thames. Lei & Armitage (1980) also found Krogh's curve is not reliable for predicting the time for embryonic development in Daphnia ambigua. McLaren (1963) demonstrated the usefulness of Belehradek's function for predicting the effect of temperature on development rates in marine copepods. Following upon this, several workers used this function to describe the relationship between the rate of development and temperature for copepods (McLaren, 1965; Corkett & McLaren, 1970; Corkett, 1972; Smyly, 1973; Landry, 1975), for cladocerans (Herzig, 1985), for rotifers (Herzig, 1983) and for marine amphipods (Steel & Steel, 1973, 1975).

In contrast to the above, Bottrell (1975a) and Lei & Armitage (1980) both found curvilinear logarithmic functions describe the relationship most appropriately. However, as Heip (1974) stresses, there is no theoretical justification for choosing one rather than another. As many relationships cannot be linearized and so tested by simple means, it is difficult to compare development-temperature relationships intraspecifically and interspecifically. Bottrell (1975a) suggests that curvilinear logarithmic equations are most generally applicable and can now be validated statistically. The same author goes on to say that a common slope or intercept or single line to describe the relationship for a number of species should not be used, unless it can be shown to be statistically valid. The invalidity of using single relationship applies also to egg size, as there is evidence in the literature that egg size is related to development time in cladocerans (Bottrell, 1975a; Bottrell et.al., 1976; Vijverberg, 1976; Lynch, 1980b) and in copepods (McLaren, 1965, 1966; McLaren et.al., 1969).

Food effect on duration of embryonic development has been ignored to date. Rocha (1983) found egg duration to be significantly affected by the nutritional condition of the mother. Munro & White (1975) and Bottrell et.al. (1976) both suggest that intraspecific variation in egg development time may arise from changes in egg size. This suggestion is confirmed by Rocha's (1983) findings that Daphnia magna produced larger eggs under food-limiting conditions. Orcutt & Porter (1984) have also recorded significant food-level effect as well as synergistic temperature-food effects on rates of embryonic development in Daphnia parvula.

1.4.5 THE EFFECTS OF FOOD CONCENTRATION AND TEMPERATURE ON REPRODUCTION

The two main effects that these environmental factors have on cladoceran reproduction are (a) alteration of fecundity through changes in the number and/or size of eggs, and (b) altering the size and age at which the female reaches maturity. In the literature factors affecting these characteristics in various species have been discussed in relation to resource availability, temperature and predator-prey interactions.

The immediate effect of increase in food concentration is increase in the number of eggs carried by the female (Hall, 1964; Kerfoot, 1974; Rocha, 1983; Orcutt & Porter, 1984). There is probably both a direct and an indirect effect since females tend to grow larger when well fed and large females can carry more eggs than smaller females (Green, 1956; Hall, 1964; Burgis, 1967; Kerfoot, 1974). In daphnids at least, food limitation not only decreases the number of eggs per female but the few eggs tend to be larger under these circumstances (Rocha, 1983).

Kerfoot (1974) also found a negative relationship between egg size and temperature in a population of Bosmina longirostris, but no such correlation in other species inhabiting the same water body. Burgis (1967) recorded a higher volume of eggs in Ceriodaphnia pulchella at 13°C than at 20°C and also showed that the same sized females had more, smaller eggs in Ceriodaphnia pulchella than in Ceriodaphnia reticulata. However, in these studies the simultaneous effects of food concentration were not measured.

When comparing the reproductive output of daphnids grown under natural and enriched (algae added to natural food) food conditions Green, (1954), Hrbáčková (1963, 1974) and Kerfoot (1974) found larger primipara in enriched food than in natural food. It is possible that the natural food may have contained inedible particles, and food quality in the natural situation may not have been so good as that provided in the experiments.

Rocha (1983) did study temperature and food concentration effects simultaneously and she found a direct relationship between the size of the primipara and food concentration in three species of daphnids. Primipara size also tended to increase with decrease in temperature when they were fed on limiting concentrations of 0.1 and 0.01 $\mu gC.L^{-1}$, but she found no simple relationship at higher non-limiting food concentrations.

There is a very extensive literature concerned with the possible effects of predation on cladoceran life-cycle characteristics. One of the earliest such studies was that of Hrbáček & Hrbáčková (1960) who, in observing different species of Daphnia from ponds with different fish stocks but reared under constant food and temperature conditions in the laboratory, found that those from the pond with a high fish stock matured at a smaller size, and produced smaller eggs, than those from the pond with a low fish stock. They suggested that the dwarf strains resulted from intense fish predation.

Since then many studies have confirmed that planktivorous fish tend to selectively remove the largest and most visible members of cladoceran communities (Dodson, 1972; Allen, 1973;

Lane, 1979; Lynch, 1979). Invertebrate predators such as copepods and the larvae of Chaoborus spp., prey upon smaller sized species and individuals of the same species (eg. Anderson, 1970; Confer, 1971; Brandl & Fernando, 1975; Kerfoot, 1975). Since number of eggs is related to body size, such predation must have an indirect effect on reproduction of the prey populations, but whether or not predation has a direct effect on the reproductive characteristics of individuals is more controversial. For example, Brambilla (1982) found Daphnia pulex reaching maturity (primipara) at a smaller size and with fewer, smaller eggs at 18°C, late in the season than earlier in the season at 10°C. He showed that early in the season mortality was due to invertebrate predation on animals <0.75 mm, which were roughly the first and second instar. Hence, larger eggs enabled these two instars to be completed more quickly than when eggs were smaller. In the late season Amb$\overset{\iota}{\text{y}}$stoma larvae preyed on the larger sizes and at the same time Diacyclops predated the animals <0.5 mm, which is about first instar. As the animals left that instar the mortality risk due to Diacyclops was removed, so he suggested that reduced primipara size may have evolved to reduce mortality by Amb$\overset{\iota}{\text{y}}$stoma. If this suggestion is correct, it seems odd, since a smaller neonate is not a prerequisite for a smaller primipara, that it does not counter the Diacyclops predation by having larger neonates (eggs), and Amb$\overset{\iota}{\text{y}}$stoma predation by producing smaller primipara instead of producing small eggs at this season. In addition, population survival would be increased by having more eggs, whereas fewer were recorded. Consideration

of possible food effects is quite impossible in this study because the cell counts given do not indicate whether the animals were food limited or not, at this particular period. Since it is evident from the literature that fecundity is food dependant it is interesting to consider Brambilla's (1982) field observations in the light of Rocha's (1983) experimental results. She showed that daphnid species exhibit an inverse relationship between temperature and egg size. Therefore the production of smaller eggs late in the season may be primarily a reaction to higher summer temperature. It is thus clear that it is important to consider not only predator-prey interactions, but other possible influences which might have operated at the same time.

Other recent studies which do combine field observations with experimental evidence show more clearly that changes in the body size of neonates and primipara are not always a result of predation even when predators are present in the environment: some species avoid predation by migration. Threlkeld (1980) showed that in spite of size-selective predation some cladocerans show compensatory trends which are sufficient to counter its effects. He demonstrated this by considering the habitat selection of larger Daphnia pulicaria and smaller Daphnia galeata in Wintergreen Lake and carrying out in situ experiments during mid-summer. The smaller Daphnia galeata were found to be inhabiting the warm epilimnon while Daphnia pulicaria inhabited the cooler hypolimnion. The former were more susceptible to size-selective predation. The author suggested that this habitat separation merely reflected the responses of the two species,

depending on their body size, which permitted maximum population growth rates at a balance-point between predation intensity (which must vary at least with light level and prey body size), and their predator-free response to food and temperature conditions. Thus the smaller Daphnia galeata can live in the epilimnion because the positive effect of warm epilimnetic temperatures on growth may be sufficient to off-set the additional mortality which results from predation in the euphotic zone. In contrast Daphnia pulicaria tends to live in deeper water because, in spite of reduced population growth rates, light intensities are lower and predation intensity may be reduced. In the same study, comparisons of life history characteristics of the two species, in epilimnetic and hypolimnetic incubations, showed that reproductive effort and size at maturation were not different in animals incubated at different depths, for either species. In view of the possibility of such compensatory mechanisms it is clearly unwise to look at predation effects in isolation.

Following the same line of investigation, Stich & Lampert (1984) experimentally tested the growth responses of the Daphnia hyalina and Daphnia galeata, which coexist in Lake Constance, to constant and fluctuating conditions of temperature and food in a flow-through system. His results confirmed that, with respect to growth, production and reproduction, it would be advantageous for both species to stay in the warm food-rich water of the lake. This experiment provided direct support for the hypothesis that mortality due to fish predation is the factor which forces

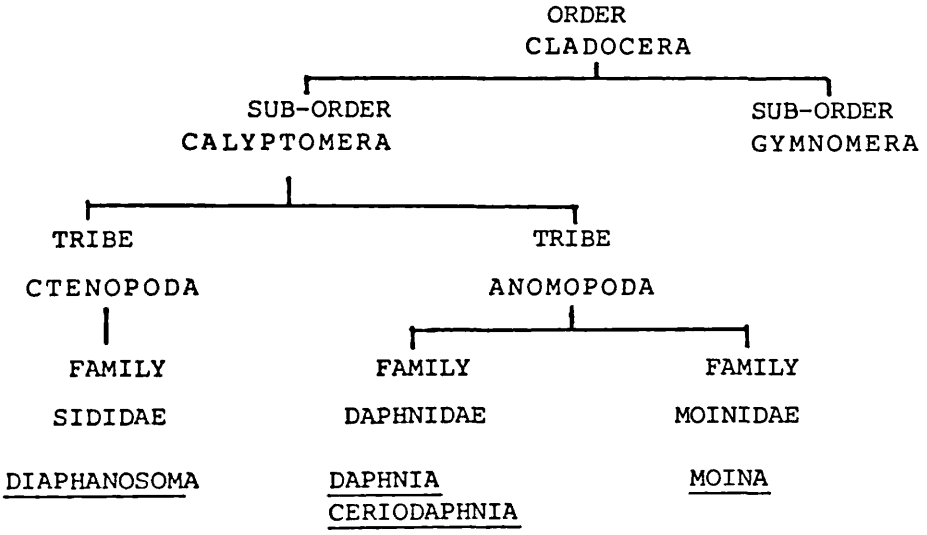
Daphnia hyalina to avoid the surface layers during the day, while Daphnia galeata does not show such diurnal migration. This suggests that some species avoid predation by migrating rather than shifting their neonate and adult body size. In such species changes in the life-cycle stages cannot be explained only by predation.

All these studies support the conclusion of Lynch (1980) that, in addition to all the basic energetic constraints, the presence of a predator in an aquatic environment may modify the advantages of a particular life history by imposing high mortality rates on a particular size-class. Therefore, interpretation of the adaptive significance of a cladoceran life history requires an evaluation of both the foraging ability, and vulnerability to predation, of different sized individuals.

1.5 FILTERING STRUCTURES OF THE CLADOCERA STUDIED

The morphology of cladoceran thoracic limbs has been extensively studied since early this century (Naumann, 1923; Cannon and Leak, 1933; Eriksson, 1935; Fryer, 1963, 1968, 1975; Smirnov, 1968, 1969, 1971; Watts & Petri, 1981; Ganf & Shiel, 1985a, b). The details of the thoracic limbs in species of Chydoridae and Macrothricidae were studied in detail by Fryer (1968 & 1975) and Smirnov (1968 & 1971). Ganf & Shiel (1985a, b) studied the functional morphology of the first three limbs of Daphnia carinata.

The thoracic limbs of different cladocera show differing degrees of complexity, and some classifications are based on these differences as shown in the diagram. Those of the tribe


ORDER
CLADOCERA
SUB-ORDER
CALYPTOMERA
SUB-ORDER
GYMNOMERA
TRIBE
CTENOPODA
TRIBE
ANOMOPODA
FAMILY
SIDIDAE
FAMILY
DAPHNIDAE
FAMILY
MOINIDAE
DIAPHANOSOMA
DAPHNIA
CERIODAPHNIA
MOINA

Ctenopoda have six pairs of thoracic limbs (Calman, 1909) the first five pairs of which have "filter" combs bearing long cylindrical setae with fine setules (Fig. 3.3) (Cannon, 1933, Geller & Muller, 1981). The filter combs are carried by the gnathobasal and distal endites of the thoracic limbs and, in the genus Diaphanosoma, these two combs are arranged in one functional unit (Cannon, 1933; Geller & Muller, 1981). The Cladocera of the tribe Anomopoda have only gnathobasal filter combs (Fig. 3.3) and in both Daphnidae and Moinidae they are situated on the third and fourth thoracic limbs (Cannon, 1933; Geller & Muller, 1981).

Although the gross morphology of cladoceran limbs has been known for a long time, studies of their ultrafine structure, using electron microscopes, have only been made within the last five years. Those in which the intersetular distance (which is assumed to determine the mesh size, Fig. 3.3) has been measured show that it differs in different species, within different populations of the same species, and in different body sizes of the same species (Korinek & Machacek, 1980; Korinek et al, 1981; Geller & Muller, 1981; Crittenden, 1981; Porter et.al., 1983; Brendelberger & Geller, 1985; Hessen, 1985; Ganf & Shiel, 1985a & b).

(a) The size of filtered particles

Geller & Muller (1981) using the size range of their filter meshes arbitrarily categorised cladocerans into three feeding groups:

(1) Those with filter meshes from 0.24 μm - 1.6 μm presumed to be highly efficient bacterial feeders;

(2) Those with filter meshes from 1.0 μm - 1.6 μm presumed to be low efficiency bacterial feeders;

(3) Presumed macrofiltrators with meshes finer than 2 μm in only a small part of their filtering areas, which would be unable to feed on suspended bacteria.

To date there is no direct evidence to support this categorization.

Since the work of Cannon (1933) until early this decade cladocerans were thought to filter food particles from the water current created by the thoracic limbs. The particles were known to be trapped by the filter combs (Fig. 3.3) whose mesh of setae and setules acted as a sieve. The "leaky sieve" model of filterfeeding put forward by Boyd (1976) implies that larger particles are trapped and smaller are lost when water containing food particles passes through the filter mesh. There is conflicting evidence as to whether or not the filtering mechanism acts in a purely mechanical sieve-like fashion or whether the animals can feed selectively.

The mechanical sieving and "leaky sieve" model of Boyd (1976), which was accepted until 1980 as a description of food capture in cladocerans was first challenged by Koehl & Strickler (1981), and subsequently by Gerriston & Porter (1982), Porter et.al. (1983) and Ganf & Shiel (1985a & b). Their calculations showed that copepods and cladocerans feed at low Reynolds Number (<1 Vogel, 1981) in a viscous environment. Their calculations indicate that the boundary layer around the setules extends across the inter-setular distance and hence predict that no water passes through the mesh. This implies that the filter combs play no role in food capture but act as solid paddles, creating water

current. In contrast, Gophen & Geller (1984) carried out feeding experiments with various size-ranges of beads, examined the gut contents of their animals and made direct observations of feeding behaviour in conjunction with electronmicroscope observations and showed that Daphnia filtering can be interpreted as a mechanical sieving. Therefore, whether or not the filter mesh plays a role in food capturing is still an unsolved problem.

Active qualitative selection of food by cladocerans is not evident since they ingest artificial particles such as plastic and latex beads, sephadex particles and micronic beads (Burns, 1968; Gerriston & Porter, 1982; Gophen & Geller, 1984; Hessen, 1985). They can thus be considered as passive feeders. The work of Hessen (1985) supports this, showing that the clearance rate of E. coli and yeast by the cladocerans he studied was similar to the clearance rate of the corresponding sized beads (1.0 & 0.5 μm) except in Bosmina. This lack of selection contrasts with the copepods where, in the same study, it was evident that Eudiaptomus gracilis avoided beads but showed a higher clearance rate with similar sized yeast, while the clearance rate with E. coli was zero. Horn (1985), aiming to find out whether copepods and cladocerans feed on algae selectively and show preference for particular food sources, provided different algae of four to seven size classes and 4-5μm nanoplankton of unknown species to Daphnia hyalina, Eudiaptomus gracilis and Cyclops vicinis. He found that the species investigated did not exhibit marked food selectivity.

There is some information on the upper size limit of particles that can be ingested by Cladocera. Burns (1968) showed that there is a direct relationship between maximum size of particles ingested and increasing body size. Gliwicz (1980) and Gliwicz & Shedlar (1980) take the view that the upper size is controlled by the animal's regulation of the carapace gape. The lower size limits of the particles ingested was not well known until recently. The size-efficiency hypothesis of Brooks & Dodson (1965) assumed that the minimum size of the food particles ingested is independent of body size. That cladocerans can retain bacteria is known from the work of Smyly & Collins (1975) on Ceriodaphnia quadrangula, McMahon & Rigler (1965) on Daphnia magna, Gophen et al. (1974) on Ceriodaphnia reticulata and Lampert (1974) on Daphnia pulex. Porter et al. (1983) and Peterson & Hobbie (1978) point out that the bacteria used in these experiments were large rods, more than 1 μm long and that natural free-living bacteria are between 0.1 - 1 μm in size. However, the experiments of both these authors, using natural bacterioplankton, showed a positive intake by daphnids.

Low efficiency of bacterial filtering rates compared to algae and yeast were demonstrated by Peterson et al. (1978) (=30% of the rate for yeast), by Porter et al. (1983) (= 26-33% of the rate for alga) and by Persson (1985) (= 1/3 - 2/3 of the rate for Chlamydomonas). In contrast to this Haney (1973) showed that Daphnia galeata and Daphnia rosea can filter out labelled bacteria (0.5 - 1.2 μm) at an average rate similar to those of yeast (3.5 μm) and algae (12-14 μm).

Gerristen & Porter (1982), Porter et.al. (1983) and Hessen (1985) recorded cladocerans feeding on particles smaller than their mesh size. Rubenstein & Koehl (1977) put forward four mechanisms by which this could be achieved (a) direct interception, (b) inertial impaction, (c) motile particle deposition, (d) gravitational deposition. The explanation given by Porter et al. (1983) was that in the presence of large particles bacteria are collected by a piggy-back mechanism. Bacteria are collected either attached to organic particles larger than the mesh size, or because the filtering appendages are already clogged, or made stickier, by the presence of larger particles. Gerristen & Porter (1982) suggest that small particles like bacteria could be captured by mechanisms such as surface charges or hydrophobic-hydrophylic interactions. On the other hand, Gophen & Geller (1984) claim that nothing more complicated than mechanical sieving is needed to explain the intake of particles greater than the mesh size. They further say that they do not neglect the possible involvement and impact of physico-chemical surface forces in particle collection, but they think that mechanical sieving is the dominant factor in filter-feeding of Daphnia and probably other Cladocera.

Egloff & Palmer (1971) were the first investigators to examine filtering area and to find a relationship between filtering area and body length. This was established for two species, Daphnia magna and Daphnia rosea and they attempted to discover to what extent difference in filtering rate relative to body length could be attributed to the differences in

relationships of filter area to body length. They used reported filtering rates for these species. Korinek & Machacek (1980), Arunda (1983), Brendelberger & Geller (1985), Ganf & Shiel (1985a) examined the relationship of filtering area to body size in daphnids generally but so far no relationship has been established for cladoceran species belonging to families other than the daphnidae. This study adds to the list of daphnid species for which such a relationship is available and also provides a relationship for species of Moina (Moinidae) and Daiphanosoma (Sididae).

CHAPTER 2

STUDY SITE AND EXPERIMENTAL SPECIES

2.1 STUDY SITE

Sri-Lanka is an island situated between 5°55' and 9° 50' North of the Equator and covers an area of 65,630 km^2 (25,352 sq miles). Kalawewa Reservoir is situated 8°25' - 7°57' N and 80°31 - 80°35'E, in the dry zone of the island at an elevation of 129m above mean sea level. (Fig 2.1). The reservoir has a total area of 28.3 km^2 and a maximum depth of 7m at full supply level. This reservoir is a unit of the Mahaweli Diversion irrigation scheme, and supplies water for agricultural purposes to certain areas in the dry zone. The reservoir receives water diverted from the Mahaweli river in addition to its catchment input and input due to precipitation. The water stored in the reservoir is distributed via 6 canals which are controlled by sluice gates. Details of the irrigation system are given in Jayatunga (1982) and a map of the reservoir in Fig 2.1.

The zooplankton fauna of this reservoir was studied for a period of over one year (Jayatunga, 1982) and recorded 4 species of Copepoda, belonging to 4 genera, 7 species of Cladocera belonging to 7 genera. 26 species of Rotifera belonging to 14 genera. In that study the dominant cladoceran species were found to be Ceriodaphnia cornuta, Diaphanosoma excisum, Moina micrura and Daphnia lumholtzi (given in the order of their general abundance). Hence these four species were selected for the present study.

Photographs of these species are given in Plates 1 and 2.

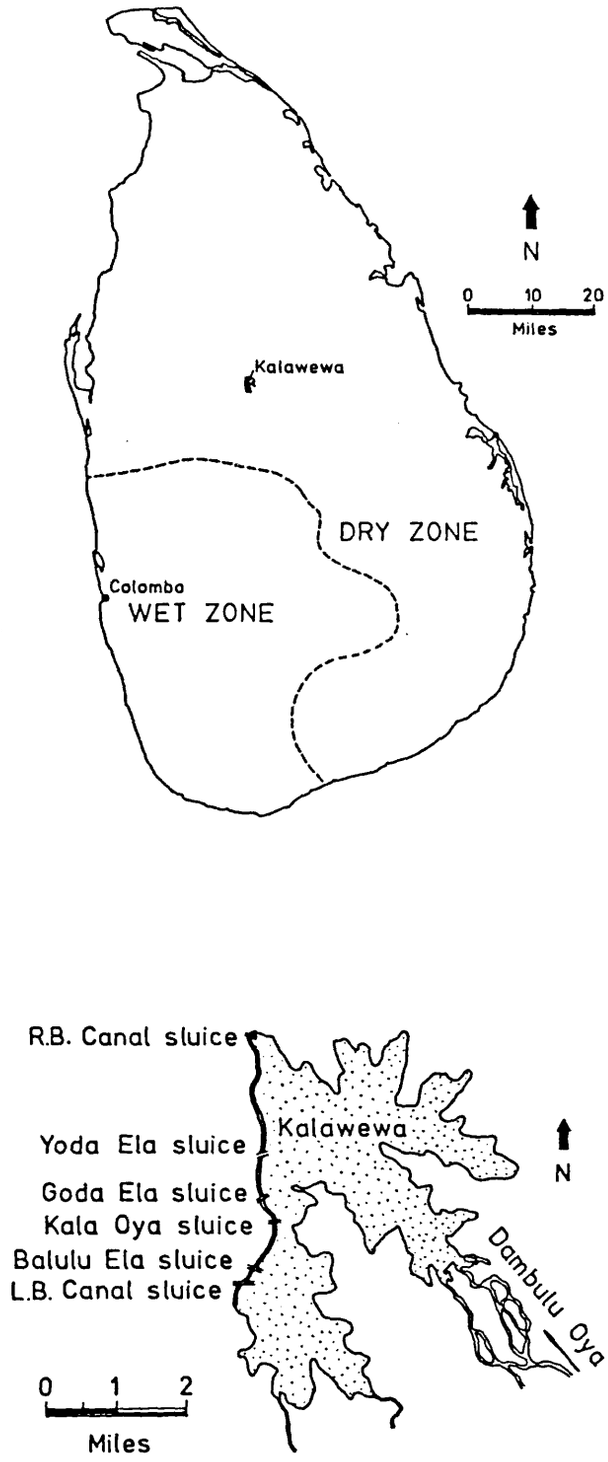


Figure 2.1 Maps of Kalawewa reservoir and its location in Sri Lanka.

2.2 IDENTITY OF THE SPECIES

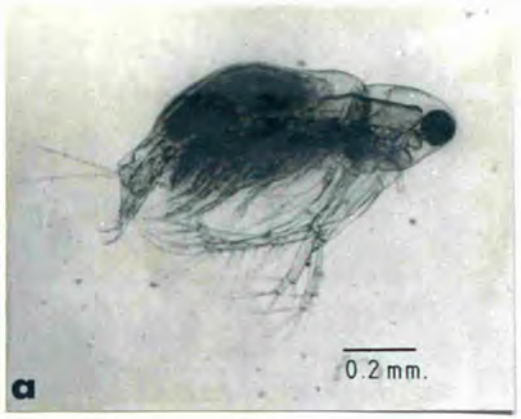
Diaphanosoma excisum

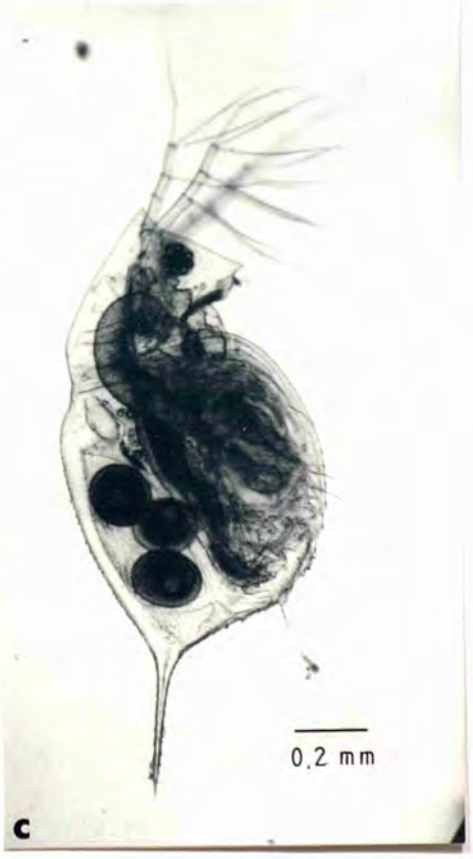
This species was originally described by Sars (1885). Rajapaksa (1983) found that Diaphanosoma excisum, collected from different localities in Sri Lanka over a period of 15 years, agreed with Sars's description. That collection included species from Kalawewa reservoir. Details of the species description are given in her paper and by Kořínek (1984). From these descriptions the species used in experiments was confirmed to be Diaphanosoma excisum.

Syn onyms

It is quoted in Rajapaksa (1983) that Daday (1898) regarded Diaphanosoma singalense from Sri Lanka as a new species. Apstein (1907 & 1910) described this species as differing from Diaphanosoma excisum due to the shape of the head, the armature of second antenna and the structure of the ventral margin of the valve. Later Bar (1924) considered Diaphanosoma singalense as a variety of Diaphanosoma excisum. In favouring Bar's conclusion, Rajapaksa (1983) noted that in Diaphanosoma excisum the number of spines of the valve and shape of the head varies greatly, even within the specimens of the same population. Kořínek (1984) also recorded changes in the dorsal shape of the head of different populations due to variable size of antennal muscles, and a variable number of marginal spines on the post-duplicature margin of the shell. According to Kořínek this latter character was used to distinguish the species of Diaphanosoma paucispinosum but he regards this species as a younger syn onym of Diaphanosoma excisum until more information is available.

Plate 1 Photographs of the species studied:
a) Diaphanosoma excisum
b) Moina micrura
c) Daphnia lumholtzi
d) Ceriodaphnia cornuta





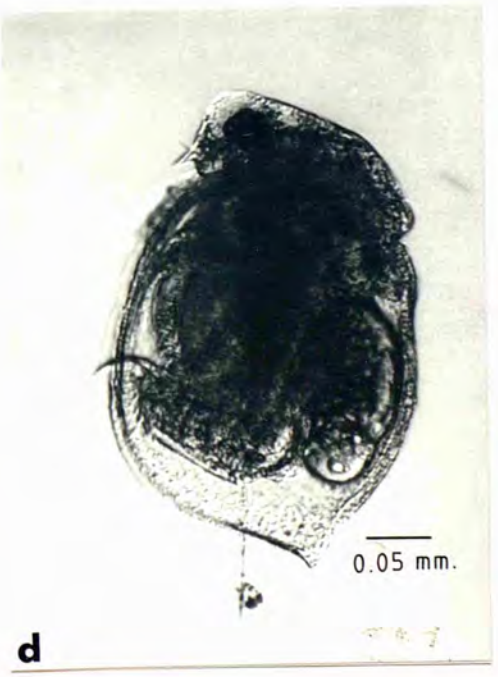
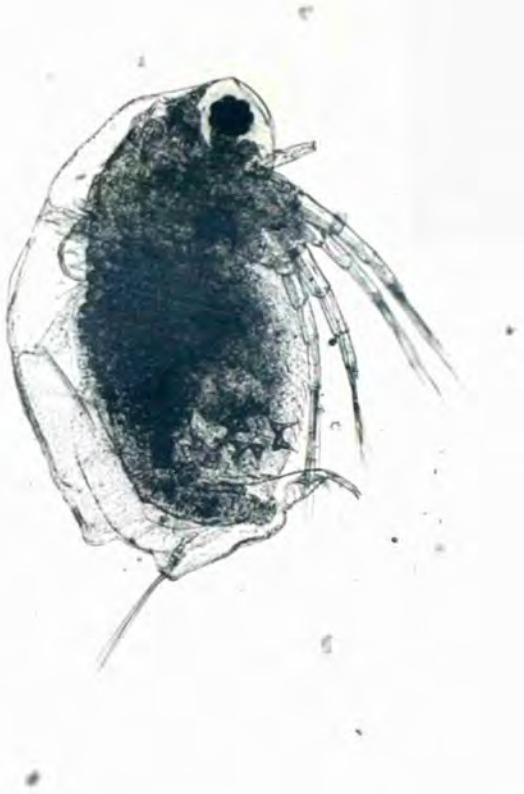


Plate 2. Moina micrura, to show the level of parasitism common in Lake Kalawewa.



Distribution

This species has been recorded from tropical and subtropical regions of the world (reviewed in Rajapaksa, 1983). In Sri Lanka it is found in all types of habitats being common in ponds and reservoirs. Diaphanosoma excisum is one of the most abundant planktonic cladocera in the tropics (Fernando, 1980) and occurs commonly in tropical regions of Asia, Africa and Australia, but not in South and Central America.

Moina micrura

This species was originally described by Kruz (1874). According to Rajapaksa (1983) material from different localities in Sri Lanka agrees with the original description of Kruz (1874) and Goulden (1968). According to the latter author Moina micrura can be confused with 6 species of the same genus: M. affinis, M. branchiata, M. flexuosa, M. hartwigi, M. minuta and M. weismanni and a comparative study of these species is given in his monograph. According to the same author Moina micrura consists of two sub-species M. m. dubia and M. m. ciliata.

Distribution

According to Goulden (1968) like other species of the genus, Moina micrura is commonly associated with small temporary water bodies found primarily in semi-arid and arid regions of the world. However, unlike the others Moina micrura may also be found in the plankton of large freshwater lakes. According to Fernando (1980) Moina micrura is one of the most abundant cladoceran species in tropical regions. Rajapaksa (1983) reviewed the evidence to show that this species is distributed world-wide except in very cold regions.

Daphnia lumholtzi

This species was originally described by Sars (1885). According to Rajapaksa (1983) samples from different localities in Sri Lanka closely agree with this original description and she found the size of the crest on the head, the length of the tail spine and shape of the rostrum to be variable within and between populations. This author also stated that this species can be easily distinguished from the other two Daphnia species (Daphnia carinata and Daphnia cephalata) found in Sri Lanka by well developed fornices, the presence of 10-14 spines on the ventral margin and by its small size. The most recent descriptions of this species are given by Rajapaksa (1983) and Kořínek (1984).

Ceriodaphnia cornuta

This species was also originally described by Sars (1885), and Rajapaksa (1983) found that samples collected from different localities in Sri-Lanka agreed with Sars's description. Some of the diagnostic characters are the horn-like projection on the anterior margin of the head, a pointed beak-like rostrum and bipartate posterior dorsal projection. It is quoted in Kořínek (1984) and Rajapaksa (1983) that R zoska (1956) discussed the relationship between Ceriodaphania cornuta and Ceriodaphnia rigaudi, where the latter lacks the projection on the head, and concluded they were synonymous. But Kořínek favours the view that they are either or polymorphic forms of one species which exhibits a remarkable degree of phenotypic variation. Kořínek (1984) examined the variations in structure of this species from different parts of the world, which are recorded in the

literature, and suggested that at least two different species are included under the name Ceriodaphnia cornuta. This is supported by Berner (1985) who in comparing fine details of the morphology of hairy morphs (hair attached to carapace) and epipphial females supports the view that Ceriodaphnia cornuta Sars is a highly variable species which is distinct from Ceriodaphnia rigaudi Richards; she also distinguishes Ceriodaphnia cf cornuta, a new species which has characteristics in common with the above two species and may possibly be related to them phylogenetically. As the specimens used in the present study are the ones which were descendants from parthenogenic females with a horn-like projection the species was considered as Ceriodaphnia cornuta. Ceriodaphnia cornuta is a very common tropical species of this genus found in almost all freshwater habitats.

2.3 TERMS AND DEFINITIONS

Neonate - The new born young released from the maternal brood pouch (first juvenile instar).

Primipara - A female carrying its first set of eggs in the brood pouch (first adult instar).

Fecundity - Number of eggs carried by a female (or number of neonates produced) in a particular instar, expressed as eggs per female.

Embryonic duration - The time taken for an egg which is laid into the maternal brood pouch to be released as a neonate.

Post-embryonic duration - The time from the release of a neonate to the onset of its primipara stage.

Threshold food concentration - The minimum food concentration required for a physiological process (eg. growth, reproduction) to occur. (Fig 2.2).

Incipient limiting food concentration - The food concentration beyond which any increase in concentration does not significantly increase the magnitude of the physiological process (Fig 2.2).

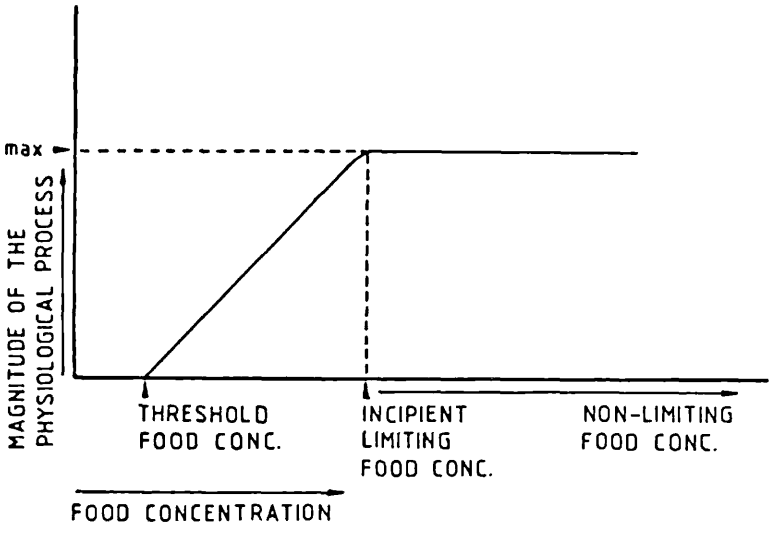
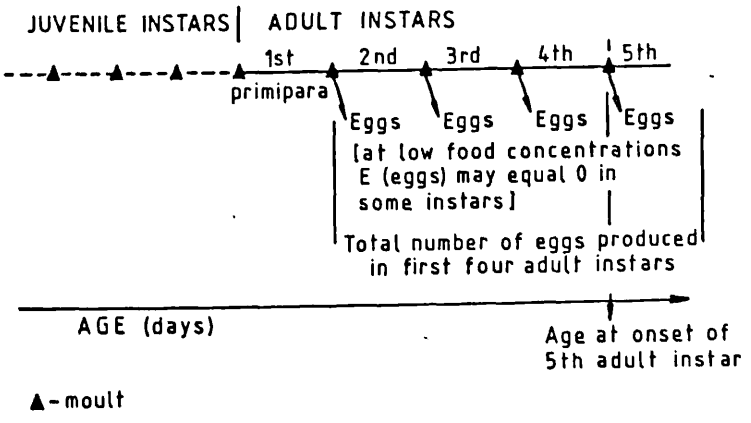


Fig 2.2. Relationship between the rate of a physiological process and food concentration to illustrate threshold food concentration and incipient limiting food level.

Initial reproduction rate - The total number of eggs produced by the first four successive adult instars, divided by the age of the female (in days) of the fifth adult instar. This is expressed as eggs per female per day and is influenced both by the fecundity of the female and the embryonic duration (or duration of any instar in which eggs are not produced).



CHAPTER 3

MATERIAL AND METHODS

3.1 EXPERIMENTAL DESIGN

The investigation had two major components, laboratory experiments to measure life-cycle parameters, and field work to examine the populations of the same species under natural conditions.

(a)Laboratory experiments

The laboratory experiments were designed to evaluate life-cycle parameters (body size, body growth, embryonic duration, time at maturity, and number and size of offspring produced) at different combinations of food and temperature. The temperatures were chosen to fall within the range of fluctuating temperatures in Kalawewa reservoir. The food concentrations were selected to fall within the range of limiting and non-limiting food concentrations for growth and reproduction of cladocerans found by previous authors (Lampert, 1978; Rocha, 1983). The experimental temperature-food combinations used are given in Table 3.1.

Table 3.1 Experimental design: animals where grown at the combinations of food and temperature indicated +

	Food $mgC.L^{-1}$						
Temp °C	0.01	0.03	0.05	0.1	0.25	0.5	1.0
22	+	+	+	+		+	+
27	+	+	+	+		+	+
32	+	+	+	+	+	+	+

When earlier experiments suggested that limiting level for growth and reproduction lay between 0.1 and 0.5mgC.L^{-1}, an additional food concentration (0.25mgC.L^{-1}) was used at 32°C in order to determine limiting level more precisely. Unfortunately it was not possible to do this at the lower temperatures also. Diaphanosoma excisum and Moina micurara were grown at all three temperatures but Daphnia lumholtzi and Ceriodaphnia cornuta were tested only at 22°C and 32°C.

Experiments were carried out in soda glass, polystop bottles. To prevent any possibility of interaction between individual animals interfering with the experimental results, only one animal was reared in each bottle. Experience with similar life-cycle studies under controlled food and temperature combinations, and especially at low food concentrations, has shown that two individuals compete with each other (Rocha, 1983; and Duncan, personal communication). Normally there were four replicate bottles for each temperature-food combination, but whenever juvenile mortality occured more than four (6-8) replicates were used.

Several methods were developed within the experimental design to keep the food concentrations constant throughout.

(a) The specific gravity of the food organism, Scenedesmus acutus, is greater than one and it is a non-motile alga so its cells sink in standing water. In order to keep food concentration and temperature homogenous within each bottle, the bottles were attached to wheels which rotated at one revolution per minute. The wheels and the manner of attachment of bottles are illustrated in Plate 3.1.

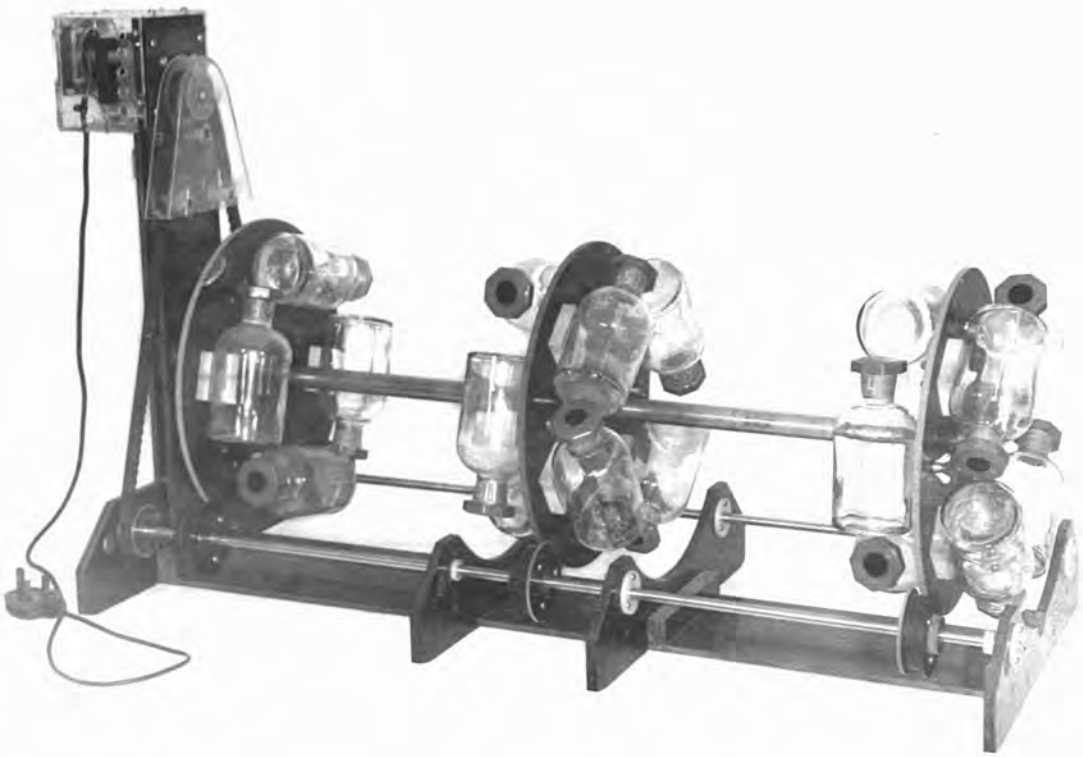


Plate 3. Experimental set-up. Rotating wheels with bottle attachments.

(b) Within each experimental period an attempt was made to keep the food concentration within 75% - 80% of the desired level by using appropriate sized bottles. This was determined by observing the feeding rate of adult Diaphanosoma excisum.

This was carried out at the lowest food concentration which planned for the experiments; a concentration of 850 cells per ml (approximately equal to 0.01 $mgC.L^{-1}$) because the percentage change in the number of cells, in a particular volume of water, after 24 hour grazing, is highest in the lowest food levels. The food medium was prepared as described in page 79 except that the concentration was determined not by the wet-oxidation method but by cell counts, using a haemocytometer. It was then diluted, using GF/F filtered Thames river water, to obtain a concentration of 850 cell ml^{-1}. A sample of the medium was preserved with a few drops of Lugol's Iodine. Six 500 ml polystop soda glass bottles were filled with this prepared food medium. One animal (1.1 - 1.2 mm) was introduced to each and the bottles were attached to the rotating wheels, immersed in a water bath kept at 26°C. After 24 hours algal samples from each bottle were preserved with a few drops of Lugol's Iodine. One ml from each of these samples (final product from bottles), and the original food samples (3 replicates) were introduced into modified Utermohl sedimentation chambers (Utermöhl, 1958) and the cells were counted using a Wild Orthoplan inverted microscope.

From these counts the filtering rate at 26°C was determined according to the following equation (Edmondson and Win burg, 1971).

$$\text{Filtering rate } (ml.ind^{-1}.h^{-1}) = \frac{V \log Co - V \log Ct}{0.4303 \cdot t}$$

Where V = volume of water per individual (ml)

Co = initial food concentration (cell ml^{-1})

Ct = final food concentration (cell ml^{-1})

t = time (h)

The initial mean food concentration determined ± SD was 839 ± 12.12 cells ml^{-1} and the mean filtering rate ± SD was 1.494 ± 0.314 ml ind^{-1} h^{-1} (Table 3.2)

Table 3.2 Experimentally determined filtering rates of adult Diaphanosoma excisum.

Initial concentration no. of cells ml^{-1}	Final concentration no. of cells ml^{-1}	Filtering rate ml ind^{-1} h^{-1}
839	772	1.600
839	785	1.279
839	784	1.304
839	788	1.128
839	757	1.976
839	769	1.675

In order to estimate filtering rates at the experimental temperatures the mid-point Q_{10} value of 1.8 determined experimentally by Burns (1969) for 4 species of Daphnia was used in the following equation.

$$R_1 = R_2 e^{0.06.t}$$

where R_1 = Rate at temperature 1

R_2 = Rate at temperature 2

t = Difference in temperature

temperature coefficient = 0.06 when Q_{10} = 1.8

Feeding rate at each temperature was then calculated according to the following equation

$$\text{Filtering rate (ml ind}^{-1}\text{ h}^{-1}) = \frac{\text{Feeding rate (cells ind}^{-1}\text{h}^{-1})}{\text{Food concentration (cell ml}^{-1})}$$

Using the values of feeding rate thus obtained, the percentage of the initial cell concentration which would be grazed by the animal during the 24 hour period, in different sized bottles, was calculated and the results are given in Table 3.3

Table 3.3 Predicted filtering and feeding rates for experimental temperatures calculated using Q_{10} = 1.8 and percentage of cells which would be consumed in different size bottles during 24 hour grazing in a food concentration of 850 cells ml^{-1} ($\simeq$ 0.01 $mgC.L^{-1}$)

Temp °C	Filtering rate ml $ind^{-1}h^{-1}$	Feeding rate cell $ind^{-1}h^{-1}$	% of cells grazed during 24 hr		
			125	250	500
22	1.05	21.36×10^3	20.15%	10.07%	5.04%
27	1.59	32.50×10^3	30.66%	15.32%	7.66%
32	2.15	43.86×10^3	41.37%	20.67%	10.34%

On the basis of these results (Table 3.3) 250 ml bottles were sufficiently large for adult Diaphanosoma excisum not to reduce their food concentration by more than 20% at all three temperatures, even at the lowest food concentration. Since juvenile Diaphanosoma excisum, and the adults of Moina micrura and Ceriodaphnia cornuta, were smaller than the animals used in this experiment they were likely to have lower filtering rates and therefore reduce the food concentration even less. On this assumption 250 ml bottles were used for all animals. Only the oldest Daphnia lumholtzi were larger than the Diaphanosoma excisum used in this experiment so the same sized bottles were used for that species also. The problems encountered in culturing Daphnia lumholtzi (page 95) were not related to bottle size.

The temperature was kept at a constant level (less than ± 0.5°C fluctuation) by immersing the rotating wheels into a water bath where temperature was controlled either by a Betta-tech CU 400 heater-chiller or by a combination of a Haak E1 immersion heater and a Techem 100 dip cooler. The system was set up two days before the beginning of each experiment and the temperature was read repeatedly to check its stability. Thereafter the temperature was checked frequently throughout the experiment.

All the experiments were carried out under normal daylight conditions (12 ± 1 hour light and dark). As mosquito breeding is a big problem in the tropics the whole experiment set-up had to be kept under mosquito netting cover.

(b) Field Investigations

Field investigations were carried out in Kalawewa reservoir (Chapter 2). The preliminary field collection was made in December 1983 to collect animals in order to culture them for the life-cycle experiments. On the other field sampling days in addition to collecting animals for cultures the investigations that were carried out are summarized in Table 3.4.

Table 3.4

The dates of the field observations and the investigations carried out on each occasion (+ indicates the factors measured)

Date	Chlorophyll "a"	Sestonic carbon	Length-carbon weight relationship			
			Diaphanosoma	Moina	Ceriodaphnia	Daphnia
May 84	+	+	+			
Aug 84	+	+		+		+
Nov 84	+	+	+		+	
Feb 84	+	+	+		+	+

3.2 ANIMAL SOURCE AND MAINTENANCE OF CULTURES

The plankton samples were collected from Kalawewa reservoir, Sri Lanka (Chapter 2) and brought to the laboratory in five gallon plastic cans. The four species were isolated into GF/F filtered tap water which had been aerated overnight. These monospecific cultures were maintained in 2 litre glass jars at room temperature (26°C - 30°C) under natural daylight conditions (12 $\pm$ 1 hour light and dark). The animals were fed daily with Scenedesmus acutus in sufficient quantity to provide a non-limiting concentration of about 1.0 $mgC.L^{-1}$. These algae were from the same cultures as those used for the experiments thus their carbon content was known (page 79). The jars were covered with fine netting to prevent mosquitoes breeding in the cultures. Sub-culturing of the cladocera was carried out once every two weeks to prevent over crowding. There were no difficulties in maintaining the stock cultures of Diaphanosoma excisum, Moina micrura and Ceriodaphnia cornuta, but, even with careful handling and sub-culturing, it was not possible to maintain a culture of Daphnia lumholtzi without production of epphipia. New cultures of all four species were started after each field collection (see Table 3.4).

3.3 FOOD SOURCE AND MAINTENANCE OF CULTURES

Monospecific cultures of Scenedesmus acutus from Max Planck Institute, Plön (maintained at Royal Holloway and Bedford New College) were taken to Sri Lanka and cultured there in two ways.

(a) on Agar plates (stock culture)

(b) in liquid medium (used in the preparation of experimental food)

(a) Agar plates:

One litre of CHU-12 medium (the formula and composition of which are given in Appendix 1) was prepared, heated to about 60°C and 10 grams of Agar was dissolved in it. This medium was sterilized for half and hour in an autoclave. Sterilized petri dishes were half filled with the medium, covered and allowed to cool. Scenedesmus acutus, from an already growing plate, was introduced to the new agar plates with the aid of a sterilized pipette. These plates were kept under continuous illumination from fluorescent lamps.

Small insects, mainly fruit flies and ants, managed to creep into the petri dishes and contaminated the agar with fungi and bacteria. This was overcome by sealing the two parts of the petri dishes with several thicknesses of cellotape (as ants can bore through one layer). These plates could thus only be used once and were therefore prepared in batches sufficient to last 3-4 weeks.

(b) Liquid medium:

CHU-12 medium was autoclaved in 1 litre flat-bottom flasks for half an hour. Already growing algae from a stock plate was suspended in sterilized distilled water and thus transfered to sterilized culture medium with the aid of a sterilized pipette. The mouths of the flasks were covered with aluminium foil, kept under the continuous illumination of fluorescent lamps and aerated with Whisper 300 aerators.

A new culture flask was started every day. It should be noted that these liquid cultures were not bacteria free. At night insects were attracted to the fluorescent lights and managed to creep into the liquid cultures. This problem was largely overcome by covering the room's ventilation holes, thus completely sealing the culture room. Even with these precautions, contamination with blue-green algae did occur on three occasions. Immediately such contamination was discovered, all liquid cultures were discarded, all glass ware was sterilized and a new series of cultures set up. When this happened the experiments had to be stopped halfway due to unavailability of food and restarted when new cultures were grown.

The period of exponential growth of Scenedesmus acutus in liquid medium was determined as follows.

Two liquid cultures were maintained (at 22°C - 24°C) in a controlled temperature room) one of which was started from an already growing liquid culture medium, and the other was started from an agar plate. A sub-sample was taken out daily from each flask, 1 ml was fixed by adding a few drops of Lugol's Iodine. The cells were settled in an Utermohl sedimentation chamber and counted using a Wild Orthoplan inverted microscope. When the cell concentration was dense an appropriate series of dilutions were done prior to fixing, so that a reasonable number of cells sedimented for counting.

The cell concentration plotted against time is shown in Fig 3.1. Algae 6-9 days old were harvested in order to get large numbers still in the exponential phase of growth for use as food for the experimental Cladocera.

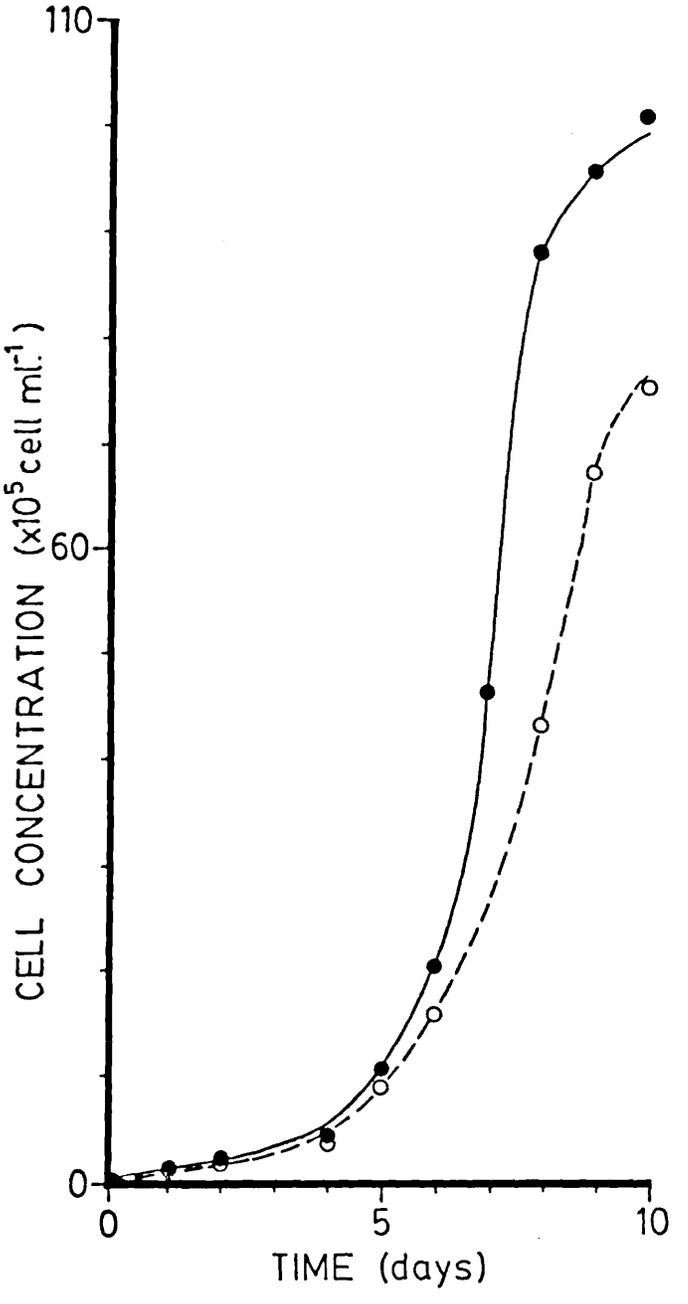


Figure 3.1 Growth of Scenedesmus acutus in two culture media.

●——● Culture medium started from an agar plate.

o— — —o Culture medium started from an existing liquid culture.

3.4 THE PREPARATION OF EXPERIMENTAL FOOD

Tap water was aerated overnight in 10 l glass containers and then filtered through double GF/F pads of 4.7 cm diameter, with the aid of a 250 ml filter unit. Filtration was done under suction using a pump but high levels of sediment particles prolonged the filtering time due to clogging of the filterpads. This water was then used for preparing the experimental food at the following defined algal concentrations: 1.0, 0.5, 0.25, 0.1, 0.05, 0.03 and 0.01 $mgC.L^{-1}$.

Scenedesmus acutus was harvested from the liquid cultures whilst still in their exponential phase of growth, usually from 6-9 day old cultures as discussed above. The liquid culture was centrifuged to sediment the algae and the supernatant of CHU-12 medium was decanted off. The algae were washed twice by re-suspension in distilled water and re-centrifugation. This ensured that the experimental animals were offered good food uncontaminated by CHU-12 medium. The Scenedesmus was then re-suspended in GF/F filtered, aerated tap water and this was used as stock food. It was more concentrated than any of the experimental food concentrations and, with experience, the approximate concentration needed could be judged by the intensity of its colour.

The carbon content of this stock food was measured using the wet-oxidation technique (or chemical oxygen demand) as described in Mackereth et.al. (1978). The principle and the detailed procedure of this method are given in Appendix 2. Food with a concentration of $1.0 mgC.L^{-1}$ was prepared by adding appropriate

amounts of GF/F filtered tap water to the stock food suspension. The other food concentrations were prepared by dilution of the 1.0 $mgC.L^{-1}$ food. The preparation of food was carried out daily during each experimental period and required 4-5 hours. Algae from one flask was not used for more than two days because of the danger of contamination.

3.5 LIFE CYCLE EXPERIMENTS: EXPERIMENTAL PROCEDURE

Before each experiment, a new sub-culture of the experimental species was started from field specimens brought from Kalawewa reservoir, and acclimatized to the experimental temperature for about two weeks. About 6 hours prior to the beginning of the experiment, animals carrying late stage embryos were isolated in order to obtain neonates of known age. The food media, prepared as described above were poured into clean experimental bottles and brought to experimental temperature. (The bottles used in the experiments were washed daily with soap and water, rinsed twice in distilled water and oven dried.) Neonates less than 6 hours old were introduced into the bottles filled with the experimental food, with one individual per bottle. The length of each neonate had previously been measured (Fig 3.2) using an Olympus microscope with a calibrated eye piece and a magnification of x100. (1 eye piece division = 0.0144mm). As the neonates were small (0.2 - 0.45mm depending on the species) and easily stuck to the surface tension layer, this procedure was tricky and clean pipettes, a steady hand and a good experienced eye were required to avoid loss of neonates. The bottles

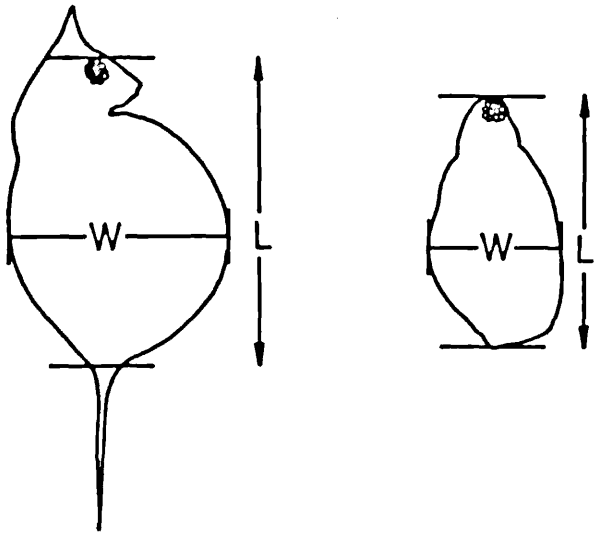


Figure 3.2 Positions at which measurements of body length and width were made on Daphnia lumholtzi and other species of Cladocera examined in this study.

containing the animals were attached to the rotating wheels in the water bath. The animal in each bottle at 27°C and 32°C was examined twice daily and at 22°C once a day until each animal reached the 5th adult instar. Hence at higher temperatures (27°C and 32°C), for each species 24 bottles were examined twice daily. The food was changed only once a day by transferring the animals to a new set of cleaned bottles with the appropriate food concentrations.

During each examination the animal was carefully placed onto a glass slide with a drop of water using a Pasteur pipette. At each observation, the length was measured, the ovarian stage assessed, and the presence or absence of eggs or embryos noted. It proved impossible to measure the egg diameters of any species as (a) they were very small, (b) any movement of the animal on the slide changed the shape of the eggs and (c) it was difficult to restrict their movements without danger of them drying up. Daphnia lumholtzi was found to be especially sensitive. Diaphanosoma tended to throw out the eggs or embryos from the brood pouch if disturbed.

During the observations each change of instar was confirmed by locating the ecdysed carapace. During the early stages this was time consuming due to its small size and transparency. When young were born, they were isolated, measured and collected on muffled GF/F pads of 0.8 cm diameter for carbon determination. These pads were kept in a desicator, away from atmospheric dust until the required number of neonates was cumulated. Once reproduction started the examination of each bottle required half to one hour as it proved very difficult to locate the tiny transparent neonates through the glass of the bottles.

Each experiment took a longer period than the actual duration from neonate to 5th adult instar because during the experiments accidental deaths and losses occured and each of these were replaced by a new neonate of known age from the stock culture. Up to about the fourth instar the handling of all four species was very difficult as the animals tended to become trapped at the air-water interface. Hence to get four sets of complete results at each temperature-food combination took much longer than expected.

3.6 CARBON CONTENT OF ANIMALS

(a) Those grown under defined food conditions

Animals were grown in a similar manner to those in the experiments described above (3.5) at 32°C, and with daily changes of food. For this purpose Diaphanosoma excisum and Moina micrura were reared at 1.0, 0.25 and 0.1 $mgC.L^{-1}$. Ceriodpahnia cornuta were reared only at 1.0 and 0.25 $mgC.L^{-1}$ as this species did not survive at lower food concentrations. It was possible to rear Daphnia lumholtzi only at 1.0 $mgC.L^{-1}$. Two methods were employed to determine the carbon content of these animals. Those reared at 1.0 $mgC.L^{-1}$ were measure according to a modified wet dichromate oxidation method (see 3.4) and the rest by dry combustion and an infra-red carbon-dioxide analyser (Salonen, 1979).

The wet oxidation carbon determination method required a minimum of 10 µgC per sample so the number of neonate animals required per sample was aobut 50-75, but this number decreased with increasing individual size. Details of the number of animals used in each sample are given in Appendix 4. The

animals of known lengths were collected on to carbon-free GF/F pads, 0.8 cm in diameter, which were kept in a desciçator until enough animals had been collected in each size category. The carbon content of the animals thus collected was determined in terms of $\mu gC\ ind^{-1}$ according to the modified wet oxidation method (Talling personal communication, unpublished) given in Appendix 2.

As the amount of atmospheric dust is normally quite high in windy tropical conditions, even in laboratories, both the transfer of animals on to muffled pads, and the micro-titrations had to be carried out in a room in which the ceiling fan was switched off and all ventilation holes were covered. This demanding work was therefore done under somewhat uncomfortable conditions since ambient tropical temperatures (and sometime humidities) are high.

For the analyses in which the infra-red carbon dioxide analyser was used animals were collected in body size classes (0.05 mm) on to carbon-free platinum pans. The sensitivity of this technique was much greater, so the number of animals required per size class was much lower than for the wet-oxidation method. The platinum pans were stored in cavities made in an aluminium block and burned in a muffle furnace to ensure that they were free of carbon. Once the animals were collected on to the pans, the blocks were wrapped with muffled aluminium foil and kept in a descicator until the carbon determinations were carried out. The platinum cups containing animals were introduced into a furnace at 950°C and the carbon dioxide liberated by the burning

of the sample was carried through the infra-red carbon dioxide analyser. This was attached to a pen recorder which gave a peak in proportion to the carbon dioxide content of the sample. The recorder was calibrated with known concentrations of oxal ic acid and, using the calibration curve, the carbon content of the samples were calculated.

(b) Field animals

The species required were sorted from the plankton samples (which had been collected from the reservoir using vertical and horizontal hauls of a 125 μm plankton net) and separated out into 33 μm filtered reservoir water. The animals were measured under an Olympus microscope with a calibrated eye piece, washed twice in double distilled water and transfered onto muffled GF/F pads according to their size classes. When enough animals in each size category were collected on the GF/F pads they were dried at 60°C and stored in a desciator until the carbon determinations were carried out. As for the experimental animals, for each carbon measurement of the lowest size class 50 -75 animals were required, and the number required decreased as size class increased. The details of numbers of animals used in each size category together with their carbon contents, are given in Appendix 4.

Several precautions were taken when analysing the carbon content of the field animals.

The sorting was done with live animals because all preservatives contain carbon and it was therefore not possible to preserve the animals prior to sorting. Sorting of the animals

was carried out in a place of close access to the reservoir because it was not possible to transfer the animals to the laboratory since the journey took 6-7 hours and by that time changes would have occurred (especially food and oxygen) in the samples crowded with unsorted animals. It was necessary to avoid the loss of carbon due to starvation. Neonates born during the journey would have been used to completely different food conditions and the lowest size category of animals would have shown a completely different carbon content from that expected from the field.

To sort out enough animals of different size classes of one species took at least 3-4 days of continuous work. As all biological processes take place rapidly at tropical temperatures it was not possible to use one sample from the reservoir for more than 5-6 hours.

Carbon determination of these samples was carried out using the modified dichromate wet-oxidation method (Talling personal communication) as given in Appendix 2B.

3.7 DETERMINATION OF SESTONIC CARBON IN KALAWEWA RESERVOIR

The sestonic carbon was measured in two fractions (1) total content (2) particles <33µm. A known amount of reservoir water was filtered on to muffled (at 500°C for 2 hours) GF/F pads using a milipore filter unit. The pads were dried at 60°C and transfered to the laboratory in a descicator. The organic carbon content was determined as $mgC.L^{-1}$ by the wet dichromate oxidation method (Mackereth et.al. 1978). Details of the method are given in Appendix 2.

3.8 FIELD CHLOROPHYLL "a" CONTENT

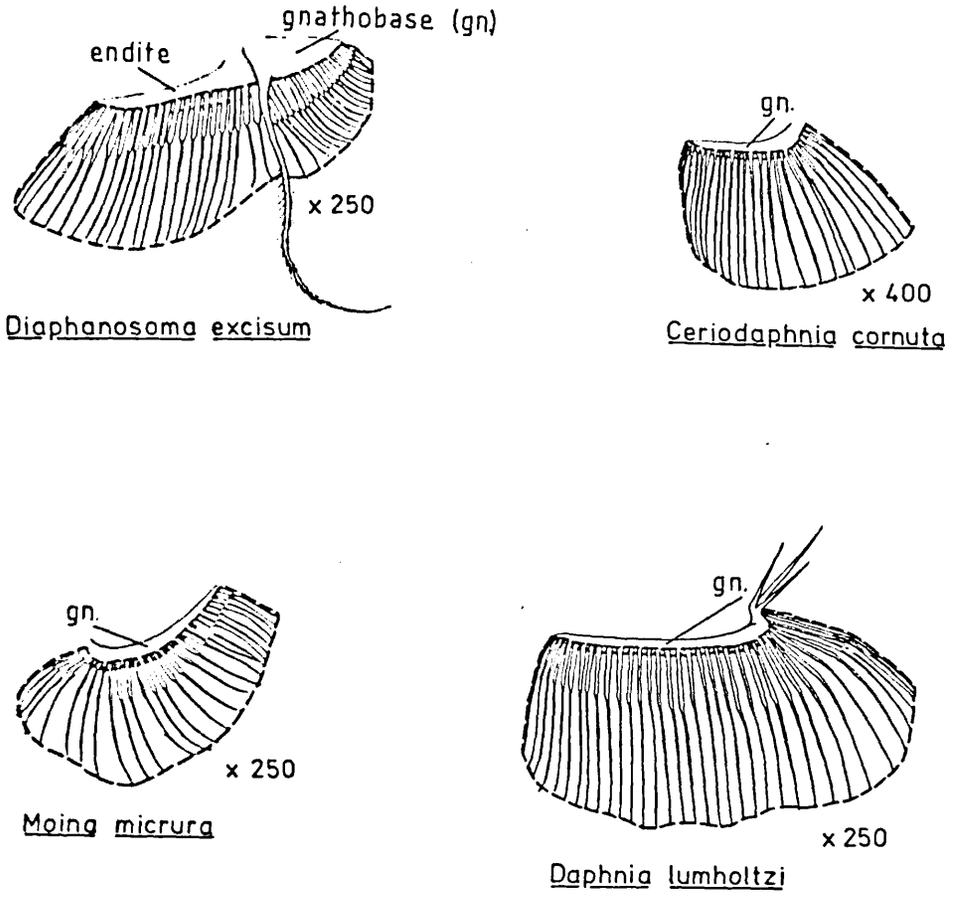
As with the sestonic carbon this too was measured in two fractions. (1) chlorophyll content of the total algae and (2) chlorophyll content of algae <33 μm diameter. A known amount of water was filtered on to GF/F pads and as much water as possible was removed by suction and the dried pads were stored in a descicator covered with black paper. The chlorophyll "a" was determined by methanol extraction method using the procedure given in Appendix 3.

3.9 FILTER LIMB STRUCTURE

The filtering limbs of the Cladocera were studied using live animals (except for Moina micrura) which had been brought from Sri-Lanka to England and kept in a constant temperature incubator. Cultures were sub-cultured frequently in GF/F filtered pond water and fed with Scenedesmus acutus daily. Moina micrura was studied using formalin preserved samples.

(a) Filtering area

For the measurement of filtering areas the appendages bearing filter structures (3rd and 4th limbs in Moina, Ceriodaphnia and Daphnia and 1st - 5th limbs in Diaphanosoma) were removed from the animals, in polvynyl lactophenol with lignin pink, from a range of sizes after body length had been measured as described previously. The limbs were mounted on glass slides with the setae in the horizontal plane. The filtering area was drawn using a Wild Orthoplan microscope with a camera lucida attachment (Fig 3.3). The areas of the drawings were measured using a grafpad with an area programme attachment to a BBC computer. Three readings were taken of each to the nearest $0.869\mu m^2$.



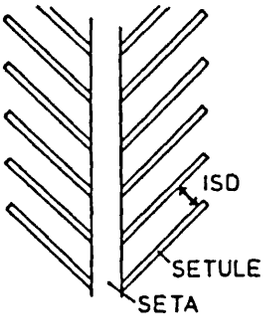


Figure 3.3 a) The filtering limbs of the four species studied. The dotted line indicates the filtering area of the comb which was measured.
b) A magnified view of a single seta of the filter comb to show the position at which the measurements of inter-setular distance were made.

(b) Inter-setular distance

A scanning electron microscope was used to measure the inter-setular distance. For the scanning electron micrographs body length was measured in a range of different sized animals from each species which were then fixed in a 2% glutaraldehyde in PO_4 buffer of pH 7.4 for 1-2 hours. Specimens were washed in buffer and post fixed in 1% aqueous Osmium tetroxide for one hour. After washing in distilled water they were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90% and absolute) for 20-30 minutes at each strength. After two more washes in absolute alcohol they were critical point dried in liquid carbon dioxide. The limbs were dissected, transfered to aluminium stubs and subsequently coated with Paladium/Gold. Their morphology was studied using a Cambridge S-400 scanning Electronmicroscope. Several points on the filtercomb were photographed from the middle region of the setae, in order to avoid the basal setae which have setules arranged with a larger gap. The photographs were enlarged and the perpendicular distances between the setules were measured as shown in Fig 3.3. Using the magnitude of each enlargement the inter-setular distance was calculated. The enlargements were made so that it was possible to measure the inter-setular distance to the nearest 0.015 μm.

3.10 ANALYSIS OF DATA

Several statistical methods and computer facilities were employed in analysing the data.

Regression analysis - (Sokal and Rholf, 1969)

This was employed to analyse the effect of an independent variable on a dependent variable to evaluate whether there is any significant relationship between the two parameters. The F value was employed in judging the level of significance. The exponential and power relationship were linearized by natural log transformation. An elementary statistical computer program was employed in analysing the regressions.

Multiple regression analysis (Sokal and Rholf, 1969)

This was used to see the effect of more than one independent variable on a dependent variable. The Minitab statistical package was used in analysing the result. This test predicts the F value due to the interacting effect of the independent variables employed as well as the F value due to each independent variable separately. This thus provided a means of judging which of the independant variables had the greatest effect on the dependent variable.

A stepwise multiple regression analysis, which was also available in the Minitab statistical package, was used to examine the interactions between a number of parameters.

Covariance analysis (Steel and Torrie, 1980)

Simple regressions predict the significant level of the relationship between two parameters. The effect of a third parameter on these relationships was examined by analysis of covariance which compared the regression lines to predict whether there were significant differences between them. For example: the significant relationships between body length and body carbon

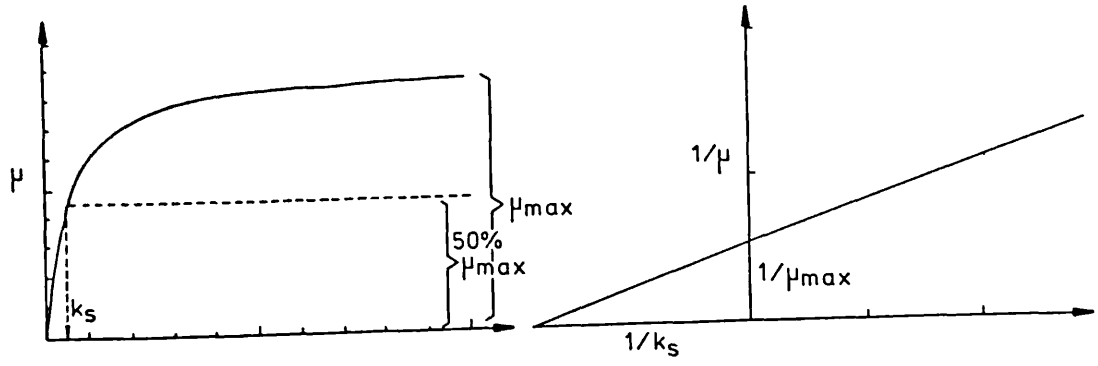
of animals grown under different food concentrations was compared by this analysis to predict whether there was a significant difference between the relationships at different food levels. Two tests were used: The Sum of Square Simultaneous Test Procedure (SS-STP test Sokal and Rholf, 1969) predicts the difference between the slopes (regression coefficients) and the Student-Newman-Keuls Test (Steel and Torrie, 1980) predicts the differences between the elevations of the regressions.

Student-t-test

When few data were available, as in the cases of Daphnia lumholtzi and Ceriodaphnia cornuta the significant differences were tested using Student-t-test (Sokal and Rholf, 1969).

Michaelis-Monod model (Uhlmann, 1979)

According to this model, the dependence of a physiological rate process (such as growth rate, developmental rate etc) of an organism on the concentration of nutrients or light intensity can be represented as a hyperbolic saturation curve as shown below:



$$\mu = \frac{\mu_{max}.S}{Ks + K} \qquad \frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{1}{\mu_{max}} \cdot \frac{1}{S}$$

μ = rate (eg. growth rate μgC $ind^{-1}d^{-1}$)

μmax = maximum possible rate

S = nutrient concentration

Ks = the nutrient concentration where μ is 50% of μmax

The Lineweaver-Bruke modification (Ulhmann, 1979) will linearize the Michaelis-Monod function giving a straight line by reciprocal transformation of the values. This relationship is as follows.

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{Ks}{\mu_{max}} \cdot \frac{1}{S}$$

in which the linear regression between $1/\mu$ and $1/s$ will result in an intercept = $1/\mu max$ and slope = $Ks/\mu max$ such that both Ks and μmax values can be predicted.

Growth curves

A computer programme written by Wroot (1984) based on Schnutes' model (1971) was used in fitting growth curves. The details are explained in Chapter 4.

CHAPTER 4

EFFECT OF FOOD CONCENTRATION AND TEMPERATURE ON GROWTH

Growth of an animal is the increase in body size (including growth of an ovary) with time. Body size is of fundamental importance in zooplankton studies because physiological rate processes such as growth itself, respiration, feeding and excretion are all related to body size and can be expressed "per unit body size". Life cycle characteristics such as number of juvenile instars, the time of first reproduction and fecundity are all related to body size, usually to weight. In addition consideration of body size is important in ecological studies due to size-selection by predators feeding on zooplankton.

In the past the body size of cladocera was usually expressed in terms of length. This has several drawbacks. It is not possible to carry out comparative studies in terms of length. Within a group of animals such as Cladocera the body shape varies and this leads to variation in weight per unit length. Even within a species this ratio will vary due to changes in body shape such as those known as cyclomorphosis. This problem can be overcome by expressing the size in terms of weight. On the other hand, during long-term experimental studies involving the same live animals, it is not possible to monitor changes in weight of animals like cladocerans during the experiment and length is the only possible measure of size. In such circumstances, prior knowledge of length-weight relationships for a species can be used to estimate weights from measured lengths.

4.1 THRESHOLDS FOR GROWTH

(a) *Diaphanosoma excisum* and *Moina micrura*

While carrying out the life cycle experiments at very low and limiting levels of 0.01 and 0.03 $mgC.L^{-1}$, the juveniles died at all temperatures. The animals did not live more than four days at 27°C and 32°C but at 22°C they survived slightly longer, up to 4-6 days and reached the 3rd juvenile instar at 0.03 $mgC.L^{-1}$. At 22°C even at 0.05 $mgC.L^{-1}$ juveniles died. In all these none of the juveniles died suddenly: the heart beat decreased over a period of one to two days before they ultimately died. At other food-temperature combinations (Chapter 3) animals reached maturity. Hence at very low food levels animals did not ingest and assimilate enough food to cover their metabolism and so were unable to continue their development to maturity.

(b) *Ceriodaphnia cornuta*

Only two experimental temperatures (22 and 32°C) were used for *Ceriodaphnia cornuta* but the animals did not thrive for more than 5 days at 22°C even at high food levels. At 32°C too this species did not grow to maturity even at 0.1 $mgC.L^{-1}$. At 0.25 $mgC.L^{-1}$, out of the four replicates, one did not mature even after 6 days and died in the 3rd juvenile instar. In the other 3 replicates animals attained maturity on the fourth day. Therefore *Ceriodaphnia cornuta* has a threshold food concentration for growth between 0.1 and 0.25 $mgC.L^{-1}$ when *Scenedesmus acutus* is the food source.

(c) Daphnia lumholtzi

At 22°C in 0.01, 0.03 and 0.05 mgC.L^{-1} eight replicates were carried out but the juveniles died after 3-5, 4-7 and 6-12 days respectively. Out of the eight replicates at 0.1 mgC.$^{-1}$ only two managed to attain maturity in 6 days, while the other prolonged juvenile growth up to 16 days. At all food levels not a single animal attained the 5th adult instar. The oldest instar that was reached was the 4th adult instar at 1.0 mgC.L^{-1}.

At 32°C animals were observed to have fungal infections on several occasions. The experiments were replicated whenever these infections were observed. The infection was a severe one, such that mycellia became distributed throughout the body, often within a day, finally killing the animal. The other peculiar feature found was that at 0.5 mgC.L^{-1} and 1.0 mgC.L^{-1}, though animals did not produce eggs, the body was filled with yellow/brown oil globules. The concentration of these globules within the body was so high that, even to the naked eye, the normally transparent animals looked yellowish-brown in colour. This is not a normal characteristic since such a condition was not observed in the animals grown at 1.0 mgC.L^{-1} for carbon determination. The carbon determination was carried out using animals collected in August but the experiments were carried out with cultured animals derived from a single female collected in November. It is clear that with this set of experiments some factor, which may be internal or external, caused the excess energy gained to be stored as reserves rather than used for reproduction. In addition, mortality was very high and animals

died even while carry developing embryos in the brood pouch. Due to these abnormalities the growth at 32°C was not considered. The threshold food concentration for this species for growth at 22°C is 0.1mgC.L^{-1}.

4.2 LENGTH:CARBON-WEIGHT RELATIONSHIPS

The literature contains references to zooplankton weights expressed in terms of wet weight, dry weight or as the weight of a particular element such as carbon or nitrogen. Wet weight is not an accurate measure since water gets trapped between carapace and limbs of Cladocera and evaporation occurs during the weighing procedure. According to Vollenweider (1984), even dried biological matter always retains very small amounts of water. However, both wet and dry weight in relation to body length are available for a number of zooplankton species and have been reviewed by Dumont et.al. (1975) and Bottrell et.al. (1976). Many recent studies have established body size-length relationships in terms of different elements such as organic carbon, nitrogen, phosphorus, or calcium (Baudoin and Ravera, 1972; Butorina, 1973; Vijverberg and Frank, 1976; Lampert, 1977b; Rocha, 1983; Duncan et.al., 1985a, 1985b).

There is consistency in these length-weight relationships either interspecifically or intraspecifically. Dumont et.al. (1975) found a tendency for limnetic species of Cladocera, Copepoda and Rotifera to have relatively lower weights per unit length than periphytic or benthic species; and within a species, populations having a more limnetic way of life weighed less than those with more littoral habits. The authors suggest that this may be due to limnetic animals consuming more energy for swimming than littoral ones.

Seasonal variation in carbon weight-length relationships within a species were recorded by Rocha (1983) for Daphnia magna, Daphnia pulicaria and Daphnia hyalina in the London reservoir, and by Duncan (1985) and Duncan et.al. (1985) for Daphnia pulicaria and Daphnia hyalina in Lake Washington. The same studies of carbon weight-length relationships were given for species of Daphnia reared under defined food concentrations and temperature conditions and the elevations of the regressions were significantly lower in limiting food levels. Because of this evidence that the nutritional condition of temperate Daphnia species from two water bodies affects their carbon weight-length relationship, an attempt was made in the present study to establish carbon weight-length relationship for each of the four tropical species studied, at defined food levels at 32°C, and these relationships were used to estimate the body sizes of experimental animals in terms of carbon content of the body; the length measurements were converted to appropriate body carbon weights to establish growth curves as well as growth rates. In addition experimentally established length-body carbon were used in establishing the nutritional condition of the reservoir animals.

In order to obtain the length-carbon weight relationship for the studied species under known conditions the animals were reared at 32°C. Both Diaphanosoma excisum and Moina micrura were reared at 1.0, 0.25 and 0.1 $mgC.L^{-1}$; Ceriodaphnia cornuta at 1.0 and 0.25 $mgC.L^{-1}$ and Daphnia lumholtzi at 1.0 $mgC.L^{-1}$. Body carbon content was measured from known length animals as described in Chapter 3.

The relationship between body carbon content and body length was found to be well described by a power function $Y = aX^b$ (Y = body carbon content and X = length) and the parameters of the natural log-transformed linear regressions are given in Table 4.1: all the regressions are statistically significant. The regressions are graphically presented in Fig 4.1. In Diaphanosoma excisum and Moina micrura the relationships at 1.0, 0.25 and 0.1 $mgC.L^{-1}$ are compared by covariance analysis in Table 4.2. This shows that the slopes of the three regressions are not significantly different but the elevation of those at 0.25 and 0.1 $mgC.L^{-1}$, which have no significant difference between them, are significantly different from the 1.0 $mgC.L^{-1}$ regression having a higher elevation. From this it is evident that at 0.25 $mgC.L^{-1}$ and below these two species are food limited. Based on this analysis, the first two regressions could be pooled and the pooled regression used to represent the length:carbon-weight regression for limiting food conditions. Though it could be pooled, the individual relationships were used in converting length to weight when analysing growth in order to see the relationship more precisely. The relationship obtained at 1.0 $mgC.L^{-1}$ was used in predicting the weights at 0.5 $mgC.L^{-1}$ as both these food concentrations are non-limiting conditions (as demonstrated in Chapter 6). In Ceriodaphnia cornuta, since the animals did not grow at 0.1 $mgC.L^{-1}$, the significant relationship at 1.0 and 0.25 $mgC.L^{-1}$ is given in Table 4.1 and Fig. 4.1. Analysis of covariance (Table 4.2) indicates a similar trend to the above two species, the two regressions being parallel, but

that at 0.25 $mgC.L^{-1}$ having lower elevation. The relationship for Daphnia lumholtzi at 1.0 $mgC.L^{-1}$ is also given in Table 4.1 and Fig. 4.1. Due to the complications which occurred in the Daphnia lumholtzi cultures (page 95) it was not possible to carry out experiments at other food levels.

In order to examine interspecific differences the significant relationships of the four species at 1.0 $mgC.L^{-1}$, given in Table 4.1, were compared by analysis of covariance (Table 4.3) and are plotted in Fig. 4.2. It is evident (Table 4.3) that the regression coefficients were not significantly different but the elevations do differ significantly, Ceriodaphnia cornuta has the highest elevation followed by Moina micrura, Daphnia lumholtzi and Diaphanosoma excisum. As the regressions are significantly different it is not possible to use a common length-carbon-weight regression for these species, (which belong to four different genera), even at one food level and temperature.

4.3 GROWTH CURVES

Growth curves provide information on how the size of an animal varies with time. According to Bertalanffy (1964), growth curves can demonstrate how environmental factors influence the time course of an increase in body size from such properties as the shape of the curve, the point of inflection and the steepness of the initial part, all of which can be changed by the temperature condition of the animal and/or its nutritional state.

A computer programme written by Wroot (1984), based on Schnute's model (1981), was used in fitting the empirical data to the following growth curves.

(a) Logistic

$$W_t = W_{max}/1 + \exp(-g(t-t_o)))$$

(b) Von Bertalanffy

$$W_t = W_{max}\ (1 - \exp(-g(t-t_o))^p$$

(c) Gompertz

$$W_t = W_{max}\ (\exp(\exp(-g(t-t_o))))$$

(d) Richards

$$W_t = W_{max}\ (1 - (b)\exp(-g(t-t_o)))^p$$

The importance of this model is that without prior knowledge of the shape of the growth curve, it predicts to which growth curve the data is best fitted. As there are differences in other growth curves, without the prior knowledge it is not easy to judge regarding the best fit; for example, (according to Schnute 1981) the parameter t_o represents the time at which the growth curve crosses the time axis in Von Bertalanffy's curve; and in contrast, in Richard's, Gompertz, and the logistic curves t_o is the time of inflection, and the latter never crosses the t-axis. On the other hand, the Von Bertalanffy's, Richard's, Gompertz and logistic curves have a theoritical limiting size, while the linear growth model (in which $W_t = g(t - t_o)$) does not have such a limitation. In addition the number of model parameters involved in the different growth curves differs and in such a situation decisions have to be made on a reasonable number of parameters that should be used.

These problems were overcome by Schnute's model (1981) and Wroot's computer programme in which the constants a and b, plus the residual sum of squares, predicts to which of the growth curves the data could best be fitted. Using the computer programme (Wroot 1984) it was found that Richard's model fitted the results well.

For certain data sets both Richard's and Von Bertalanffy's curves predicted similar residual mean squares and very close $\hat{Y}$ values, and for comparative purposes Richard's model was used in all cases. Richards (1959) growth model expresses the relationship between weight and time as follows:-

$$W_t = W_{max} [1 - b \exp(-g(t-t_o))]^p \text{----------(1)}$$

where

W_t = weight at time t ($\mu gC.ind.^{-1}$)

W_{max} = asymptotic weight ($\mu gC.ind.^{-1}$)

g = growth constant

t_o = the time of inflection (days)

b = model parameter

p = the exponent and equal to reciprocal of b

The best fitting Richard's growth curves are illustrated in Fig. 4.3 for each temperature condition and for every food concentration for which a curve could be obtained. Note that these are semi-logrithmic plots with body weight on a log scale. The parameters of the Richard's growth equations are given in Table 4.4. On each growth curve, the time of appearance of the primipara, and her size, is indicated by an arrow which gives a visual picture of whether or not the curve inflects before the onset of maternity.

In Diaphanosoma excisum it is clear from the initial straight portion of the growth curve that the early stage of development shows exponential growth under all experimental conditions. Comparing the value of t_o, which is the time of inflection (Table 4.4), and the ages of the primipara (Table 4.5) it is evident

that exponential growth occurs up to, or just before, the primipara stage at low te mperatures and for limiting food concentrations but goes beyond the primipara stage at higher temperatures and non-limiting food concentrations.

There were differences between Moina micrura and Diaphanosoma excisum. Comparing the t_o values given in Table 4.4 and the ages of primipara in Table 4.5, it is evident that in Moina micrura growth is exponential up to the primipara stage only at non-limiting food levels and higher temperatures (except at 0.5 $mgC.L^{-1}$ and 32°C). At limiting food levels (0.1 and 0.05 $mgC.L^{-1}$) no exponential growth is evident as t_o values are negative, except at 0.1 $mgC.L^{-1}$ and 32°C. At the lowest temperature, even at non-limiting food levels, the exponential growth is restricted to only a very short period.

Both Daphnia lumholtzi at 22°C and Ceriodaphnia cornuta at 32°C show similar trends to Diaphanosoma excisum showing exponential growth up to or just before the primipara stage.

Body growths at different food levels but one temperature are plotted together in Fig. 4.4 to illustrate the effect of food concentration. It is clear that below 1.0 and 0.5 $mgC.L^{-1}$ body growth is related to the amount of food since the curves for these two food concentrations are very similar. Food concentration has an effect on the size of the primipara and the 5th adult. It is also evident that the time taken to attain the 5th adult instar increases, as indicated by the decreased slope in low food concentrations.

The effect of temperature at each particular food level is illustrated in Fig. 4.5 by comparing growth curves of one food but at different temperatures. The temperature effect is mainly on duration and is more pronounced at low food concentrations. From these growth curves it would appear that temperature does not affect the size of the primipara, but when this is analysed statistically it becomes apparent that, in Moina micrura, the primipara tends to be smaller at higher temperatures. This will be discussed in more detail in Chapter 5. There is no clear effect of temperature on the size of the 5th adult instar at 22°C and 27°C in Diaphanosoma excisum but at 32°C the 5th adult instar tends to have a greater body size. The 5th adult instar of Moina micrura tends to have a similar body size at all temperatures except at the highest food level but at non-limiting food levels it tends to have a greater carbon content (see Fig. 4.5).

4.4 GROWTH RATES

The growth curves show the pattern of growth under the defined experimental conditions but it is not possible to compare them statistically between conditions in one species or between species especially because they cannot be linearized by simple transformations. Hence, the growth curves give only a visual comparison. On the other hand, growth rates can be used in comparisons and can be expressed in several ways.

It is clear from the growth curves (eg. Fig. 4.3) that the initial phase of growth is exponential and during this phase of growth which usually co-incides with the juvenile phase of development, the instantaneous growth rate can be computed from the equation

$$W_t = W_o \, e^{g(t-t_o)} \text{ ---------- (2)}$$

where

W_t = weight at time t ($\mu gC.ind.^{-1}$)

W_o = initial weight ($\mu gC.ind.^{-1}$)

t = final time (days)

t_o = initial time (days)

g = daily instantaneous growth rate

This relationship can be linearized by transformation to natural logarithms, resulting in

$$\ln W_t = \ln W_o + g\Delta t \text{ ----------(3)}$$

where

$$\Delta t = t - t_o$$

in which the regression relating $\ln W_t$ with Δt has a slope which corresponds to the instantaneous growth rate. The advantage is that linear regressions can be compared statistically by covariance analysis and this was used to investigate the effect of food concentration on the values obtained for the instantaneous growth rate (g) which is the slope of the regression, and the elevation of the curve at a particular temperature, as well as the effect of temperature on these parameters at a particular food level.

According to Ricker (1975) growth is not in practice, usually exponential over a very long period of the life cycle but only in the immature phase, but any growth curve can be treated in this way if it is divided up into short segments of time (ie where $t \rightarrow o$). Taking this into consideration, absolute growth rate, which is the increase in weight per unit time ($\mu gC.ind.^{-1}d^{-1}$) was calculated as $\frac{\Delta W}{\Delta t}$ where Δt was less than one day, and ΔW the change in weight within that period.

In order to compare the growth rates intraspecifically within treatments, and interspecifically, the relative or weight specific growth rates was computed as a percentage, according to

$$G\% = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{1}{W_1} \text{ ----------(4)}$$

where

W_1 = initial weight at t_1

W_2 = weight at t_2

$$G\% = \frac{W}{t} \cdot \frac{1}{W_1} \cdot 100$$

Considering the exponential growth of Moina micrura it is evident from the t_o values given in Table 4.4 that at low food levels no exponential growth occurred at all. But assuming that growth within the first day was exponential, instantaneous growth rates were computed for this short period.

The daily instantaneous growth rates at different temperature-food combinations were obtained by regressing natural log body weight on age and the regression statistics are given in Table 4.7. All regressions are statistically significant. The slope or the b value represents the daily instantaneous growth rate (g, d^{-1}). The values obtained were very high, particularly at the higher temperatures and in the two non-limiting food levels (1.0 and 0.5 $mgC.L^{-1}$). Below 0.5 $mgC.L^{-1}$ and in the lower temperature of 22°C the rate decreased quite markedly. The values for Moina micrura were almost double those for Diaphanosoma excisum under the same experimental conditions.

In order to determine whether these growth rates were different or not, the regressions in Table 4.7 were compared by covariance analysis in two ways: (1) by comparing all the regressions for one food level but different temperatures (Table 4.8.) and by comparing all the regressions for one temperature but different food levels (Table 4.9).

Examining Table 4.8 the SS-STP test comparing the regression coefficients (or instantaneous growth rate) shows that in non-limiting food (1.0 $mgC.L^{-1}$) and low food concentrations 0.05 $mgC.L^{-1}$) the regression coefficients for 32°C and 27°C are not significantly different for Diaphanosoma excisum. In Moina micrura, the slopes for the 32°C and 27°C regressions were not significantly different at any food level and there was a tendency, at the limiting food levels of 0.1 and 0.05 $mgC.L^{-1}$ for all the slopes to be very similar. The S-N-K test which compares the elevations of the regressions, by adjusted mean values of body weight, shows that in Diaphanosoma excisum these were significantly different for all temperatures at each food level except the lowest (0.05 $mgC.L^{-1}$) in which the elevation for 32°C and 27°C were not different. In contrast Moina micrura does not show a significant difference in elevation with temperature at non limiting food levels but when food is limited the highest temperature shows a significantly higher elevation at the lowest food level (0.05 $mgC.L^{-1}$) while the two higher temperatures have a significantly higher elevation at 0.1 $mgC.L^{-1}$. The comparisons in Table 4.9 provide similar results. The SS-STP test shows that, in Diaphanosoma excisum the slopes for the upper two food

concentrations are not significantly different at all temperatures. In addition at 32°C, the regression coefficients for the lower two, limiting food levels (0.1 and 0.05 $mgC.L^{-1}$) are also not different. Again in Moina micrura there were differences between non-limiting and limiting food concentrations at 27°C and 32°C, but not within these groups of food concentrations. At 22°C none of the slopes were statistically different. The S-N-K test also demonstrates differences in elevation between the regressions for the two upper non-limiting and two lower, limiting food levels.

4.5 COMPARISON OF GROWTH AT DIFFERENT PHASES IN THE LIFE CYCLE

In order to compare growth rates at different phases in the life cycle three stages were selected in Diaphanosoma excisum and Moina micrura

(1) The second juvenile instar in which development and body growth are the predominant processes.

(2) The primiparous female in which reproductive growth is added to body growth. This stage will be growing an ovary for the second brood of eggs whilst carrying the first brood in her brood pouch. Also she is often at the end of exponential body growth.

(3) The fifth adult instar, carrying her fifth brood of eggs and growing an ovary for the sixth brood. She is still capable of body growth but at a greatly reduced rate.

Table 4.5 shows the age in days, absolute growth rates in $\mu gC.ind.^{-1}d^{-1}$, and the relative growth rate in percentage of initial weight, of the three life cycle stages: the second instar juvenile, the primiparous female and the fifth adult instar. It should be noted here that the 2nd juvenile instar represents only the body growth but the other two represent body and reproductive growth.

(a) Absolute growth rate

Fig 4.6 illustrates the values in Table 4.5 as three dimensional diagrams of absolute growth rate at each temperature-food concentration combination. There is a general tendency for absolute growth rate to decrease with decreasing temperature and food concentration in all three life cycle stages and in both species, Diaphanosoma excisum and Moina micrura. In Diaphanosoma excisum at any one temperature-food concentration combination the absolute growth rate differs in the three life cycle stages, being low in the juvenile, increasing to the primipara, and decreasing to the fifth adult instar, with the exception of those at 32°C and the two highest (non-limiting) food levels where the growth rates of the primipara and the 5th adult are high and rather similar. This is shown more clearly in Fig 4.7, which is a composite, three dimensional diagram in which the growth rates of all three stages are plotted for all the experimental conditions measured. In Moina micrura it is the juveniles and the primipara which have similar growth rates and the 5th adult has a considerably lower absolute growth rate. As the 3rd instar is the primipara in Moina micrura, in most

temperature-food combinations, the close resemblance of the growth rate between 2nd juvenile and primipara is not unexpected. Again the exception lies at 32°C and the two non-limiting food levels (1.0 and 0.5 $mgC.L^{-1}$) in which the primiparous females have the highest growth rate.

(b) Relative growth rate

The relative or percentage growth rate also decreased with decrease in temperature and food concentration in both species. Fig 4.8 is a composite, three-dimensional diagram in which the relative growth rates of all three life cycle stages are plotted for all measured experimental combinations. Both species show the same pattern of highest values for juveniles, then primipara, and lowest for the 5th adult instar.

4.6 RELATIONSHIP OF WEIGHT SPECIFIC GROWTH RATE OF PRIMIPARA TO FOOD CONCENTRATION AT DIFFERENT TEMPERATURES

In order to examine the effect of food concentration on relative growth rates (weight specific growth rates) the Michaelis-Monod relationship (Chapter 3) was used. This relationship was also employed to examine any interspecific variations in growth rates. The relationship used was as follows:

$$G\% = (G\%_{max} \cdot S)/(Ks \cdot S) \text{----------} (5)$$

where G% is the percentage weight specific growth rate, $G\%_{max}$ is the maximum percentage weight specific growth rate, and Ks the food concentration ($mgC.L^{-1}$) at which 50% of the maximum weight specific growth rate is attained.

The linearized Michaelis-Monod function was used to obtain linear regressions with reciprocal transformed values of both relative growth rate and food concentration. The results of the regressions with calculated $G\%_{max}$ and Ks are given in Table 4.6 and graphically presented by the back transformed values in Fig. 4.9. It is clear from this that the relative growth rate increased with increasing temperature in both species but that in Diaphanosoma excisum the effect between 22°C and 27°C is more or less similar to that between 27°C and 32°C. In contrast, in Moina micrura the effect between 22°C and 27°C is greater than that between 27°C and 32°C. A 1.5 fold increase was found in the maximum relative growth rate ($G\%_{max}$) between 22°c and 27°C as well as between 27°C and 32°C in Diaphanosoma excisum. In Moina micrura while there was a two fold increase between the former two temperatures, the increase was only about one quarter between the latter two temperatures. In any case it is evident from Table 4.6 and Fig 4.9 that the maximum relative growth rate attained by Moina micrura, even at 27°C, was greater than that at 32°C in Diaphanosoma excisum. The latter can attain 50% G_{max} at a lower food concentration (Ks) in 32°C and 27°C than Moina micrura but the values of $G\%_{max}$ were lower. Moina micrura needs a higher Ks but, if food is available, attains higher values of $G\%_{max}$.

4.7 APPORTIONING OF GROWTH FOLLOWING MATURITY

As reproductive growth occurs in addition to body growth after the onset of maturity, an attempt was made to evaluate the absolute growth rate both for body growth and reproductive growth in terms of $\mu gC.ind.^{-1}d^{-1}$ during the period from just before the animal attained maturity (during the last juvenile phase) to the end of the fourth adult instar. The rate of body growth was calculated according to

$$\frac{[W_A - (n.w)] - W_j}{t_A - t_j}$$

where

W_A = weight of the 4th adult carrying its 4th set of eggs in the brood pouch. ($ugC.ind.^{-1}$)

W_j = weight of the animal just before the onset of maturity ($\mu gC.ind.^{-1}$)

each W_A and W_j are means of the four replicates predicted according to the Richard's model.

n = number of neonates produced in the 4th brood (mean of the four replicates).

w = carbon weight of the neonate derived from length weight relationship (mean weight of the neonates produced) assuming that this is the same as the weight of an egg in the brood pouch.

t_A = Age at the end of the 4th adult instar (days)

t_j = Age of the animal just before onset of maturity (days).

Rate of reproductive growth was computed as

$W_n \cdot N$

where

N = number of neonates produced in the first four successive broods (the mean of the four replicates)

W_n = the carbon content of the neonate derived from length-carbon weight relationship (mean of all neonates produced at each food-temperature combination).

t_p = age of the primipara (days)

t_A = age at the end of the 4th adult instar (days)

The amount of carbon channeled towards body growth and reproductive growth, computed according to the method described above, in Diaphanosoma excisum and Moina micrura is given in Table 4.10. It is evident from the results for both species that the amount of carbon channeled towards reproductive growth decreases with decreasing food concentration at any one temperature, as well as with decreasing temperature at any one food concentration. In Diaphanosoma excisum a similar trend was found with body growth at each temperature, but at the two lower temperatures the body growth remained similar at each food level below 1.0 $mgC.L^{-1}$ irrespective of temperature. Further, it is evident that in Diaphanosoma excisum, not only at non-limiting food levels but also at limiting food levels, body growth occurs even after the onset of maturity, but always (at any temperature-food combination) the rate of reproductive growth is

greater (2-10 times) than the rate of body growth. In contrast to Diaphanosoma excisum, Moina micrura gave negative results for body growth at three food-temperature combinations, using the above calculation procedure. These negative results could be due to two reasons: (a) The calculations were carried out on the assumption that the carbon content of the eggs was equivalent to the carbon content of the neonates. In Moinidae, in contrast to other cladocerans, after the egg is passed to the brood pouch the eggs are nourished by a placenta-like structure (Goulden 1968). Therefore the embryos will have a higher carbon content at the later part of their development compared to that of eggs and early embryos. The length-body carbon content relationships for this species were obtained by using the same length animals but carrying different stages of developing eggs or embryos. Therefore there is a possibility that the total body and reproductive carbon content of the animal is underestimated when derived from these length-carbon weight relationships. This may result in an underestimation of the body growth rate. (b) There is a possibility that the animal uses up its stored body carbon after the onset of maturity resulting in negative body growth. In order to evaluate body growth properly for Moina micrura it is necessary to obtain a length-carbon weight relationship of animals which are carrying embryos at their last stage of development, so that the carbon content of neonates can be deduced from the predicted values of the animals from such length-carbon weight relationships, and can be used to evaluate the rate of body growth according to this method with less error.

However, in spite of the above mentioned effects the results given in Table 4.10 for Moina micrura reveal that, except for the three occasions in which negative results were obtained for the rate of body growth, after the onset of maturity carbon is still channelled towards body growth but the amount is much lower than that channeled toward reproductive growth. Ignoring the three anomalous results, reproductive growth expressed as a percentage of total growth is similar in both Moina micrura (66-99%) and Diaphanosoma excisum (63-96%).

4.8 THE CARBON WEIGHT OF THE FIFTH ADULT INSTAR

The interacting effect of food and temperature on growth was examined by measuring the body weights of the fifth adult instar at the end of each experiment. These females had been reared in constant but different conditions until they attained their 5th adult instar. The carbon weight obtained is given in Fig 4.11 in a three dimensional diagram. At non-limiting food levels (1.0 and 0.5 $mgC.L^{-1}$) the body carbon content remained more or less the same at a particular temperature, was greatest at 32°C but decreased as temperature decreased. This pattern was shown by both Diaphanosoma excisum and Moina micrura. At any particular temperature, the decrease in body carbon content became evident below 0.5 $mgC.L^{-1}$.

The interacting effect of food and temperature on the carbon content of the 5th adult instar was examined statistically by multiple regression analysis (Chapter 3) and the results are given in Table 4.11. The relationship was found to be well

described by natural log of body weight to both natural log of food and temperature. From the results given in Table 4.11 considering the F values due to temperature and food separately it is evident that the effect of food is greater than the effect of temperature.

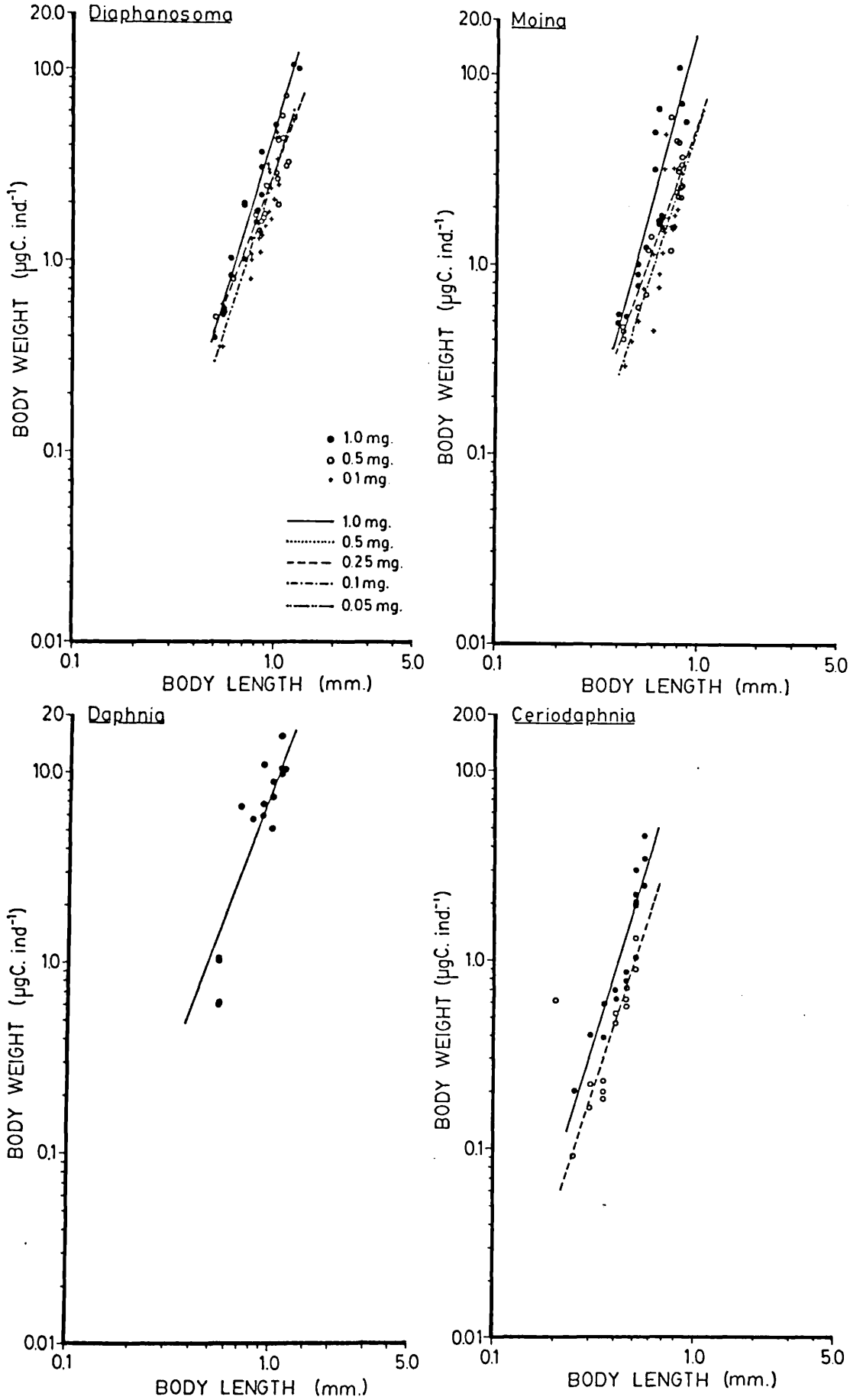


Figure 4.1 Body weight (µgC. ind^{-1}) plotted against body length (mm) on logarithmic axes for animals grown at 32° C in different food concentrations. The statistics of the regression lines are given in Table 4.1

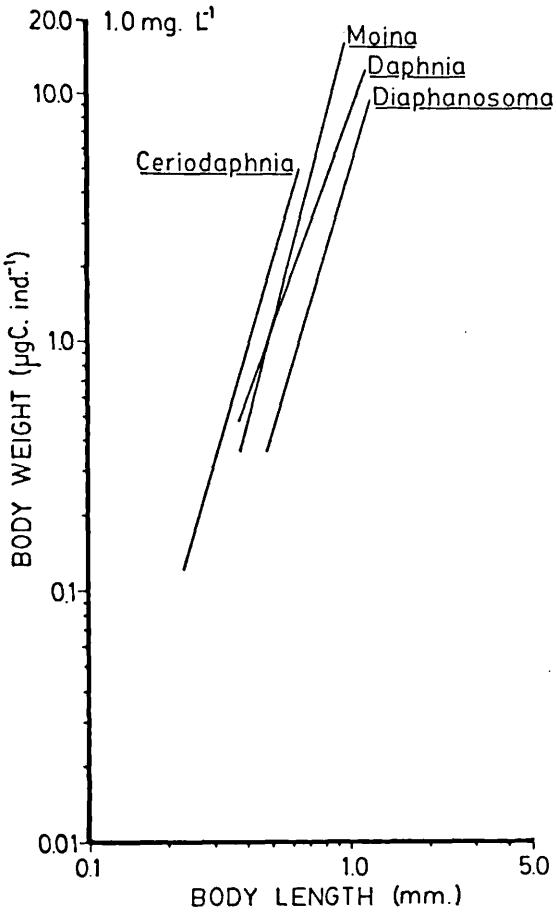
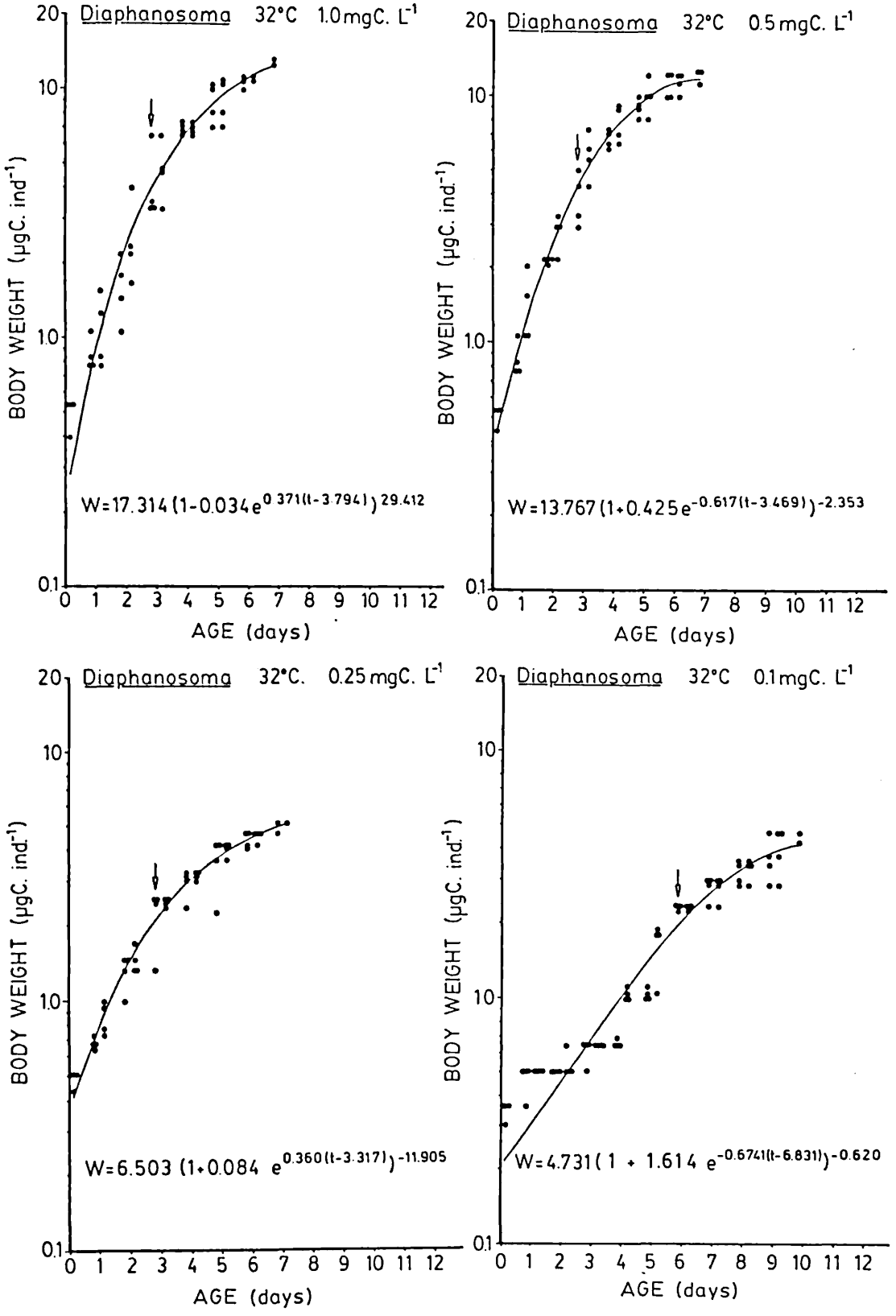


Figure 4.2 Comparison of the length/weight regressions for the four species reared at 32°C in 1.0 mgC. L^{-1} food concentration.

Figure 4.3 Growth curves showing body weight (as ugC) of individual animals against age in days at different combinations of food concentration and temperature. The equations given are those of the lines fitted using Richards model as explained in the text. The arrow indicates the size and age of primipara.

These graphs are presented in the following order:-

Diaphanosoma excisum:	32°C and 5 food levels
	27°C and 4 food levels
	22°C and 3 food levels
Moina micrura:	32°C and 5 food levels
	27°C and 4 food levels
	22°C and 4 food levels
Ceriodaphnia cornuta:	32°C and 3 food levels
Daphnia lumholtzi:	22°C and 4 food levels



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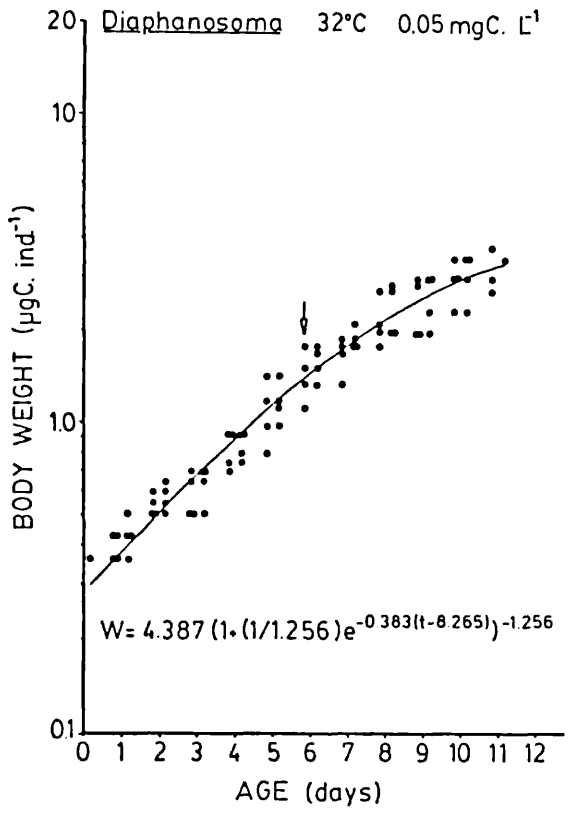

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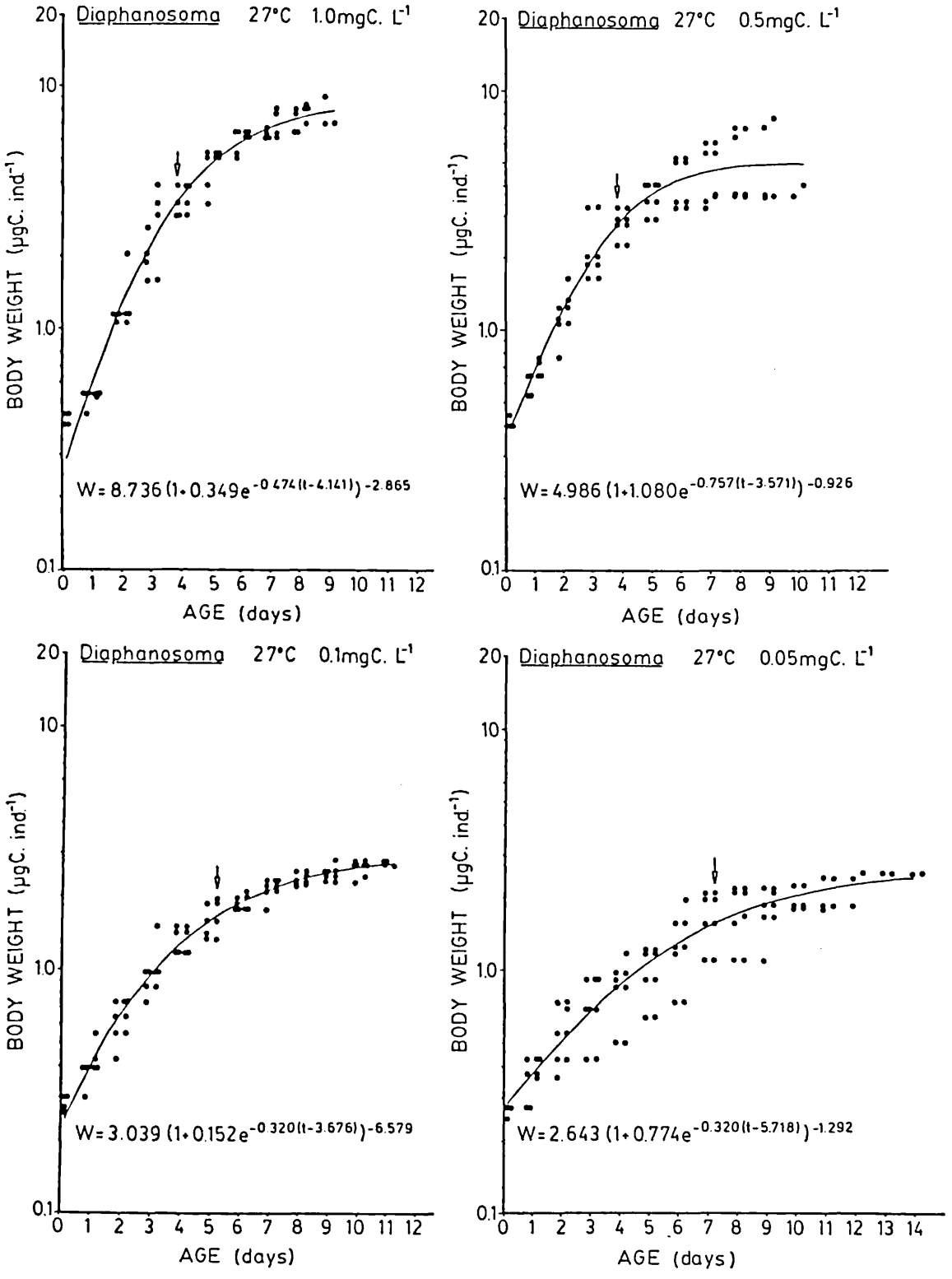

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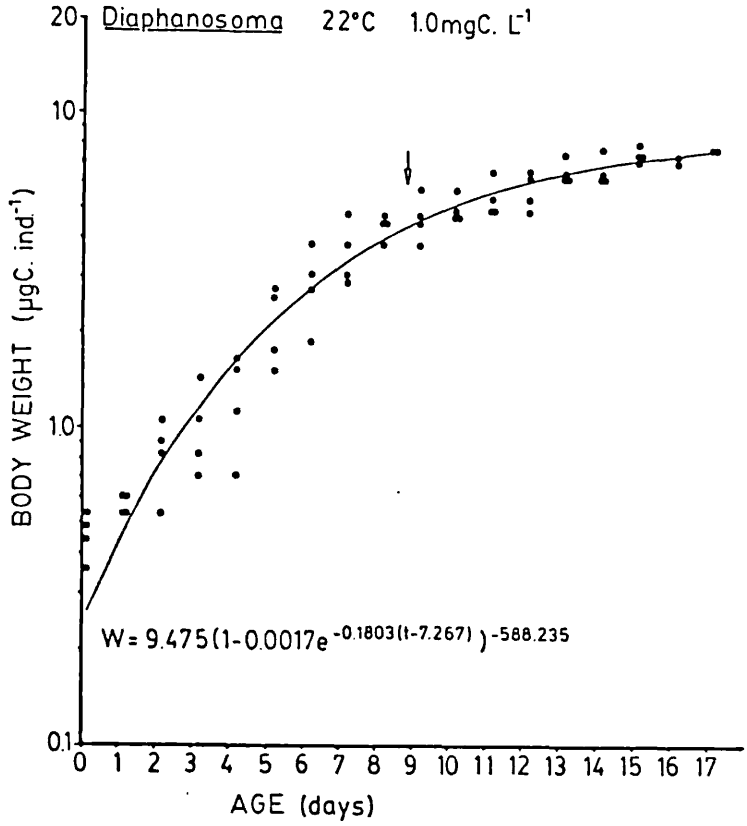

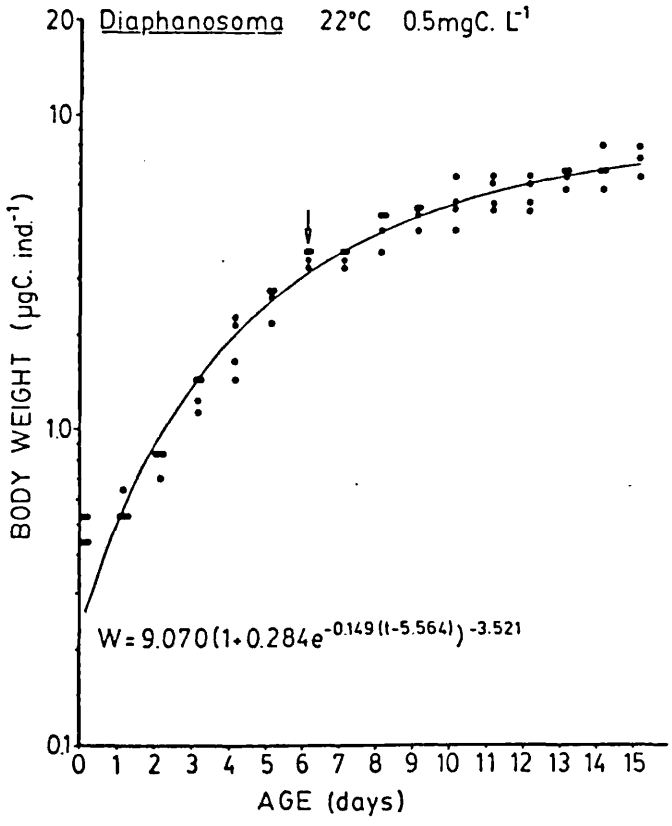

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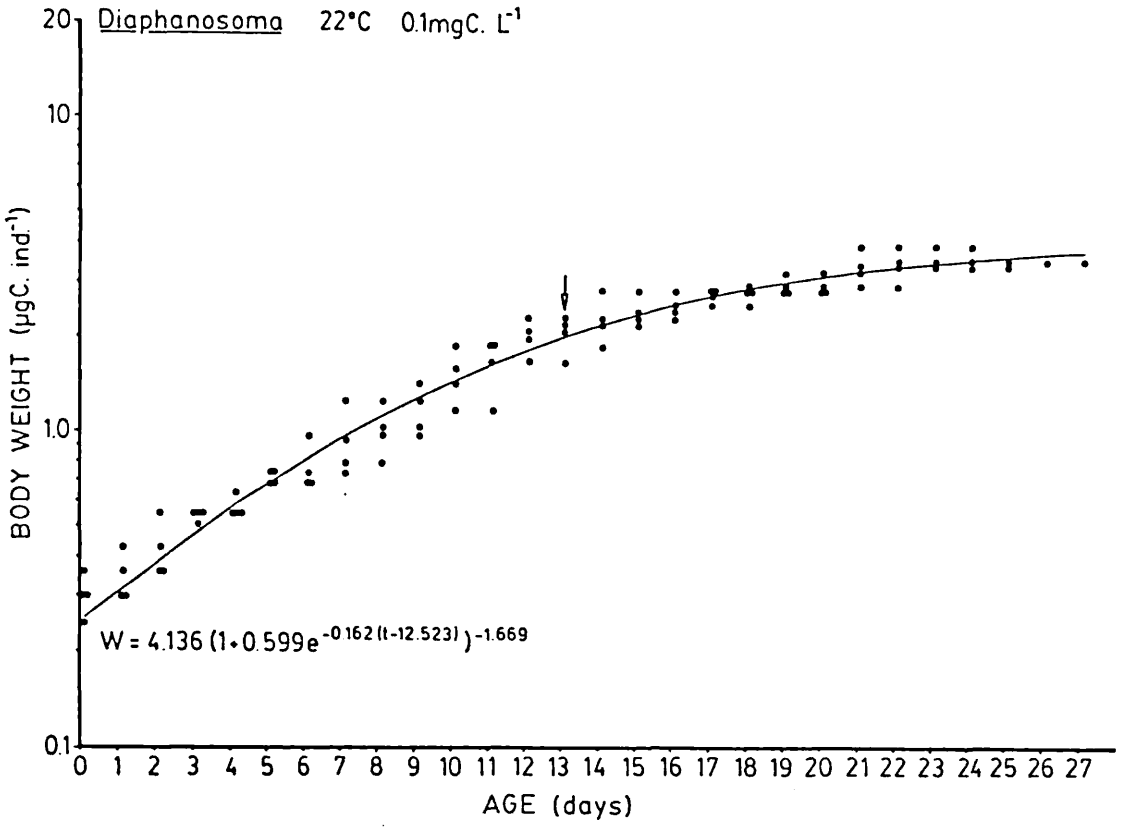

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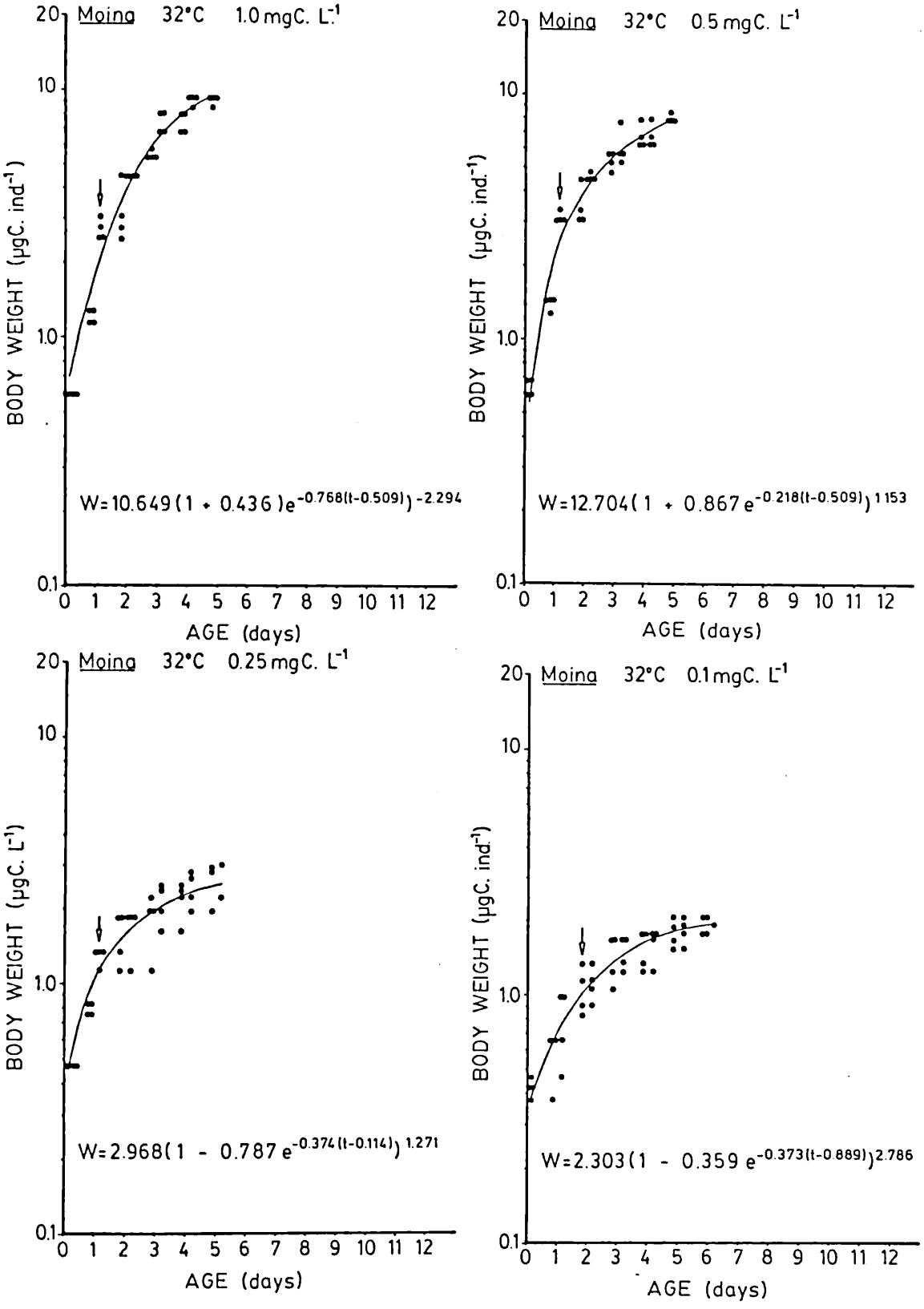

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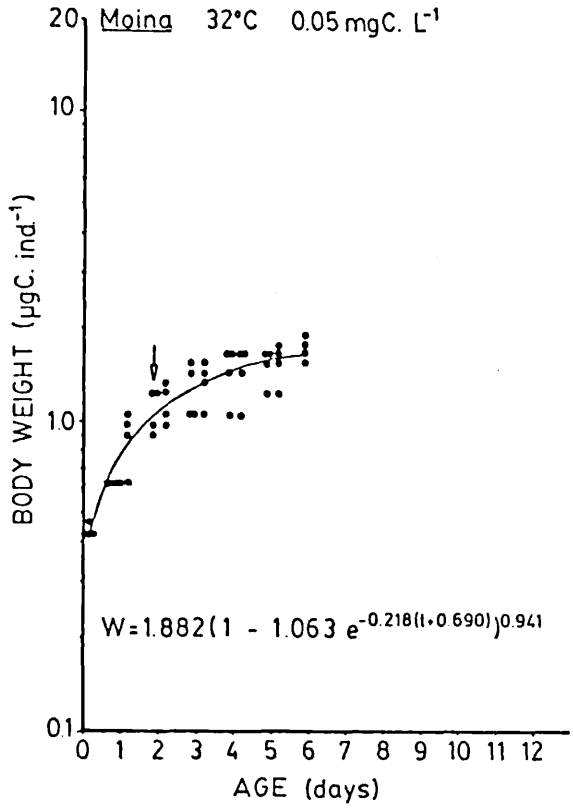

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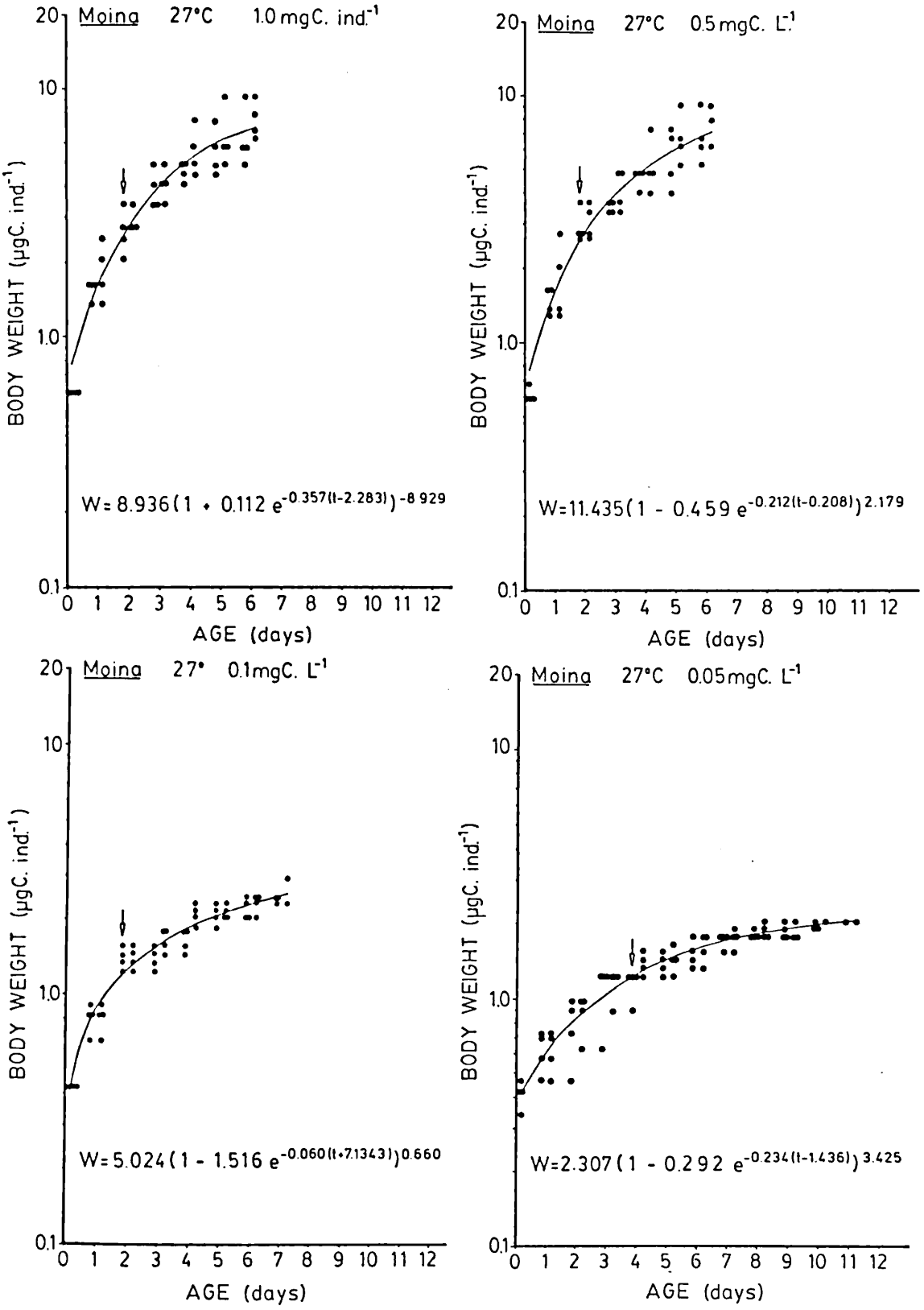

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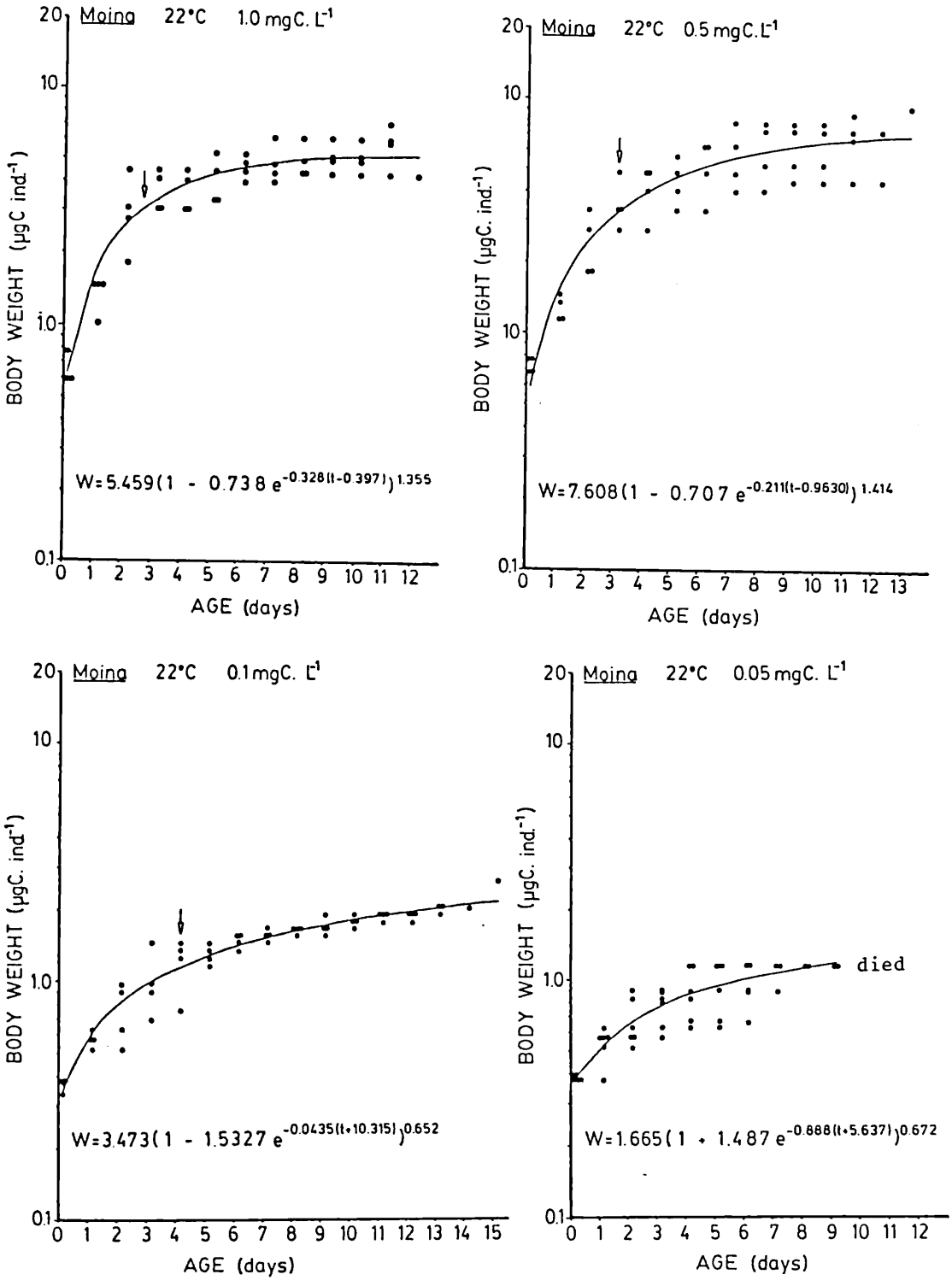

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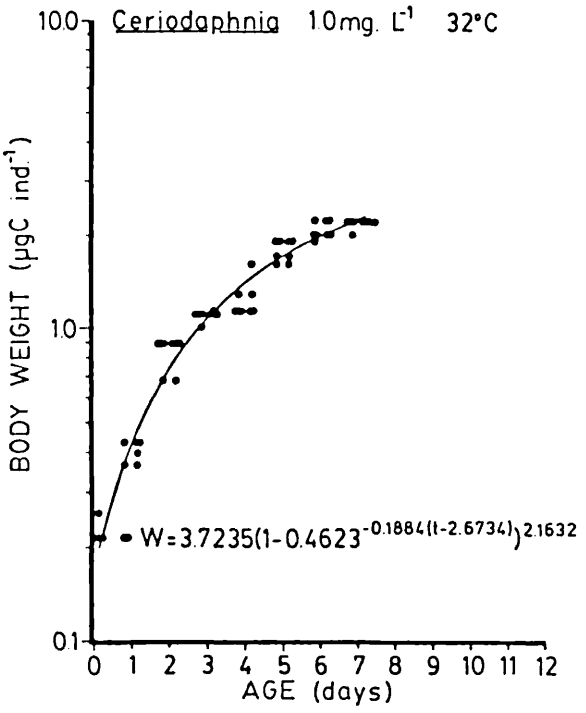

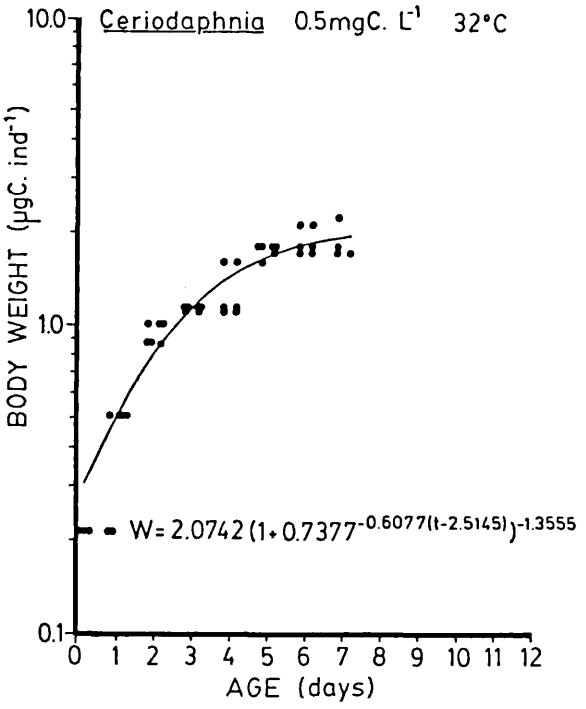

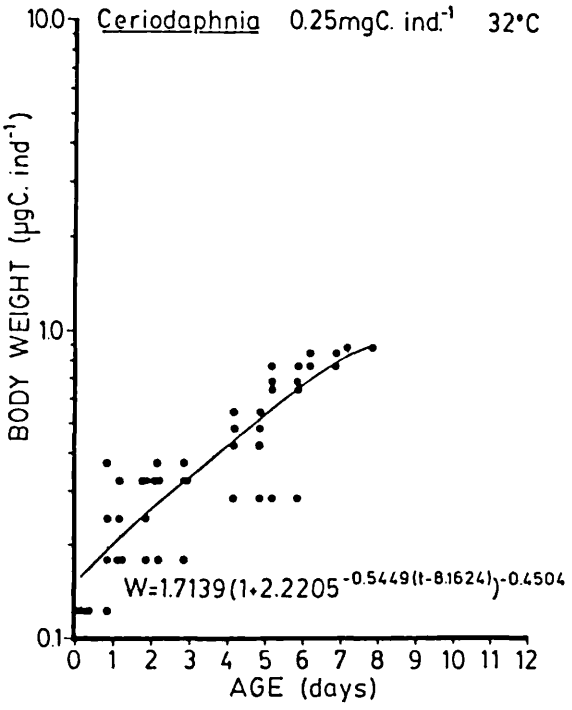

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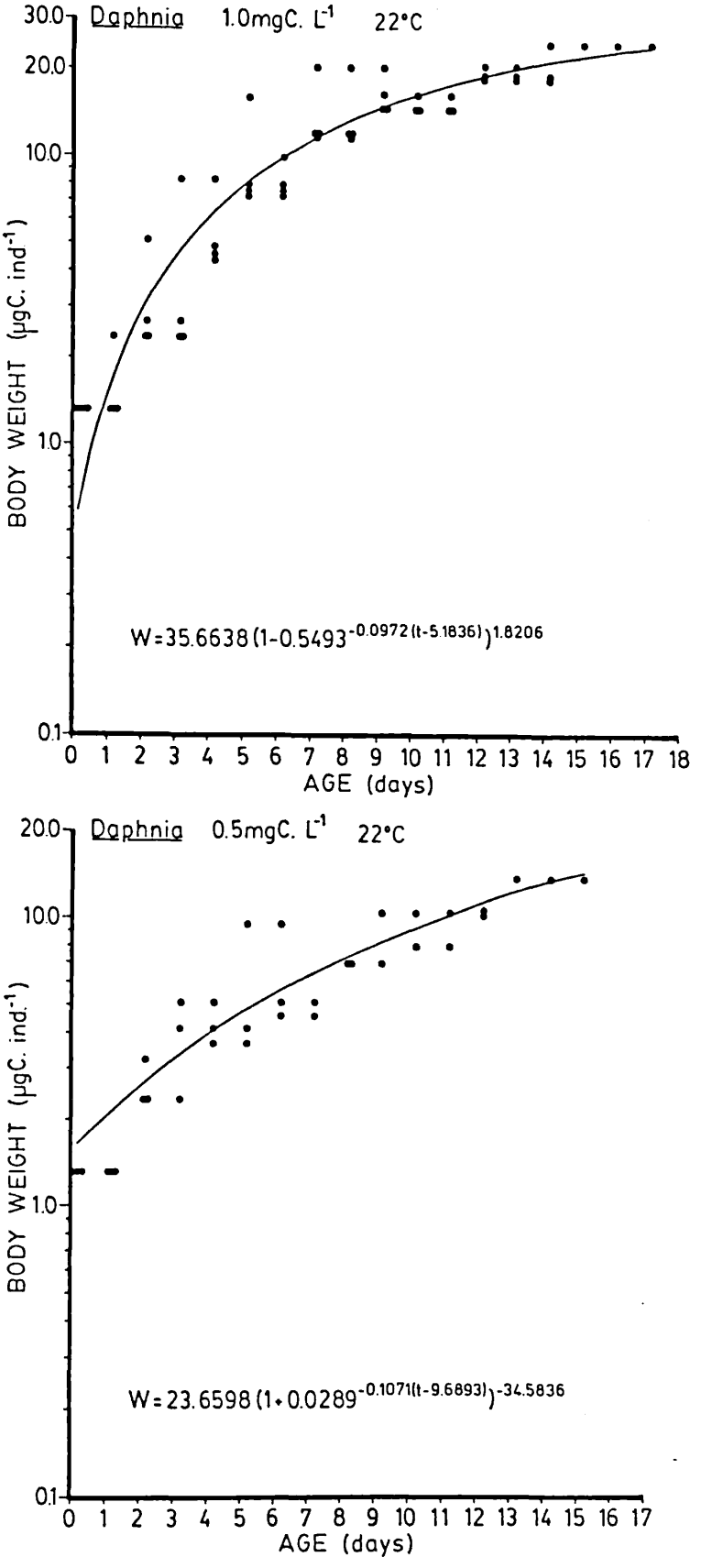

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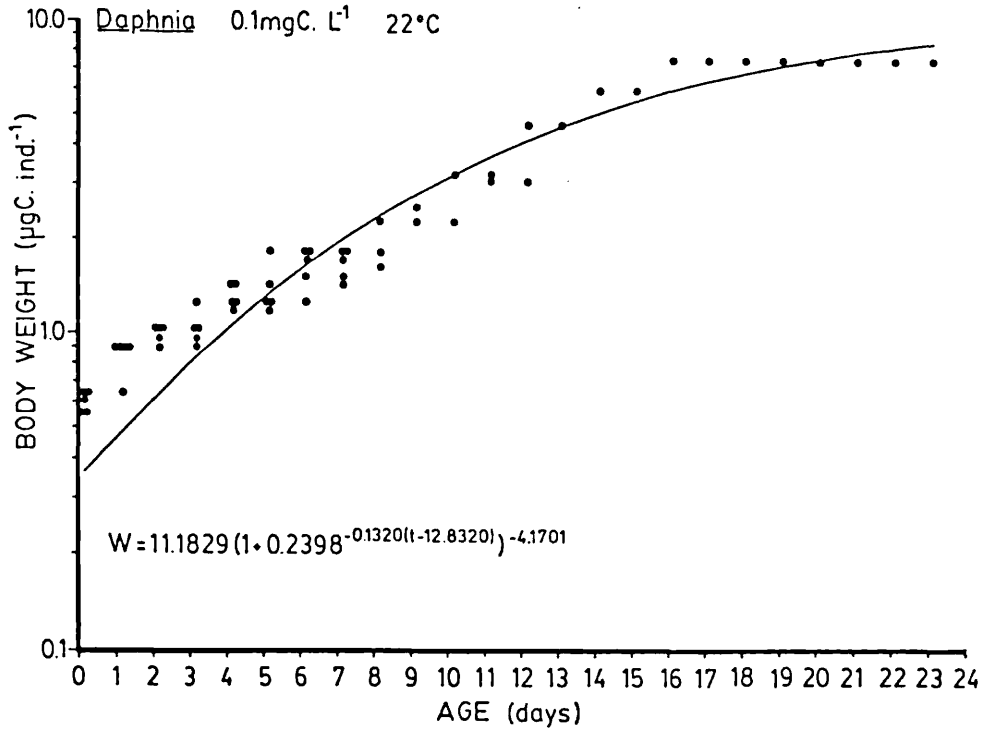

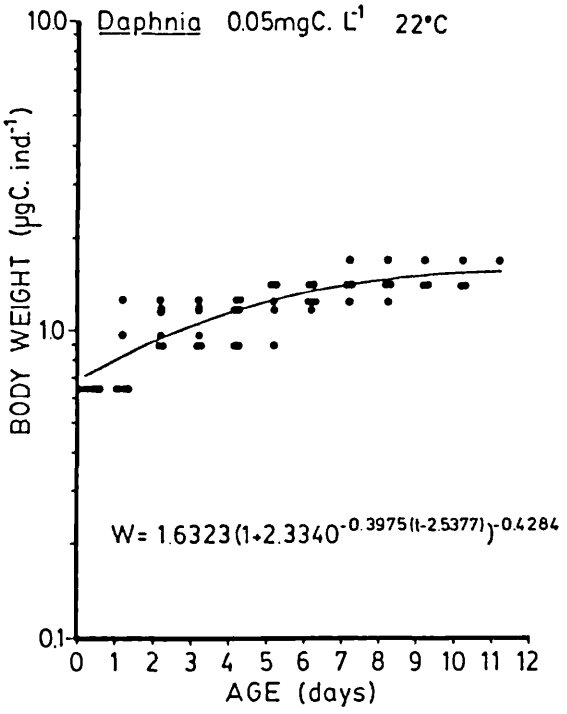

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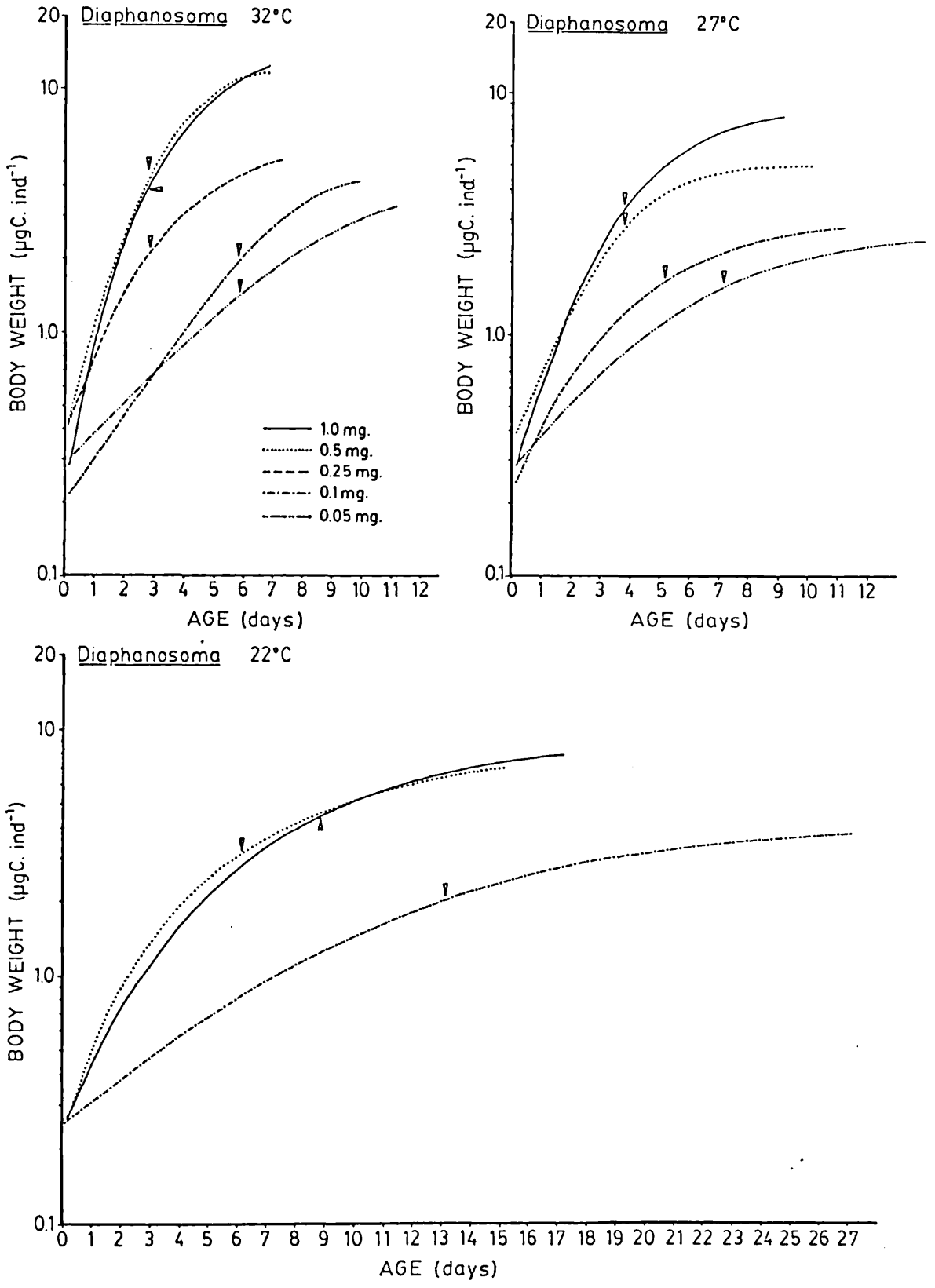


Figure 4.4 Comparison of growth curves for *Diaphanosoma excisum* and *Moina micrura* (from Figure 4.3) obtained at the same temperatures, but at different food concentrations.

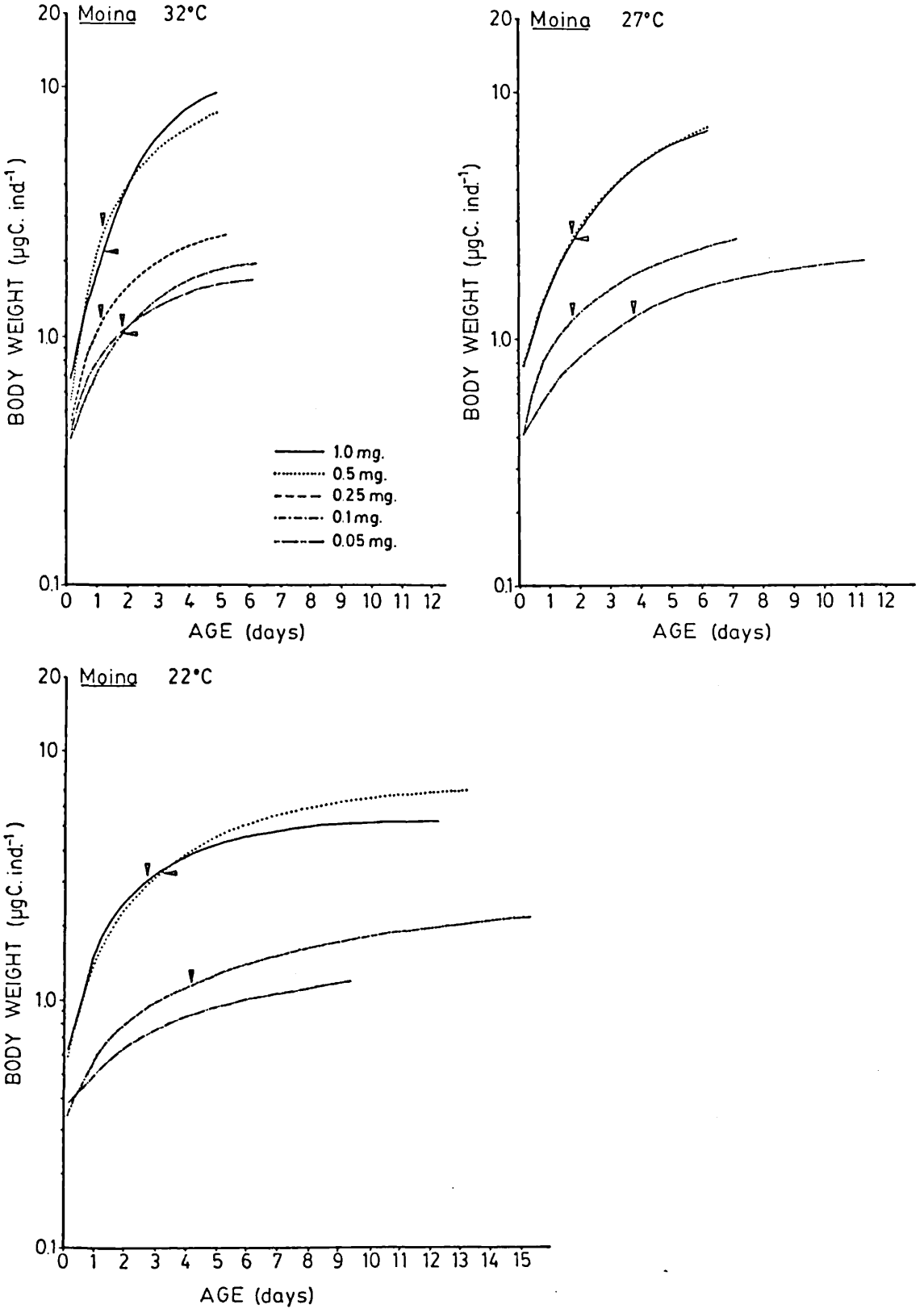
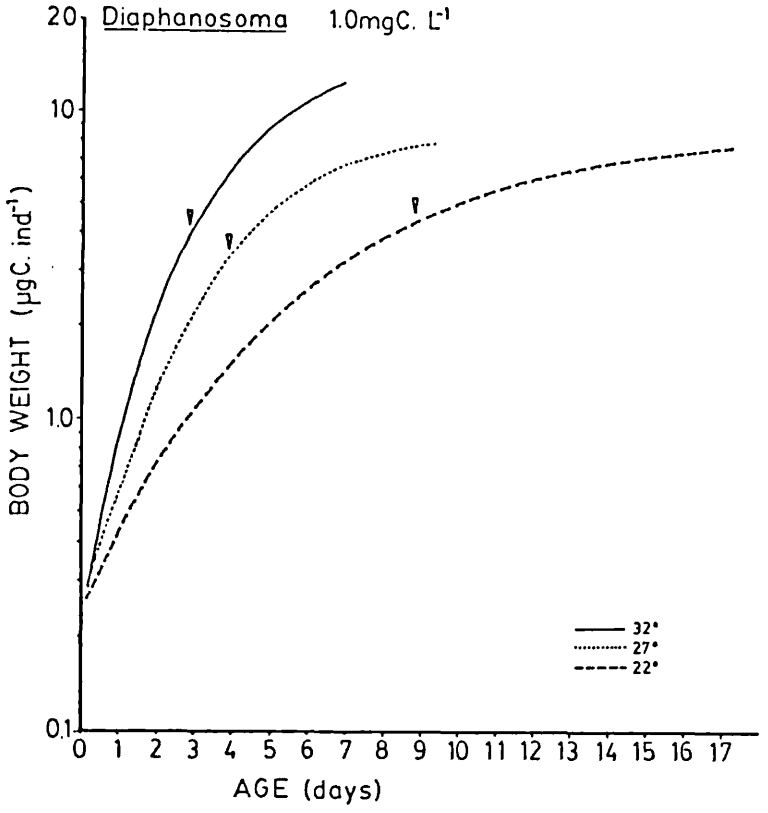


Figure 4.4 continued



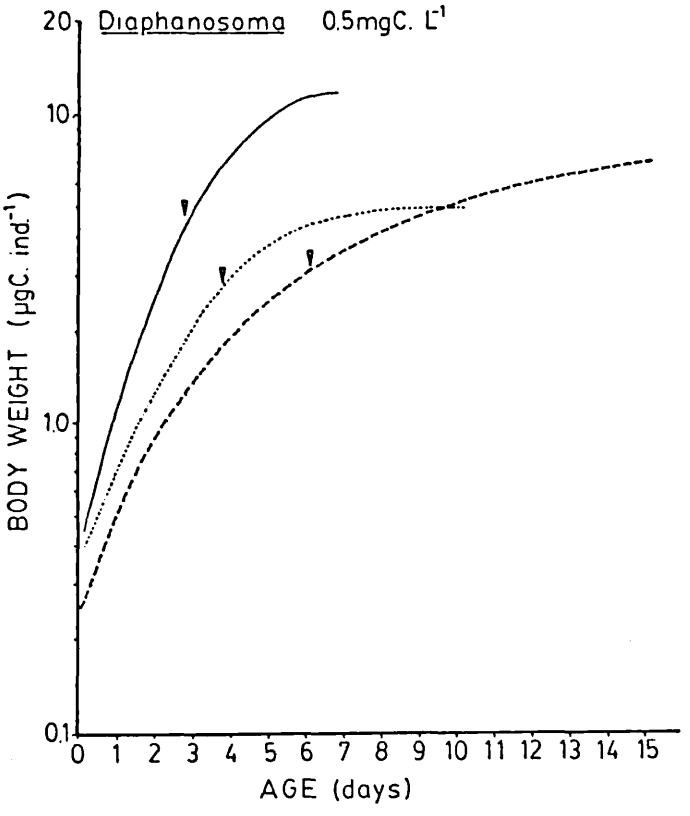
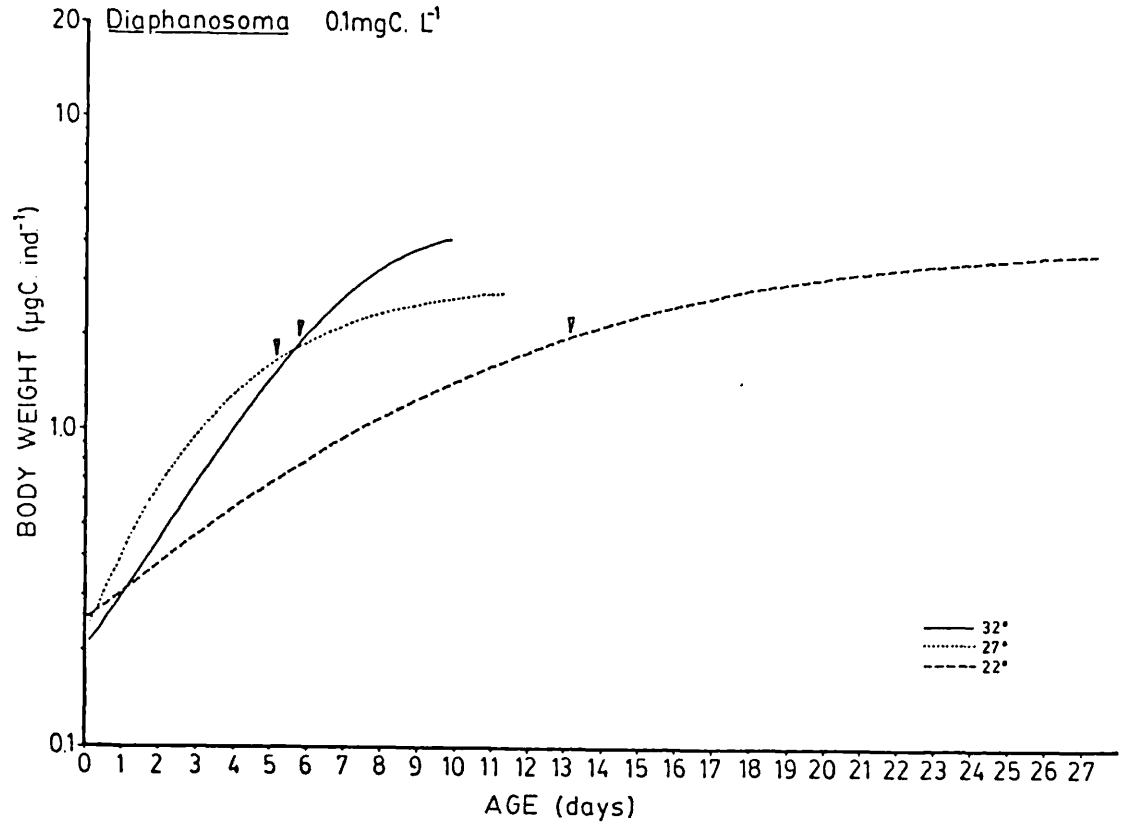
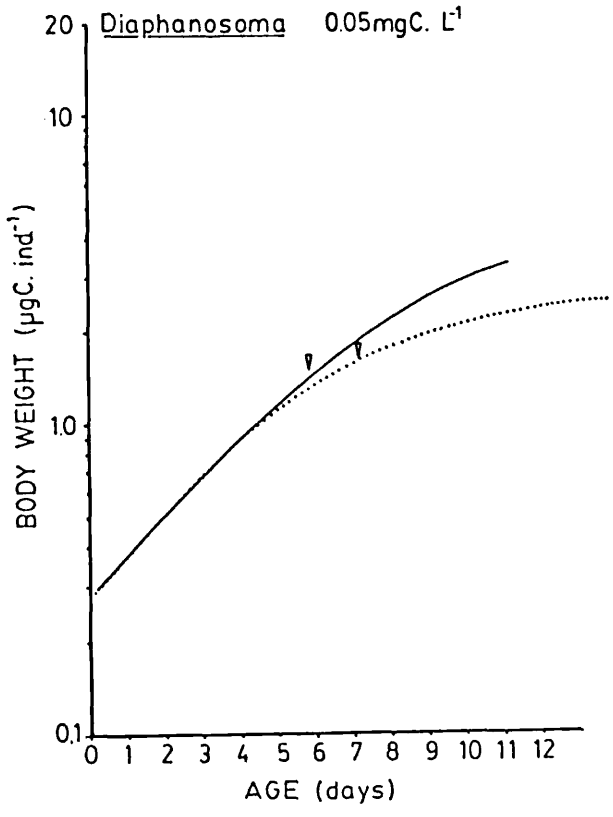


Figure 4.5 Comparison of growth curves for Moina micrura and Diaphanosoma excisum (from Figure 4.3) obtained at different temperatures but at the same food concentration.





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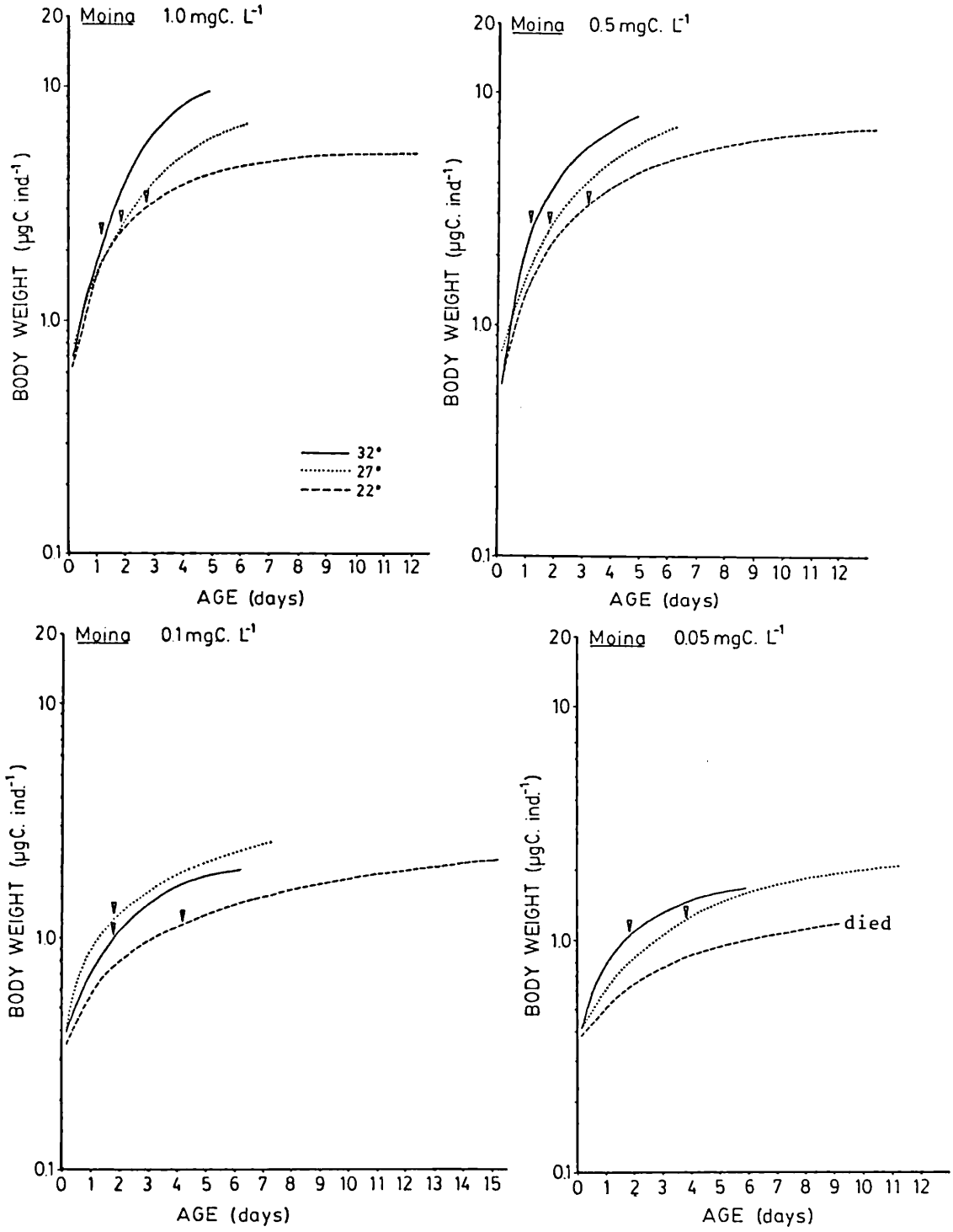

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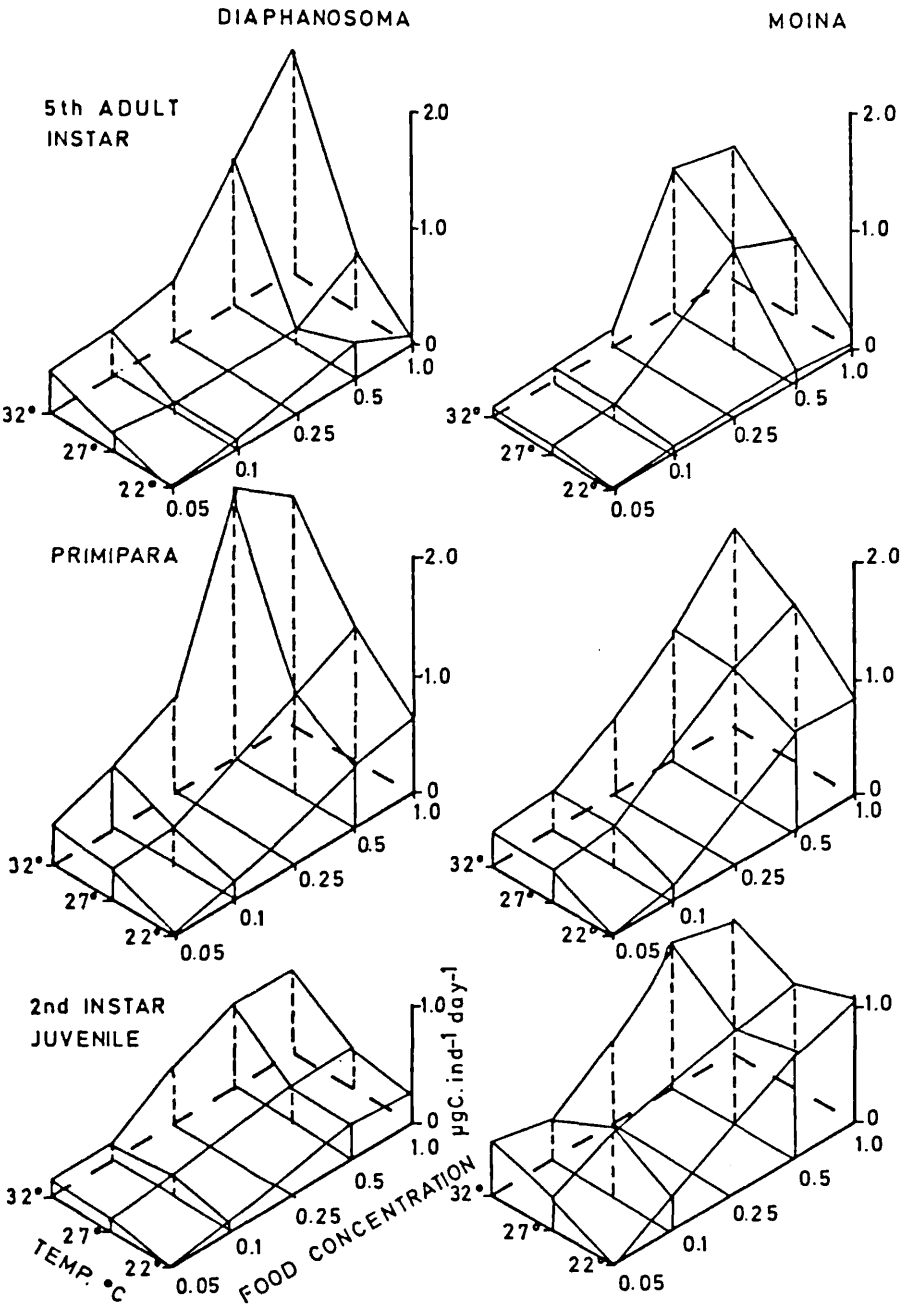


Figure 4.6 Three-dimensional plots to show the effects of temperature and food concentration on three stages of development (2nd juvenile instar, primipara, and 5th adult instar) in Diaphanosoma excisum and Moina micrura.

The vertical axis is absolute growth rate ($ugC.ind^{-1}.day^{-1}$).

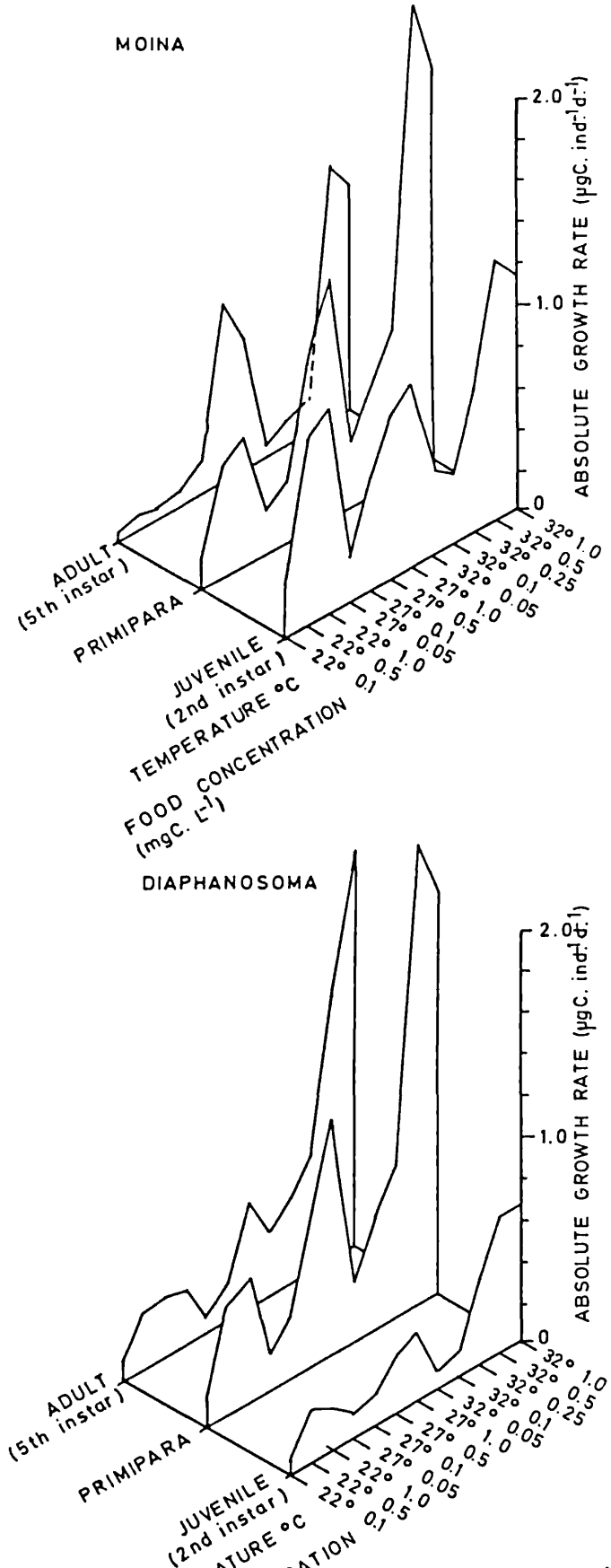


Figure 4.7 Composite diagrams comparing the effects of temperature and food concentration on the absolute growth rates of the 2nd juvenile instar, the primipara, and the 5th 5th adult instar of Diaphanosoma excisum and Moina micrura..

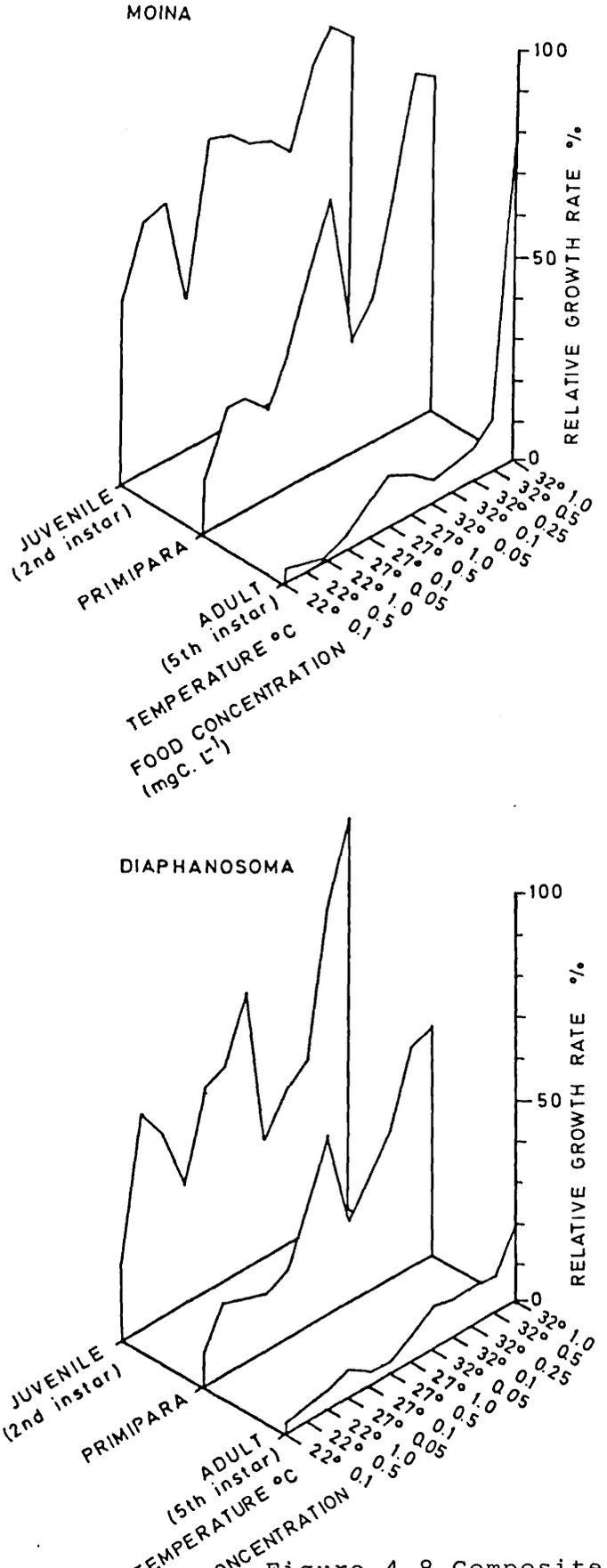


Figure 4.8 Composite diagrams comparing the effects of temperature and food concentration on the relative growth rate of three life cycle stages (2nd juvenile instar, primipara, and 5th adult instar) in Diaphanosoma excisum and Moina micrura.

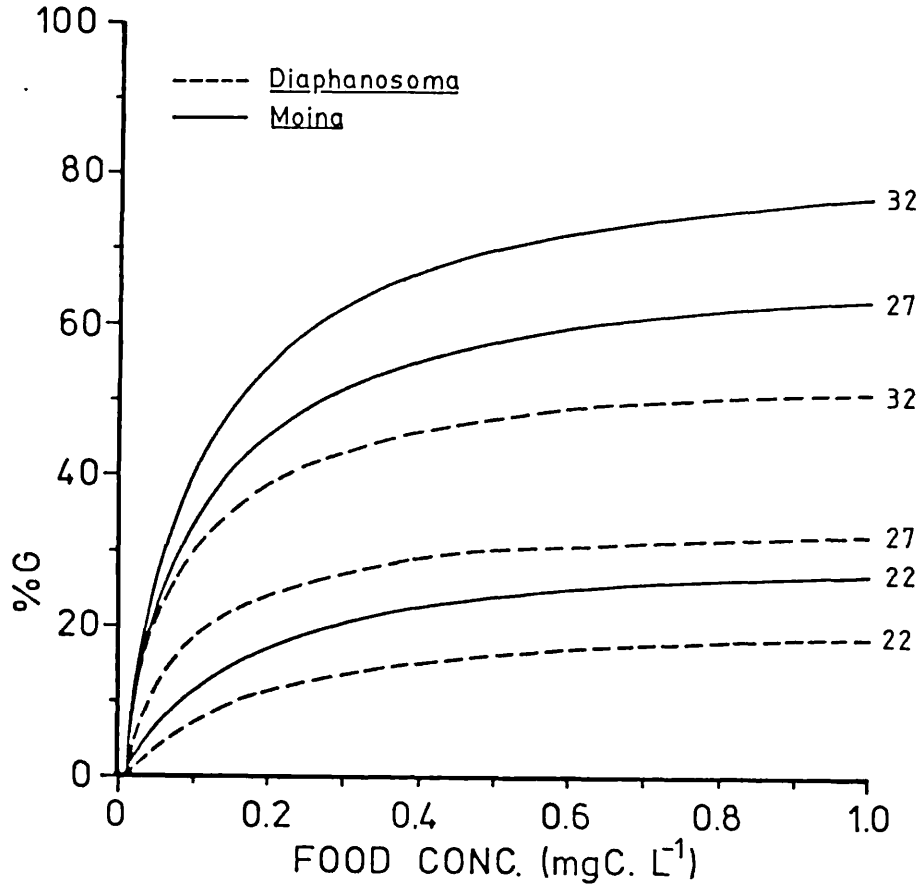


Figure 4.9 Relationships between the relative growth rate of the primipara stage and food concentration at different temperatures.

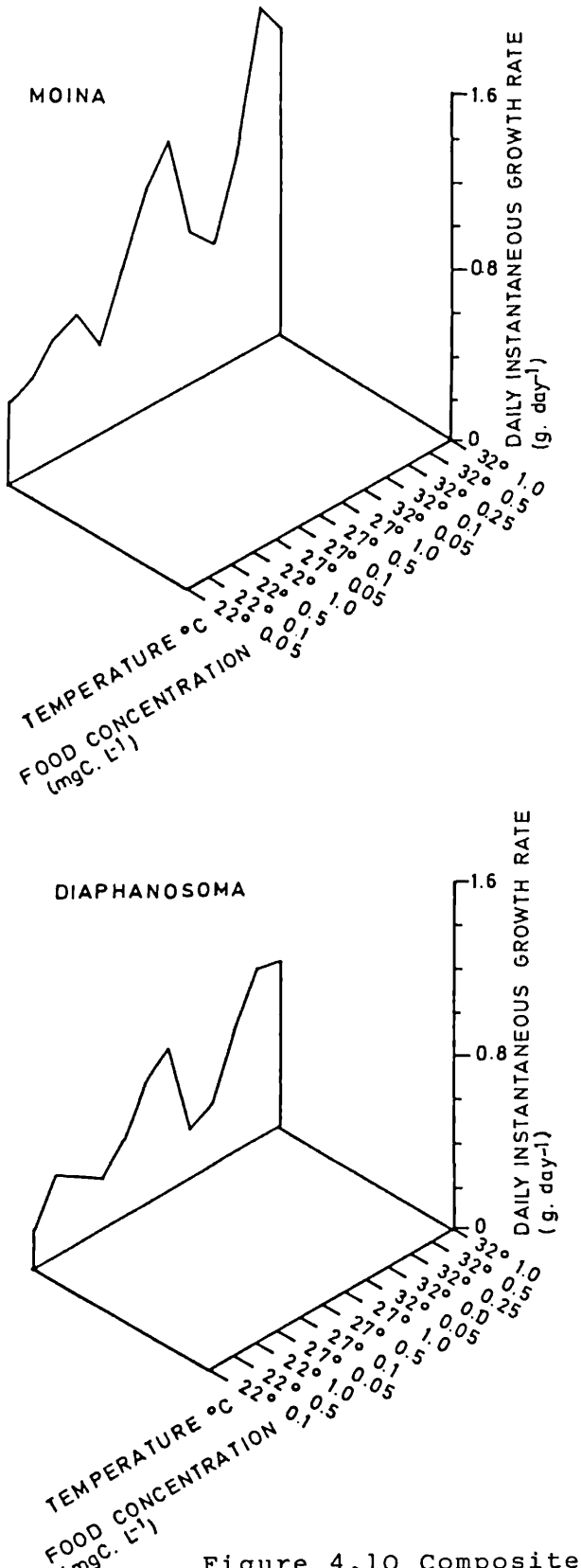


Figure 4.10 Composite digrams showing instantaneous growth rate of Diaphanosoma excisum and Moina micrura in all experimental conditions.

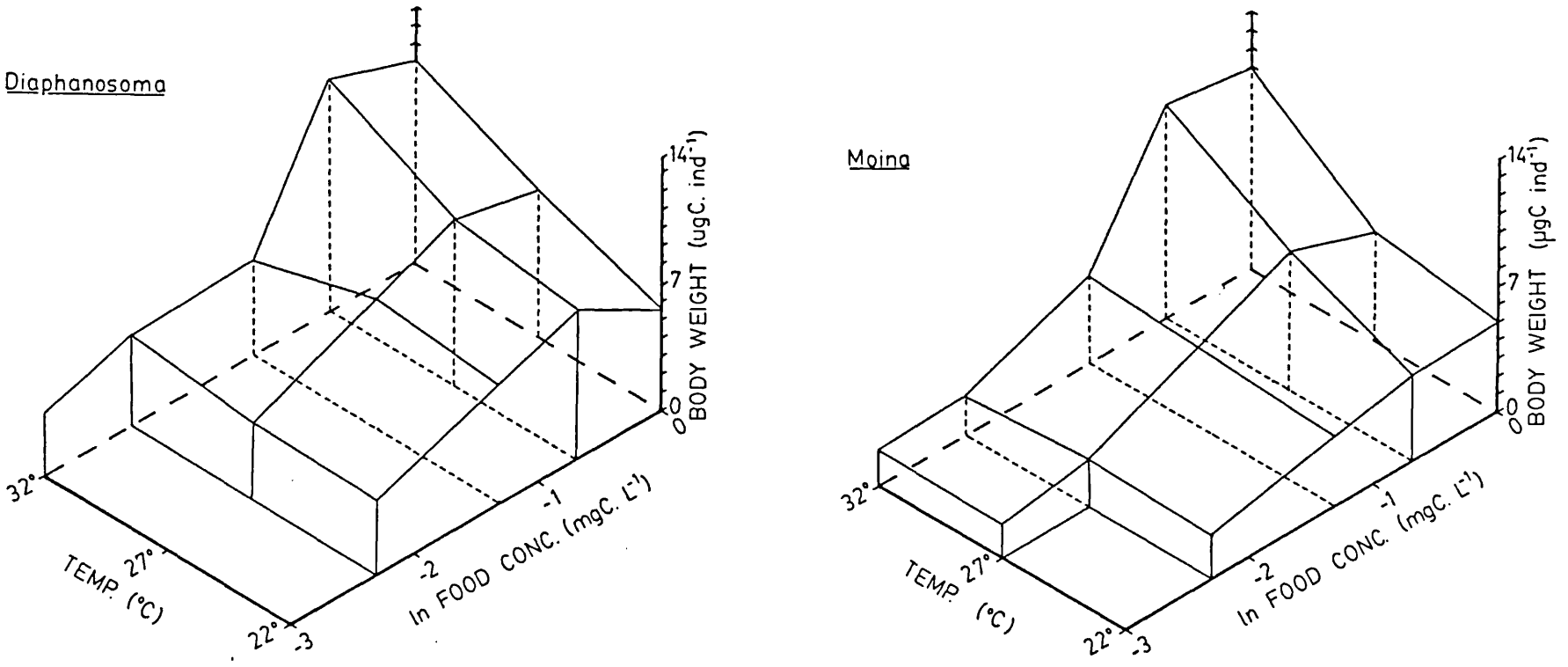


Figure 4.11 Three-dimensional plots to show the carbon weight per individual (µgC ind^{-1}) of the 5th adult instars of Diaphanosoma excisum and Moina micrura reared at different combinations of temperature and food concentration.

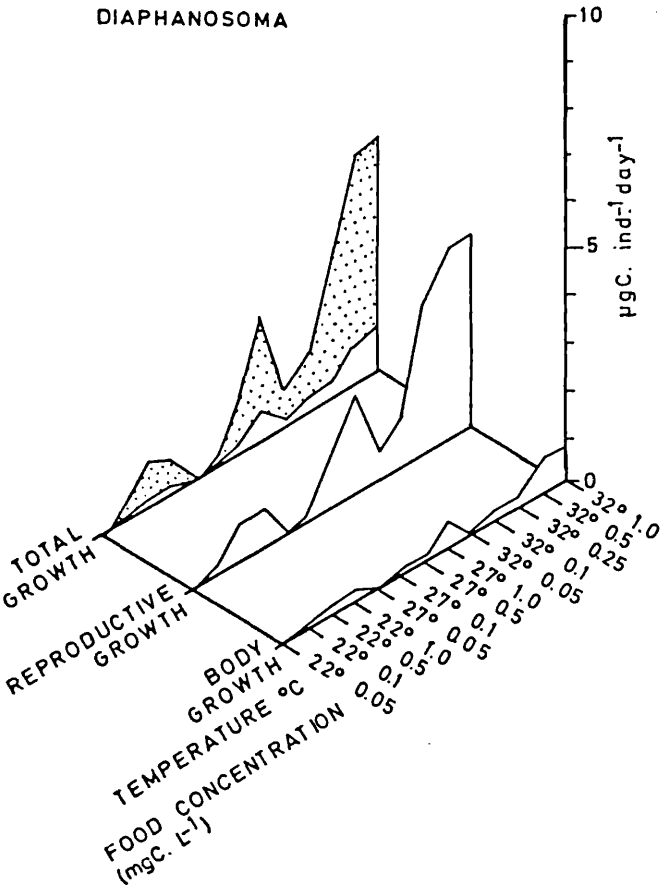


Figure 4.12 Composite diagram showing adult body growth and reproductive growth (accumulated for the period from immediately before the primipara to the end of the 5th adult instar) for Diaphanosoma excisum.

Table 4.1 Parameters of the linear regressions relating organic body carbon content ($\mu gC.ind^{-1}$) to length (mm) of (a) Diaphanosoma excisum, (b) Moina micrura, (c) Ceriodaphnia cornuta and (d) Daphnia lumholtzi reared in the laboratory at 32°C.

Regression equation: $\ln Y = \ln a + b \ln X$
Y = organic carbon content ($\mu gC.ind.^{-1}$) X = length (mm).
df = degrees of freedom; F = variance ratio; P = level of significance.

Food conc $mgC.L^{-1}$	ln a	b	df	F	P
(a) Diaphanosoma excisum					
1.0	1.5904	3.518	1,14	245.3	<0.0001
0.25	1.0739	2.782	1,18	119.2	<0.0001
0.1	1.0664	3.304	1,23	148.9	<0.0001
(b) Moina micrura					
1.0	3.0731	4.292	1,12	55.54	<0.0001
0.25	1.7912	3.025	1,23	104.15	<0.0001
0.1	1.7683	3.396	1,19	46.47	<0.0001
(c) Ceriodaphnia cornuta					
1.0	3.2300	3.683	1,13	98.50	<0.0001
0.25	2.3917	3.438	1,14	141.07	<0.0001
(d) Daphnia lumholtzi					
1.0	2.0521	3.219	1,14	63.81	<0.0001

Table 4.2 Analysis of covariance comparing the carbon weight-length regressions (given in Table 4.1) of (a) Diaphanosoma excisum, (b) Moina micrura and (c) Ceriodaphnia lumholtzi reared in the laboratory at 32°C. Differences between regression coefficients was tested by the SS-STP test and those between the elevations of the curves by the S-N-K test. Lines connect treatment numbers whose regressions are not significantly different at P = 0.05 level. Group numbers are given in ascending order of magnitude.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

(a) Diaphanosoma excisum

Comparison between regression coefficients.

Food conc mgC.L	Group no.	Regression coeff. ± SE	df	F	P	SS-STP test
1.0	1	3.518 ± 0.225	2,55	2.304	0.1094	2 3 1
0.25	2	2.781 ± 0.255				
0.1	3	3.316 ± 0.273				

Comparison between the elevation of the curve.

Food conc mgC.L	Group no.	Adjusted mean ± SE	df	F	P	S-N-K test
1.0	1	0.8892 ± 0.2257	2,58	13.807	<0.0001	3 2 1
0.25	2	0.5565 ± 0.2255				
0.1	3	0.4671 ± 0.1578				

(b) Moina micrura

Comparison between regression coefficients.

Food conc mgC.L	Group no.	Regression coeff. ± SE	df	F	P	SS-STP test
1.0	1	4.292 ± 0.576	2,54	2.261	0.114	2 3 1
0.25	2	3.025 ± 0.296				
0.1	3	3.396 ± 0.498				

Comparison between the elevations of the curve.

Food conc mgC.L	Group no.	Adjusted mean ± SE	df	F	P	S-N-K test
1.0	1	1.0168 ± 0.229	2,54	16.226	<0.0001	3 2 1
0.25	2	0.3991 ± 0.227				
0.1	3	0.2250 ± 0.227				

(c) Ceriodaphnia cornuta

Comparison between regression coefficients.

Food conc mgC.L	Group no.	Regression coeff. ± SE	df	F	P	SS-STP test
1.0	1	3.682 ± 0.3710	1,27	0.268	0.609	2 1
0.25	2	3.440 ± 0.2893				

Comparison between the elevation of the curve.

Food conc mgC.L	Group no.	Adjusted mean ± SE	df	F	P	S-N-K test
1.0	1	-0.1139 ± 0.2184	1,29	29.48	<0.0001	2 1
0.25	2	-0.7343 ± 0.2183				

Table 4.3 Analysis of covariance comparing the carbon weight-length regressions to the four species studied reared in the laboratory at 32°C and 1 $mgC.L^{-1}$ food level. The difference between regression coefficients were tested by the SS-STP test and those between the elevation of the curves by the S-N-K test. Lines connect group numbers whose regressions are not significantly different at P = 0.05 level. Group numbers are given in ascending order of magnitude.

df = degrees of freedom; F = variance ratio; P = level of significance.

(a) Comparison between regression coefficients.

Species	Group no.	Regression coeff. ± SE	df	F	P	SS-STP test
Daphnia lumholtzi	1	3.219 ± 0.399	3,53	1.159	0.334	1 3 2 4
Ceriodaphnia cornuta	2	3.683 ± 0.371				
Diaphanosoma excisum	3	3.518 ± 0.224				
Moina micrura	4	4.292 ± 0.576				

(b) Comparison of the elevation of the curve.

Species	Group no.	Adjusted mean ± SE	df	F	P	S-N-K test
Daphnia lumholtzi	1	0.4838 ± 0.2019	3,57	26.77	<0.0001	3 1 4 2
Ceriodaphnia cornuta	2	1.5118 ± 0.2076				
Diaphanosoma excisum	3	-0.0192 ± 0.1964				
Moina micrura	4	1.0289 ± 0.1949				

Table 4.4 Parameters of the Richards growth equation for fitting growth curves to a) Diaphanosoma excisum, b) Moina micrura, c) Ceriodaphnia cornuta, d) Daphnia lumboltzi.

Equation: $W_t = W_{max} (1 - b \exp(-g(t-t_o)))^P$

Wt = weight ($\mu gC.ind.^{-1}$) and time t; W_{max} = maximum weight attained; t_o = time of inflection of the curve; g = growth constant; P = exponent.

Temp °C	Food conc ($mg.C.L^{-1}$)	W_{max}	g	t_o	b	P
(a) Diaphanosoma excisum						
32	1.0	17.3193	0.3712	3.7935	0.0338	29.8558
	0.5	13.7672	0.6170	3.4690	0.4254	2.3507
	0.25	6.5026	0.3597	3.3170	-0.0837	-11.9474
	0.1	4.7308	0.6741	6.8321	-1.1136	-0.6197
	0.05	4.3863	0.3834	8.2645	-1.2560	0.7962
27	1.0	8.7360	0.4738	4.1409	-0.3490	-2.8653
	0.5	4.9858	0.7575	3.5715	-1.0802	-0.9258
	0.1	3.0391	0.3198	3.6760	-0.1521	-6.5746
	0.05	*2.6430	0.3203	5.7184	-0.7735	-1.2928
22	1.0	9.4747	0.1803	7.2670	-0.0017	-588.2353
	0.5	9.0699	0.1803	7.2670	-0.2835	-3.5273
	0.1	4.1357	0.1620	12.5218	-0.5993	-1.6686
(b) Moina micrura						
32	1.0	10.6487	0.7684	2.3022	-0.4358	2.2946
	0.5	12.7036	0.2175	0.5085	0.8663	1.1543
	0.25	2.9679	0.3736	0.1139	0.7867	1.2711
	0.1	2.3028	0.3729	0.8894	1.0633	0.9405
27	1.0	8.9363	0.3566	2.2828	-0.1124	-8.8968
	0.5	11.4347	0.2166	2.0820	0.4890	2.0450
	0.1	5.0244	0.0610	-7.1343	1.5161	0.6596
	0.05	2.3069	0.2344	-1.4362	0.2919	3.4258
22	1.0	5.4590	0.3276	0.3971	0.7381	1.3548
	0.5	7.6078	0.2106	0.9630	0.7069	1.4146
	0.1	3.4731	0.0435	-10.3149	1.5327	0.6524
	0.05	*1.6646	0.0888	-5.6366	1.4868	0.6726
(c) Ceriodaphnia cornuta						
32	1.0	3.7236	0.1884	2.6734	0.4623	2.1632
	0.5	2.0742	0.6077	2.5144	-0.7377	-1.3555
	0.25	1.7139	0.5449	8.1624	-2.2205	-0.4504
(d) Daphnia lumholtzi						
22	1.0	35.6638	0.0972	5.1836	0.5493	1.8206
	0.5	23.6598	0.1071	9.6893	-0.0289	-34.5835
	0.1	11.1829	0.1320	12.8320	-0.2398	-4.1701
	0.05	*1.6323	0.3975	2.5377	-2.3340	-0.4284

* juveniles died

Table 4.5 Age (days), Absolute growth rate ($\mu gC.ind^{-1}\ day^{-1}$) and Relative growth rate (as a percentage of body weight per day) of the three stages in life cycle: 2nd juvenile instar, primipara instar and 5th adult instar of (a) Diaphanosoma excisum, (b) Moina micrura, (c) Ceriodaphnia cornuta and Daphnia lumholtzi.

Temp.	Food conc $mg.C.L^{-1}$	2nd Juvenile			Primipara			5th Adult		
		Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD
(a) Diaphanosoma excisum										
32°C	1.0	0.833 ± 0.00	0.674 ± 0.00	92.17 ± 0.00	2.750 ± 0.00	1.906 ± 0.49	55.23 ± 8.89	6.167 ± 0.81	1.884 ± 0.28	17.14 ± 4.93
	0.5	0.833 ± 0.00	0.695 ± 0.00	75.65 ± 0.00	2.834 ± 0.47	2.289 ± 0.29	53.46 ± 8.41	6.667 ± 0.33	1.301 ± 0.14	9.45 ± 1.50
	0.25	0.833 ± 0.00	0.439 ± 0.00	42.47 ± 0.00	2.917 ± 0.17	0.797 ± 0.01	37.00 ± 1.47	6.917 ± 0.16	0.516 ± 0.03	10.40 ± 0.76
	0.1	1.334 ± 0.33	0.133 ± 0.00	37.99 ± 1.37	5.833 ± 0.00	0.583 ± 0.00	29.84 ± 0.00	9.500 ± 0.38	0.401 ± 0.04	9.86 ± 1.48
	0.05	1.167 ± 0.47	0.109 ± 0.01	29.01 ± 2.81	6.750 ± 0.42	0.353 ± 0.01	20.60 ± 1.01	10.750 ± 0.41	0.361 ± 0.04	11.24 ± 1.23
27°C	1.0	0.833 ± 0.00	0.354 ± 0.00	67.60 ± 0.00	3.417 ± 0.50	1.196 ± 0.07	43.89 ± 6.06	8.417 ± 0.50	0.496 ± 0.09	6.50 ± 1.29
	0.5	0.833 ± 0.00	0.321 ± 0.00	52.28 ± 0.00	4.333 ± 1.00	0.810 ± 0.01	27.51 ± 9.84	9.250 ± 1.03	0.151 ± 0.15	3.09 ± 3.11
	0.1	0.917 ± 0.16	0.198 ± 0.01	50.89 ± 0.09	5.417 ± 0.50	0.305 ± 0.01	17.55 ± 2.39	10.750 ± 0.41	0.097 ± 0.01	3.55 ± 0.45
	0.05	1.000 ± 0.19	0.114 ± 0.01	30.26 ± 0.44	7.250 ± 1.31	0.211 ± 0.02	13.75 ± 3.48	*11.667 ± 2.08	*0.170 ± 0.10	*7.57 ± 4.14
22°C	1.0	1.167 ± 0.00	0.211 ± 0.00	44.59 ± 0.00	7.667 ± 0.57	0.625 ± 0.01	16.82 ± 1.59	16.417 ± 0.95	0.293 ± 0.04	3.76 ± 0.65
	0.5	1.167 ± 0.00	0.298 ± 0.00	53.03 ± 0.00	6.167 ± 0.00	0.580 ± 0.00	18.44 ± 0.00	14.917 ± 0.50	0.296 ± 0.01	4.24 ± 0.35
	0.1	1.417 ± 0.50	0.031 ± 0.02	19.25 ± 0.30	13.167 ± 1.15	0.178 ± 0.02	7.94 ± 2.35	24.667 ± 2.08	0.080 ± 0.02	2.23 ± 0.67

Temp.	Food conc	2nd Juvenile			Primipara			5th Adult		
		Age ± SD	Absolute growth rate + SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD
(b) Moina micrura										
32°C	1.0	0.833 ± 0.00	1.185 ± 0.00	78.86 ± 0.00	1.167 ± 0.00	1.693 ± 0.00	81.84 ± 0.00	4.167 ± 0.00	1.185 ± 0.00	18.11 ± 0.00
	0.5	0.833 ± 0.00	1.241 ± 0.00	83.29 ± 0.00	1.167 ± 0.00	1.179 ± 0.00	84.59 ± 0.00	4.833 ± 0.00	1.242 ± 0.00	13.36 ± 0.00
	0.25	0.833 ± 0.00	0.701 ± 0.00	76.04 ± 0.00	1.167 ± 0.00	0.635 ± 0.00	56.01 ± 0.00	4.750 ± 0.41	0.192 ± 0.01	7.82 ± 0.75
	0.1	0.833 ± 0.00	0.379 ± 0.00	59.05 ± 0.00	1.944 ± 0.19	0.368 ± 0.01	35.24 ± 2.94	5.917 ± 0.16	0.132 ± 0.01	6.75 ± 0.61
	0.05	0.833 ± 0.00	0.456 ± 0.00	63.45 ± 0.00	1.750 ± 0.41	0.303 ± 0.02	28.27 ± 3.40	5.833 ± 0.00	0.081 ± 0.00	4.85 ± 0.00
27°C	1.0	0.833 ± 0.00	0.917 ± 0.00	65.77 ± 0.00	1.500 ± 0.38	1.395 ± 0.37	64.43 ± 3.63	6.167 ± 0.00	0.668 ± 0.00	9.64 ± 0.00
	0.5	0.833 ± 0.00	0.809 ± 0.00	70.15 ± 0.00	1.667 ± 0.33	1.163 ± 0.02	49.38 ± 8.24	6.167 ± 0.00	0.881 ± 0.00	12.32 ± 0.00
	0.1	0.833 ± 0.00	0.574 ± 0.00	72,44 ± 0.00	1.833 ± 0.00	0.398 ± 0.00	33.90 ± 0.00	6.722 ± 0.50	0.194 ± 0.01	7.89 ± 0.71
	0.05	0.917 ± 0.16	0.235 ± 0.00	37.02 ± 1.74	2.833 ± 0.00	0.225 ± 0.00	21.89 ± 0.00	9.917 ± 0.95	0.072 ± 0.01	3.64 ± 0.86
22°C	1.0	1.167 ± 0.00	1.088 ± 0.00	63.21 ± 0.00	2.667 ± 0.57	0.830 ± 0.11	28.30 ± 7.85	11.667 ± 0.50	0.054 ± 0.01	1.00 ± 0.19
	0.5	1.167 ± 0.00	0.947 ± 0.00	61.60 ± 0.00	3.167 ± 0.81	0.838 ± 0.08	26,67 ± 8.09	11.917 ± 0.96	0.174 ± 0.03	2.55 ± 0.52
	0.1	1.167 ± 0.00	0.271 ± 0.00	44.23 ± 0.00	4.417 ± 1.25	0.150 ± 0.02	13.13 ± 3.89	13.417 ± 1.26	0.073 ± 0.01	3.55 ± 0.42

continued

Table 4.5 continued

Temp	Food conc $mgC.L^{-1}$	2nd Juvenile			Primipara			5th Adult		
		Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD	Age ± SD	Absolute growth rate ± SD	Relative growth rate ± SD
(c) *Ceriodaphnia cornuta*										
32°C	1.0	1.000 ± 0.19	0.282 ± 0.02	66.62 ± 4.07	3.167 ± 0.471	0.337 ± 0.01	28.80 ± 4.96	6.750 ± 0.419	0.255 ± 0.01	11.58 ± 1.12
	0.5	1.056 ± 0.19	0.254 ± 0.02	48.99 ± 0.00	2.833 ± 0.00	0.342 ± 0.00	31.35 ± 0.00	6.833 ± 0.00	0.342 ± 0.00	5.00 ± 0.00
	0.25	0.833 ± 0.19	0.044 ± 0.00	22.49 ± 0.00	4.167 ± 0.00	0.142 ± 0.00	44.93 ± 0.00	7.167 ± 0.00	0.081 ± 0.04	9.61 ± 0.00

Temp	Food conc $mgC.L^{-1}$	2nd Juvenile			Primipara			5th Adult		
		Age ± SD	Absolute growth rate + SD	Relative growth rate + SD	Age ± SD	Absolute growth rate + SD	Relative growth rate + SD	Age ± SD	Absolute growth rate + SD	Relative growth rate + SD
(d) *Daphnia lumholtzi*										
32°C	1.0	1.917 ± 0.00	1.358 ± 0.16	50.54 ± 9.70	6.667 ± 1.00	1.723 ± 0.03	16.50 ± 3.35	**14.167 ± 0.00	**1.2121 ± 0.00	**5.46 ± 0.00
	0.5	1.917 ± 0.05	0.551 ± 0.30	21.57 ± 0.99	8.167 ± 2.64	0.868 ± 3.30	12.27 ± 3.30			
	0.1	2.167 ± 0.00	0.108 ± 0.00	13.19 ± 0.00						
	0.05	2.167 ± 0.00	0.127 ± 0.00	25.88 ± 0.00						

* did not attain 5th Adult
** only up to 4th Adult instar

Table 4.6 Parameters of the linearized Michaelis-Monod regressions of percentage relative growth rate (G) of primipara instar on food concentration for Diaphanosoma excisum and Moina micrura at different temperatures.

Equation $\frac{1}{G} = \frac{1}{G_{max}} + (Ks/G_{max}) \cdot 1/S$

$Y = a + b\,X$

G = percentage relative growth rate of primipara; S = food concentration ($mgC.L^{-1}$); G_{max} = maximum percentage absolute growth rate; Ks = food concentration at which growth rate is half of maximum.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

Temp °C	a	b	df	F	P	Ks	G_{max}
(a) Diaphanosoma excisum							
32	0.0177	0.00157 ± 0.0001	1,18	377.9	<0.0001	0.087	56.50
27	0.0281	0.00257 ± 0.0005	1,14	30.0	<0.0001	0.091	35.59
22	0.0435	0.00930 ± 0.0021	1,10	19.1	0.0014	0.214	22.99
(b) Moina micrura							
32	0.0116	0.00131 ± 0.0001	1,18	154.7	<0.0001	0.112	86.21
27	0.0141	0.00159 ± 0.0000	1,14	107.9	<0.0001	0.112	70.90
22	0.0312	0.00509 ± 0.0013	1,10	15.9	0.0026	0.163	32.05

Table 4.7 Parameters of the linear regression equations relating weight to age during the period of exponential growth at different temperature-food combinations.
Equation: $\ln W = \ln a + bX$
W = weight ($\mu gC.\ ind.^{-1}$); X = age (days)
d = degrees of freedom; F = variance ratio; P = probability level; r = correlation coefficient; SE = standard error

Diaphanosoma excisum

Temp °C	Food conc ($mgC.L^{-1}$)	range of weight ($\mu gC.ind.^{-1}$)	lna	b ± SE	df	F	P	r
32	1.0	0.398 - 6.432	-0.8577	0.7727 ± 0.0587	1,22	306	<0.0001	0.9421
	0.5	0.440 - 5.045	-0.7328	0.7767 ± 0.0478	1,22	469	<0.0001	0.9607
	0.25	0.435 - 2.541	-0.8203	0.5575 ± 0.0374	1,22	397	<0.0001	0.9540
	0.1	0.300 - 3.838	-1.1990	0.2945 ± 0.0200	1,34	281	<0.0001	0.9066
	0.05	0.306 - 1.789	-1.1419	0.2483 ± 0.0106	1,50	554	<0.0001	0.9577
27	1.0	0.399 - 2.028	-1.1952	0.6740 ± 0.0448	1,26	214	<0.0001	0.9471
	0.5	0.399 - 3.294	-1.0046	0.5602 ± 0.0304	1,30	318	<0.0001	0.9587
	0.1	0.272 - 1.996	-1.2427	0.3646 ± 0.0153	1,42	539	<0.0001	0.9649
	0.05	0.246 - 2.102	-1.2134	0.2460 ± 0.0109	1,54	180	<0.0001	0.8807
22	1.0	0.357 - 4.802	-0.9193	0.3060 ± 0.0197	1,30	209	<0.0001	0.9429
	0.5	0.440 - 3.686	-0.8840	0.3561 ± 0.0125	1,26	755	<0.0001	0.9845
	0.1	0.246 - 2.333	-1.2011	0.1540 ± 0.0058	1,30	396	<0.0001	0.9665

Moina micrura

Temp °C	Food conc ($mgC.L^{-1}$)	range of weight ($\mu gC.ind.^{-1}$)	lna	b ± SE	df	F	P	r
32	1.0	0.589 - 3.048	-0.8349	1.4575 ± 0.1115	1,10	171	<0.0001	0.9720
	0.5	0.589 - 3.357	-0.7715	1.5431 ± 0.1000	1,10	237	<0.0001	0.9796
	0.25	0.474 - 1.312	-0.9362	0.9504 ± 0.0609	1,10	243	<0.0001	0.9801
	0.1	0.379 - 0.978	-1.0752	0.5825 ± 0.0957	1,10	41	<0.005	0.8976
	0.05	0.422 - 0.629	-0.9716	0.6836 ± 0.0960	1,10	356	<0.0001	0.9859
27	1.0	0.589 - 2.496	-0.6795	1.1762 ± 0.1318	1,10	49	<0.005	0.9426
	0.5	0.589 - 2.742	-0.6843	1.0494 ± 0.2128	1,10	24	<0.005	0.8418
	0.1	0.422 - 0.900	-0.9307	0.6791 ± 0.1055	1,10	41	<0.005	0.8976
	0.05	0.339 - 0.723	-0.9333	0.4160 ± 0.1271	1,10	11	<0.05	0.7192
22	1.0	0.589 - 1.449	-0.4213	0.6011 ± 0.1143	1,10	46	<0.005	0.9065
	0.5	0.678 - 1.449	-0.4125	0.5519 ± 0.0713	1,10	100	<0.0001	0.9534
	0.1	0.339 - 0.629	-1.0481	0.4186 ± 0.0544	1,10	99	<0.0001	0.9529
	0.05	0.379 - 0.572	-1.0119	0.3884 ± 0.0134	1,10	142	<0.0001	0.9965

Table 4.8 Results of the covariance analysis comparing the instantaneous growth rates at different temperatures for each food level. Group 1, 2 and 3 corresponds to the temperatures 32°C, 27°C and 22°C respectively. The regression coefficients were compared by the SS-STP test and the difference between the elevations by the S-N-K test. The group numbers underlined are not significantly different at P = 0.05 level, and group numbers are given in the ascending order of magnitude.
df = degrees of freedom; F = variance ratio; P = level of significance.

(a) Diaphanosoma excisum

	Food concentration $mgC.L^{-1}$			
	1.0	0.5	0.1	0.05
SS-STP test	3 <u>2 1</u>	3 2 1	3 1 2	<u>2 1</u>
	df2,78; F49.8; P<0.0001	df2,78; F52.7; P<0.0001	df2,139; F80.3; P<0.0001	df1,104; F 0.007; P=0.932
S-N-K test	3 2 1	3 2 1	3 1 2	1 2
	df2,81; F35.3; P<0.0001	df2,81; F63.8; P<0.0001	df2,142; F63.3; P<0.0001	df1,106; F3.4; P=0.068

(b) Moina micrura

	Food concentration $mgC.L^{-1}$			
	1.0	0.5	0.1	0.05
SS-STP test	3 <u>2 1</u>	3 <u>2 1</u>	<u>3 1 2</u>	<u>3 2</u> 1
	df2,26; F12.98; P=0.0001	df2,29; F1.145; P=0.3321	df2,26; F 0.987; P=0.3862	df2,26; F3.570; P=0.0417
S-N-K test	<u>3 2 1</u>	<u>3 2</u> 1	3 <u>1 2</u>	<u>3 2</u> 1
	df2,29; F1.464; P=0.2479	df2,29; F4.120; P=0.0266	df2,29; F8.075; P=0.0016	df2,29; F7.375; P=0.0026

Table 4.9 Results of the covariance analysis comparing the instantaneous growth rates at different food levels for each temperature. Groups 1, 2, 3, 4 and 5 correspond to the food concentrations of 1.0, 0.5, 0.25 0.1 and 0.05 $mgC.L^{-1}$ respectively. The regression coefficients were compared by the SS-STP test and the difference between the elevations by the S-N-K test. The underlined group numbers are not significantly different at P = 0.05 level, and group numbers are given in ascending order of magnitude.

df = degrees of freedom; F = variance ratio; P = levels of significance.

(a) Diaphanosoma excisum

	Temperature °C		
	22°C	27°C	32°C
SS-STP test	4 <u>1 2</u>	5 4 <u>2 1</u>	<u>5 4</u> 3 <u>1 2</u>
	df2,106; F90.1; P<0.0001	df2,152; F47.3; P<0.0001	df4,162; F46.5; P<0.0001
S-N-K test	4 <u>1 2</u>	5 4 <u>1 2</u>	<u>5 4</u> 3 <u>1 2</u>
	df2,109; F137.3; P<0.0001	df3,156; F77.8; P<0.0001	df4,167; F93.4; P<0.0001

(b) Moina micrura

	Temperature °C		
	22°C	27°C	32°C
SS-STP test	<u>5 4 2 1</u>	<u>5 4</u> 2 1	<u>5 4</u> 3 <u>1 2</u>
	df3,24; F1.978; P=0.1441	df3,40; F5.371; P=0.0033	df4,46; F29.34; P<0.0001
S-N-K test	<u>5 4 2 1</u>	<u>5 4 2 1</u>	<u>5 4</u> 3 <u>1 2</u>
	df2,28; F124.506; P<0.0001	df3,44; F30.73; P<0.0001	df4,51; F31.87; P<0.0001

Table 4.10 Body growth and reproductive growth of *Diaphanosoma excisum* and *Moina micrura* at different food and temperature combinations from just before onset of maturity to the end of the fourth adult instar.

Temp °C	Food conc mg.C.L^{-1}	body growth from just before primipara to end of 4th adult instar (µg.C ind^{-1})	rate of body growth (R) (µg.C ind^{-1} d^{-1})	reproductive growth to the end of the 4th brood (µg.C ind^{-1})	rate of reproductive growth (B) (µg.C ind^{-1} d^{-1})	$\frac{R}{R+B}$ %
Diaphanosoma excisum						
32	1.0	2.808	0.717	14.00	4.10	85
	0.5	3.024	0.700	15.13	3.95	85
	0.25	0.136	0.303	12.46	3.11	96
	0.1	1.159	0.348	3.71	1.01	74
	0.05	0.486	0.139	2.71	0.68	83
27	1.0	2.450	0.525	9.59	2.03	79
	0.5	0.486	0.120	5.72	1.16	90
	0.1	0.309	0.064	0.73	0.14	68
	0.05	Did not complete 4 broods				
22	1.0	2.364	02317	7.54	0.86	79
	0.5	1.231	0.126	7.88	0.90	88
	0.1	0.868	0.069	1.33	0.12	63
Moina micrura						
32	1.0	-0.545	-0.164	21.22	7.07	102
	0.5	0.172	0.023	22.58	6.15	99
	0.25	-1.919	-0.590	10.53	2.91	104
	0.1	-0.362	-0.107	6.77	1.70	107
	0.05	0.313	0.089	1.74	0.43	82
27	1.0	1.146	0.275	12.94	2.77	91
	0.5	0.410	0.102	15.16	3.37	97
	0.1	0.833	0.197	4.72	0.97	83
	0.05	0.662	0.103	1.44	0.20	66
22	1.0	0.854	0.107	10.20	1.13	91
	0.5	2.371	0.306	9.29	1.06	78
	0.1	0.306	0.038	2.92	0.32	89

Table 4.11 Parameters of the multiple regression analysis relating body carbon weight of the fifth adult instar to both temperature (°C) and food concentration ($mgC.L^{-1}$).

Regression equation. $\ln W = -a \pm b \ln S + c \ln T$
W = body carbon weight ($\mu gC.ind.^{-1}$); S = food concentration ($mgC.L^{-1}$); T = temperature °C.
df = degrees of freedom; F = variance ratio; P = level of significance.

(a) Diaphanosoma excisum

a	b	c	df	F	P	r
-0.43	0.342	0.816	2,8	11.29	<0.005	0.859

F due to food concentration	F due to Temperature
19.8	2.77

(b) Moina micrura

a	b	c	df	F	P	r
-1.96	0.525	1.25	2,9	30.6	<0.001	0.934

F due to food concentration	F due to Temperature
55.21	6.06

CHAPTER 5

THE EFFECT OF TEMPERATURE AND FOOD CONCENTRATION ON DURATION OF DEVELOPMENT

The time from the laying of a cladoceran egg into the maternal brood pouch, through its subsequent development up to the laying of its first set of eggs, can be divided into two major periods:

(a) The duration of embryonic development (De) which occurs in the maternal brood pouch,

(b) The duration of post-embryonic, or juvenile development (Dj) which takes place outside the maternal body, using nutritive material available in the environment.

According to Bottrell et.al. (1976), there is a fundamental difference in the major factors affecting embryonic and post-embryonic development in many poikilotherms in that the former is affected by temperature only, while the latter depends on both temperature and food. Food limitation of both embryonic and post-embryonic development of tropical cladocerans has not been studied and even the effect of temperature on these two parameters has seldom been investigated. In the present study an attempt was made to examine the effects of various combinations of three temperatures (22, 27, 32°C), and four food concentrations (0.05, 0.1, 0.5, 1.0 $mgC.L^{-1}$) on both embryonic and post-embryonic duration of the four species.

While carrying out life-cycle experiments (Chapter 3) the age and the reproductive stage were recorded twice daily at 27° and 32°C and once a day at 22°C. An additional food concentration of 0.25 $mgC.L^{-1}$ was tested at 32°C. Daphnia lumholtzi and Ceriodaphnia cornuta were tested only at 22°C and 32°C. The former species suffered complications due to infections at 32°C which the latter did not grow at 22°C. Both Diaphanosoma excisum and Moina micrura did not attain maturity at 22°C and 0.05 $mgC.^{-1}$.

5.1 DURATION OF EMBRYONIC DEVELOPMENT (De)

The durations of embryonic development in all four species, at different temperature-food combinations, are summarized in Table 5.1. There is a decrease in duration with increasing temperature at all food levels. In Diaphanosoma excisum and Moina micrura, this relationship is best described by a power function, $Y = aX^b$, where Y is embryonic duration in hours, X is temperature in degrees Celsius, and "a" and "b" are constants. Transformation of both variables to natural logarithms permitted the calculation of linear regressions, together with regression statistics, and the results are presented in Table 5.2. The regressions for all four food concentrations were statistically significant and had negative slopes as expected. There is a suggestion of increased elevation in the regressions, which appear more or less parallel, with decreases in food level but the results of covariance analysis given in Table 5.3. demonstrate that in Diaphanosoma excisum there are no significant

differences in either the slopes or elevations of the regressions measured at the different food levels tested. Thus, the pattern of decrease in embryonic duration with temperature is similar irrespective of food level and for this species the relationship can be expressed by a pooled common regression

$\ln Y = 11.2332 - 2.3486 \ln X$ (Y = embryonic duration in hours; X = temperature in °C). In the case of Moina micrura, there are also no significant differences between the regressions except that at the lowest food level of 0.05 $mgC.L^{-1}$, which has a higher elevation compared to the rest. This indicates that in this species embryonic duration was prolonged at the lowest food concentration. Hence, for this species the relationship of embryonic duration to temperature, except at very low food level (0.05 $mgC.L^{-1}$), can be expressed by a pooled regression

$\ln Y = 11.1861 - 2.3554 \ln X$ (Y = embryonic duration in hours; X = temperature in °C). As explained earlier in Ceriodaphnia cornuta, it was not possible to do the experiments at 27°C with this species and the animals did not grow at 22°C. Thus, the embryonic development time of this species was only recorded at 32°C and found to be 24 hours irrespective of food level (Table 5.1.). In Daphnia lumholtzi, the development at 32°C must be discounted due to infections (page 95) and it was not possible to conduct the experiments at 27°C so results are available only for 22°C. At this temperature the embryonic development was found to be 60 $\pm$ 13.165 hours and 51.04 $\pm$ 8.591 hours at 1.0 and 0.5 $mgC.L^{-1}$ respectively. At 0.1 $mgC.L^{-1}$ only three readings were obtained because the animals either died prior to reproduction, or produced only one or two broods before death. With these few readings, embryonic duration at 0.1 $mgC.L^{-1}$ was found to be 64 $\pm$ 13.86 hours.

The relationship between embryonic duration and food concentration is also well described by the same power function: $Y = aX^b$ where, in this case, Y is embryonic duration in hours and X is food concentration in $mgC.L^{-1}$ and "a" and "b" are constants. Transformation of both variables to natural logarithms again permitted the calculation of linear regressions together with regression statistics, and the results are presented in Table 5.4. In Diaphanosoma excisum, embryonic development was found to be significantly affected by food concentration only at 27°C (Table 5.4.). This relationship has to be interpreted carefully because the differences in duration between food levels are only a couple of hours in each case (Appendix 5). However, the predicted values for embryonic duration calculated using the statistically significant relationship given for 27°C in Table 5.4. fell within the standard deviation of the mean values for 27°C given in Table 5.1. In Moina micrura the duration of embryonic development was found to be affected significantly by food concentration at 27°C and 32°C but not at 22°C (Table 5.4.). As for Diaphanosoma excisum these results must be interpreted with care. At 32°C the duration of embryonic development varied only between 18-24 hours at all food levels. Since the animals were examined twice daily, the 6 hour difference may be only an artefact of the time interval between observations. The mean values given in Table 5.1. also support this interpretation. Thus, since the time between observations was too long at this temperature, it is not correct to judge significance only by the statistical analysis. On the other hand, at 27°C the variation

is within a comparatively greater range; at 1.0 and 0.5 $mgC.L^{-1}$ duration was within 24-32 hours, at 0.1 $mgC.L^{-1}$ it was within 24-40 hours, and at 0.05 $mgC.L^{-1}$ between 32-40 hours; this resulted in a high mean value (38.28h) at this lowest level. Thus the variation at this temperature had a broader range compared to 32°C which is masked by the statistical results which could be accepted irrespective of experimental error. There may, therefore, be a tendency for embryonic development of both of these species to increase in duration with reduced food availability to the mother at 27°C, which is the normal field temperature for these species in Sri-Lanka. The lack of significant effect at 22°C in both species may be due to the lack of development at lowest food level of 0.05 $mgC.L^{-1}$ which influenced the analysis.

5.2 DURATION OF POST-EMBRYONIC DEVELOPMENT (Dj)

The results for the post-embryonic duration of the four species, at different temperature-food combinations, are summarized in Table 5.5. It is immediately clear from this table that post-embryonic development time is influenced by food concentration as well as temperature. In Diaphanosoma excisum the shortest development time overall was attained at the highest temperature (32°C) and the highest food concentration 1.0 $mgC.L^{-1}$). This is the same for the other two temperatures, that post-embryonic duration was shortest at the highest food concentration. In fact, at 22° and 27°C there is a steady increase in development time with decrease in food concentration

but, at 32°C, the increase is most marked between food concentrations of 0.25 and 0.1 $mgC.L^{-1}$. The results for Moina micrura were almost the same as those for Diaphanosoma excisum, except for the fact that, at both 27°C and 32°C, in Moina micrura the shortened post-embryonic duration is attained not only at the highest food concentration (1.0$mgC.L^{-1}$) but also at 0.5 $mgC.L^{-1}$. In Ceriodaphnia cornuta at 32°C, although the lowest mean duration was found at 0.5 $mgC.L^{-1}$, it lies within the standard deviation of the mean at 1.0 $mgC.L^{-1}$. Prolongation of post-embryonic duration is evident as food level decreased to 0.25 $mgC.L^{-1}$. In Daphnia lumholtzi, duration at the highest food concentration was found to be greater than that at 0.5 $mgC.L^{-1}$. At 0.1 $mgC.L^{-1}$, one animal matured after 148 hours while another prolonged its duration up to 384 hours. The means indicate a general tendency which seems to prolong juvenile duration at 0.1 $mgC.L^{-1}$ but it is not possible to reach any conclusion due to the paucity of results.

Table 5.5 also shows that, in Diaphanosoma excisum, there is a marked increase in the number of instars within the juvenile period at different temperatures within any one food level. There is a much less marked but real tendency within one temperature for there to be more juvenile instars at the lower food levels. Thus it appears that both age and stage of development is affected by temperature and food concentration, particularly at the lower food levels. In Moina micrura, the temperature effect on the number of instars is not as marked as in Diaphanosoma excisum, and the temperature effect is not

evident between 27°C and 32°C except at the lowest food concentration (0.05 $mgC.L^{-1}$) at 27°C. It should be noted here that at 0.05 $mgC.L^{-1}$ the higher value of juvenile duration with a large SD and the higher instar number is due to one animal which behaved in an unusual manner by prolonging its juvenile period until the 6th instar. Thus it appeared that, in Moina micrura, the age of development is affected by both temperature and food concentration as in Diaphanosoma excisum but, in contrast, the stage at maturity is affected only at low temperature (22°C) and low food concentration (0.1 and 0.05 $mgC.L^{-1}$).

Closer examination of this prolongation of juvenile duration (Table 5.5.) reveals that the increase in juvenile duration at lower food levels, in any particular temperature, is not only a result of having increased number of instar, but also due to prolongation of instar duration. This is evident from comparing the results from 1.0 $mgC.L^{-1}$ and 0.1 $mgC.L^{-1}$ at 22°C for Diaphanosoma excisum, in which juvenile duration in the latter concentration is about twice that in the former, but instar number remains about the same. A similar effect is seen at 32°C and the same two food concentrations. In Moina micrura, similarly, a condition is evident at 32°C and 1.0 $mgC.L^{-1}$ and 0.5 $mgC.L^{-1}$, in which the duration up to the third instar is 28 hours while at 0.1 and 0.05 $mgC.L^{-1}$ it is prolonged to about 45 hours.

The inverse effects upon post-embryonic duration of both temperature and food concentration, applied in various combinations, were well described by the power function, $Y = aX^b$. An attempt was made to quantify separately the effect of

temperature at different food concentrations (Table 5.6.) and effect of food concentrations at different temperatures (Table 5.7.) for both Diaphanosoma excisum and Moina micrura. Table 5.6 shows that statistically significant regressions were obtained between post-embryonic duration and temperature at all food levels for both species except at 0.05 $mgC.L^{-1}$ for Diaphanosoma excisum. At this food level the lack of results at 22°C, in which animals did not reach maturity affected the relationship. In addition the non-significant effect may have been due to the fact that, at very low food concentrations, the juvenile duration is prolonged irrespective of temperature. In these conditions the food available was probably so low that the animals could not gather enough food to mature faster even at higher temperature. This probably explains why the relationship is not statistically significant.

In general, (for both Diaphanosoma excisum and Moina micrura) duration decreased with increase in temperature (except for Diaphanosoma excisum at the lowest food level) and durations were longer at low than at high food levels (as is illustrated in Fig 5.1.). Covariance analysis of the regressions presented in Table 5.8 gives evidence that there are no significant differences between the slopes and elevations of the regressions 1.0 and 0.5 $mgC.L^{-1}$ for both species. The elevations are significantly higher at 0.1 $mgC.L^{-1}$ in both species, and higher still at 0.05 $mgC.L^{-1}$ for Moina micrura. That is, the juvenile duration is markedly and significantly prolonged at these lower food concentrations. Because there was no significant relationship

between temperature and post-embryonic duration for Diaphanosoma excisum at 0.05 $mgC.L^{-1}$ (Table 5.6.) this food concentration was not included in the covariance analysis for that species. As it is evident that in both Diaphanosoma excisum and Moina micrura juvenile duration is independent of food concentration at and above 0.5 $mgC.L^{-1}$, but prolonged below 0.1 $mgC.L^{-1}$, the limiting food level for juvenile duration falls between 0.1 and 0.5 $mgC.L^{-1}$.

All the regressions in Table 5.7, which relate post-embryonic duration to food concentration at each of the three experimental temperatures, are statistically significant. As is indicated by the low values for the regression coefficient b, the slopes are rather flat but the elevations of the regressions do appear to differ (See Fig 5.1.). Covariance analysis of the regressions was undertaken to test whether these regressions differed and the results are presented in Table 5.9. From this it is clear that the slopes are not different but all three elevations are significantly different in both Diaphanosoma excisum and Moina micrura. Hence, it is evident that decreasing temperature prolongs post-embryonic duration in a similar manner in both species.

The influence of food concentrations (S) on the rate of juvenile development (1/Dj or the proportion of development occurring per day) was further examined by applying the Linearized Lineweaver-Burke (Chapter 3) modification of the Michaelis-Monod function (Chapter 3).

$$\frac{1}{\mu} = \frac{1}{\mu max} + \frac{Ks}{\mu max} \cdot \frac{1}{S} \qquad (1)$$

As duration is not a rate, it was necessary to substitute 1/Dj into this equation:

$$\frac{1}{1/D_j} = \frac{1}{1/D_{jmax}} + \frac{Ks}{1/D_{jmax}} \cdot \frac{1}{S} \quad \text{(2)}$$

$$Dj = Djmax + Ks.\ Djmax \cdot \frac{1}{S} \quad \text{(3)}$$

This represents a straight line equation

$$Y = a + b.X$$

where a is the intercept at X = 0 give by Djmax

b is the slope give by (Ks.Djmax)

The linearized Michaelis-Monod regressions are given in Table 5.10 and illustrated in Fig 5.2. Significant regressions could be fitted to the data at all these temperatures and this permitted the back calculation of the values for the maximal juvenile developmental rate (1/Djmax) and the half saturation substrate concentration (Ks), both of which are given in Table 5.10. It is evident from the results that the rate of juvenile development is influenced by temperature and decreases in lowered temperatures. It also proved possible to calculate the temperature coefficients and Q_{10} values for the maximal juvenile developmental rate and these are given in Table 5.11. These values fall within those normally expected (between 2 and 3) for biological rate processes and have a tendency to decrease at the higher temperature ranges. The values are somewhat higher and lower in Moina micrura, although not when the whole 10°C range is considered. The animal which behaved in a different manner was reared at 27°C in 0.05 $mgC.L^{-1}$ and has been excluded from the final results (Table 5.10).

Table 5.10 also provides values for Ks (the food concentration at which half the maximum development takes place). In both species these are highest at 22°C and similar at 27°C but at 32°C there is a difference between the two species, the Ks being lowest for Moina micrura but not in Diaphanosoma excisum. A lower value for Ks at a temperature as high as 32°C seems surprising since the maximal juvenile developmental rate is the highest at this temperature. A plot of 1/Djmax on Ks (Fig 5.3) shows that the relationship is inverse in both species but with a much steeper, negative slope in Moina micrura than in Diaphanosoma excisum. However this figure illustrates clearly that the range of Ks values are lower for Moina micrura than Diaphanosoma excisum in the same temperature range with overlapping values at 27°C the normal field temperature for these species.

These relationships for the two species are illustrated in Fig 5.2 showing the higher maximal juvenile development of Moina micrura at all temperatures as well as the steeper initial slopes at the lower food levels.

Since it was found that both temperature and food concentration have an effect on juvenile (= post-embryonic) duration the interaction of the two parameters was examined using a three-dimentional plot (Fig 5.4.) and statistically by multiple regression analysis (Table 5.12). Fig 5.4 shows the general trend of increasing juvenile duration at low food concentrations and low temperatures with a very prolonged duration at the lowest temperature and food combination (0.1 $mgC.L^{-1}$ and 22°C). Comparing the two species, the increase in duration of juvenile development with decrease in food is not as pronounced in Moina micrura as that seen in Diaphanosoma excisum.

Various transformations were tried for the multiple regression analysis and the highest F values for both species were obtained with the relationship of natural log of juvenile duration to the reciprocal of temperature and natural log of food concentration (Moina micrura; F = 7564; df 2,45. Diaphanosoma excisum; F = 103; df 2,45). But when the values were predicted using this relationship the predicted values did not agree closely with the experimental results. Then the other relationships were tested and the predicted values were found to fall within the range of experimental results when the relationship of the reciprocal of juvenile duration to reciprocal of temperature and reciprocal of food concentration was considered. The results of this relationship are given in Table 5.11. The F values given separately due to food concentration and temperature, for Diaphanosoma excisum reveal that both these factors equally influence juvenile duration, although the effect of food is slightly greater. In contrast, the effect of temperature is greater than the effect of food concentration on juvenile duration in Moina micrura.

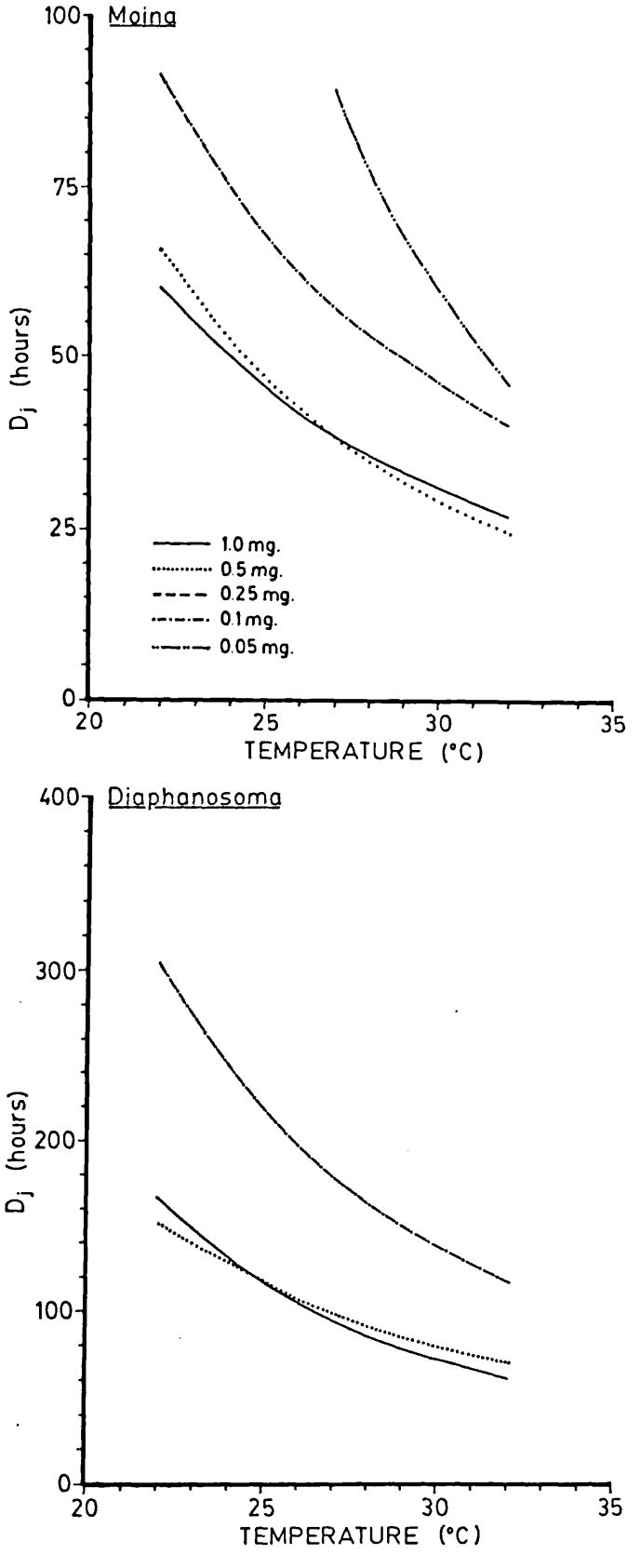


Figure 5.1 Relationships between post-embryonic duration and temperature for Moina micrura and Diaphanosoma excisum reared at different food concentrations. Food concentrations are given as mgC. L^{-1}. The statistics of the curvilinear regressions are given in Table 5.6.

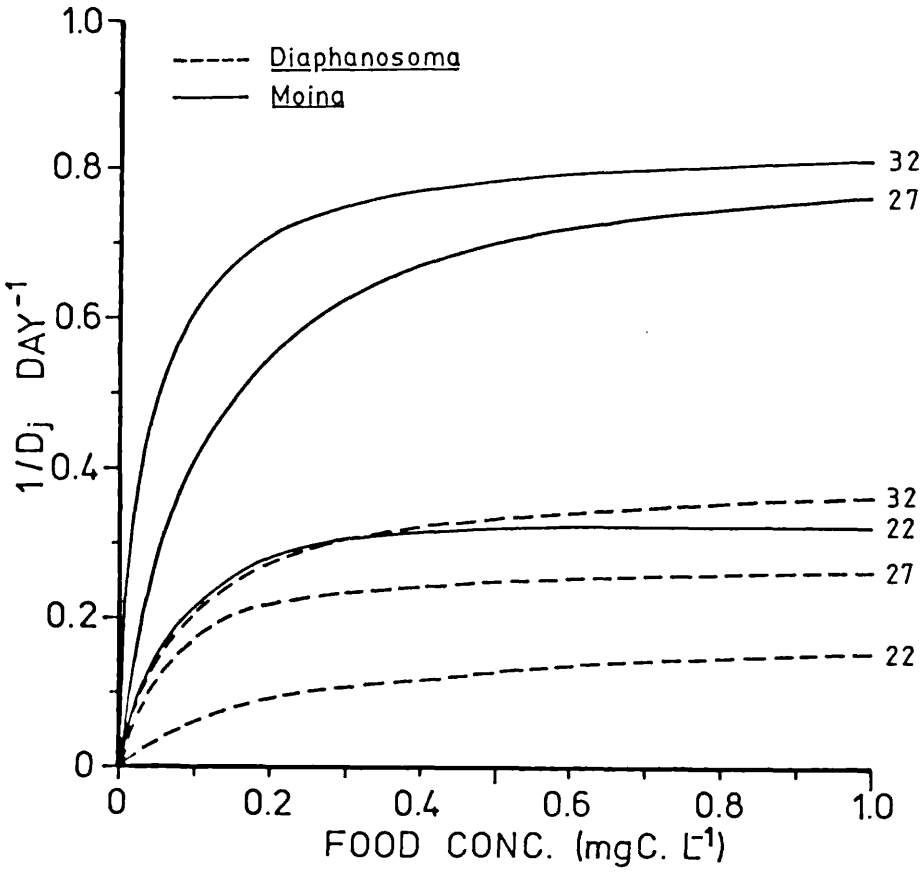


Figure 5.2 The effect of food concentration on the rate of post-embryonic development in Diaphanosoma excisum and Moina micrura reared at different temperatures. These curves were predicted using the linearized Michaelis-Monod regressions whose statistics are given in Table 5.10.

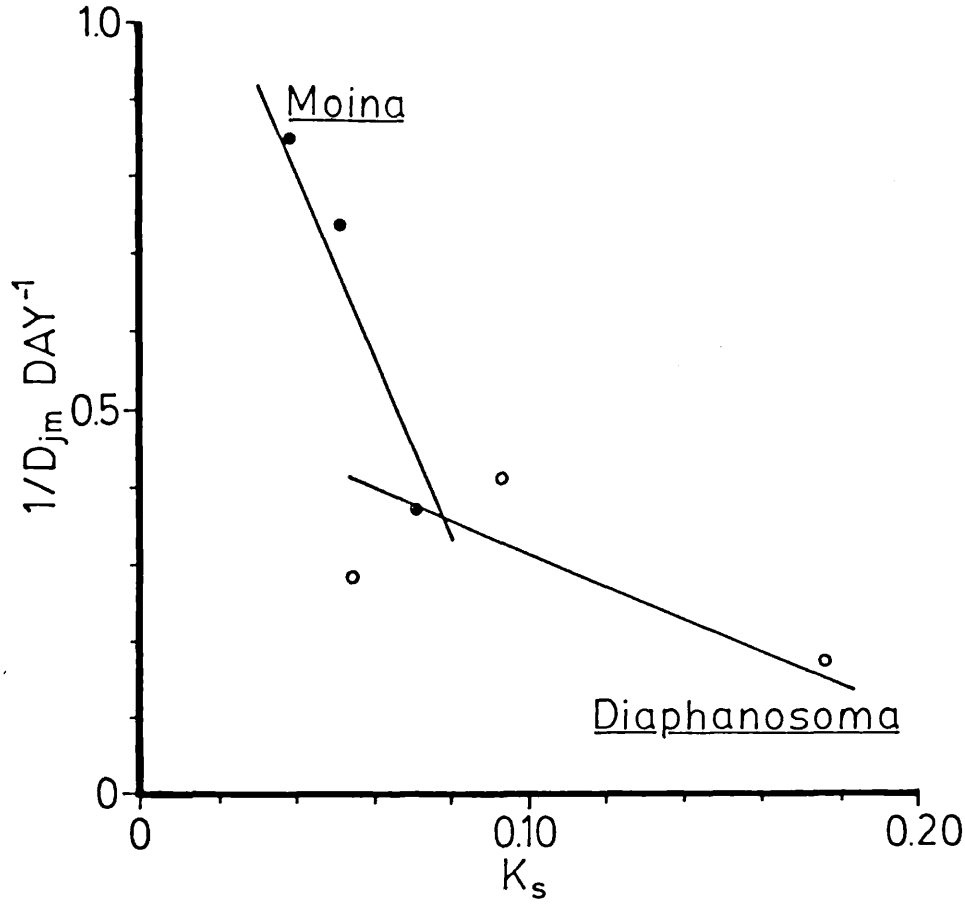


Figure 5.3 Maximum rate of juvenile development in Moina micrura and Diaphanosoma excisum plotted against k_s, the food concentration at which half the maximum rate is obtained.

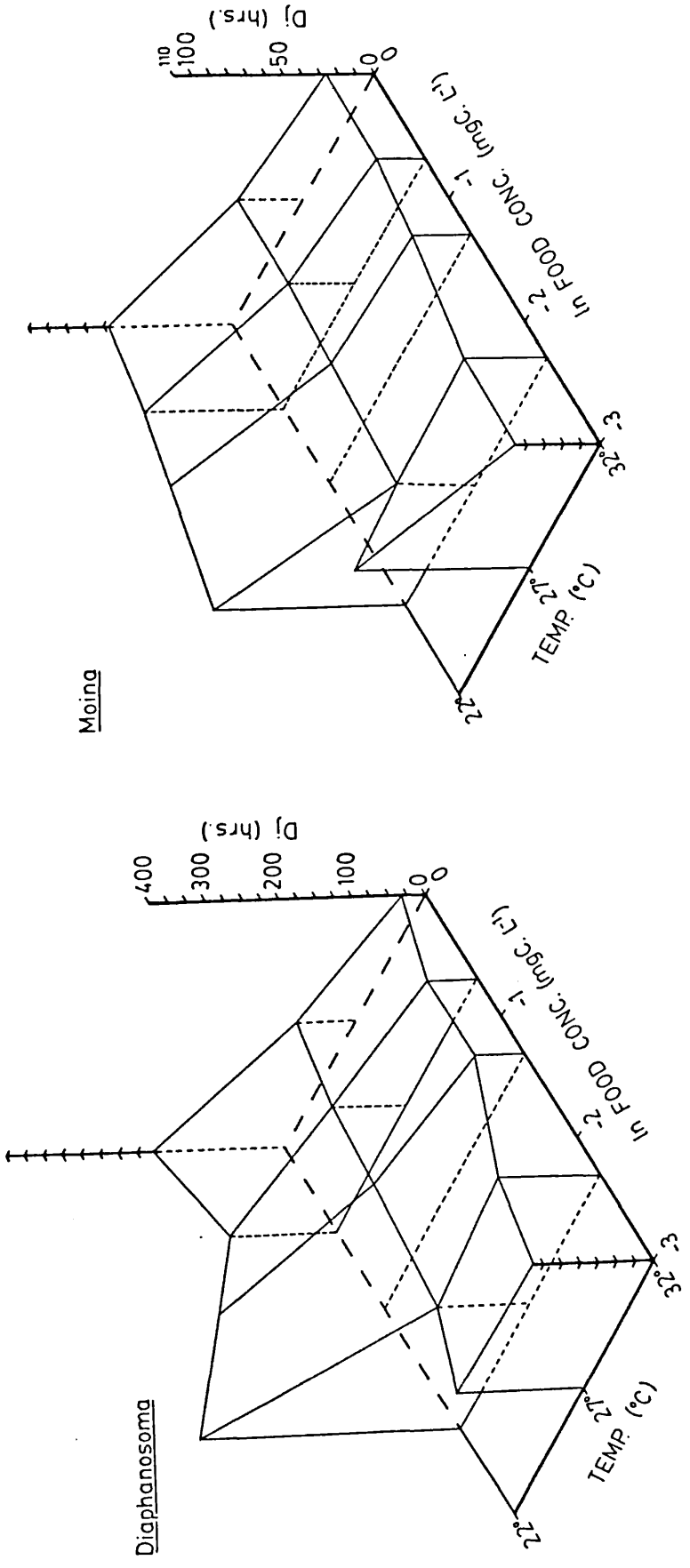


Figure 5.4 Three dimensional diagrams to show the effects of temperature and food concentration on the duration of juvenile development in Diaphanosoma excisum and Moina micrura.

Table 5.1 The duration of the embryonic development (De) of Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi in various combinations of temperature and food concentrations. (Duration in hours: mean ± standard deviation; number of observations in parenthesis).

Temp °C	Food concentration mg.C.L^{-1}				
	1.0	0.5	0.25	0.1	0.05
(a) Diaphanosoma excisum					
22	54.00 ± 6.19 (16)	54.02 ± 6.20 (16)	-	53.00 ± 9.52 (12)	juveniles died
27	29.94 ± 6.01 (16)	33.35 ± 6.97 (16)	-	37.88 ± 5.82 (16)	35.67 ± 4.80 (6)
32	22.25 ± 3.42 (16)	22.21 ± 3.10 (16)	22.39 ± 2.94 (16)	21.75 ± 3.00 (16)	22.63 ± 4.18 (16)
(b) Moina micrura					
22	53.25 ± 6.15 (16)	51.00 ± 5.37 (16)	-	53.25 ± 6.15 (16)	juveniles died
27	28.00 ± 5.84 (16)	25.88 ± 2.87 (16)	-	29.86 ± 6.39 (15)	38.28 ± 3.41 (16)
32	20.63 ± 3.07 (16)	22.50 ± 2.68 (16)	22.13 ± 2.87 (16)	22.88 ± 2.42 (16)	22.88 ± 2.42 (15)
(c) Ceriodaphnia cornuta					
32	24.00 ± 0.00 (16)	24.00 ± 0.00 (14)	24.00 ± 0.00 (09)	juveniles died	juveniles died
(d) Daphnia lumholtzi					
22	60.25 ± 13.17 (12)	51.04 ± 8.59 (12)	-	64.13 ± 13.86 (3)	juveniles died

Table 5.2 Curvilinear regressions relating the duration of embryonic development to temperature at various food concentrations for Diaphanosoma excisum and Moina micrura.

Regression equation $\ln Y = \ln a - b \ln X$
Y = duration of embryonic development in hours, X = temperature in °C.
df = degrees of freedom; F = variance ratio; P = level of significance.

Diaphanosoma excisum

Food conc $mgC.L^{-1}$	ln a	b	df	F	P
1.0	11.58	-2.467	1,46	274.9	<.0001
0.5	11.42	-2.408	1,46	251.3	<.0001
0.1	11.37	-2.382	1,42	179.9	<.0001
0.05	11.80	-2.498	1,20	66.3	<.0001

Moina micrura

Food conc $mgC.L^{-1}$	ln a	b	df	F	P
1.0	11.85	-2.563	1,46	266.8	<.0001
0.5	10.74	-2.228	1,46	226.5	<.0001
0.1	10.96	-2.274	1,45	221.8	<.0001
0.05	14.02	-3.044	1,28	164.5	<.0001

Table 5.3 Covariance analysis of the regressions comparing the duration of embryonic development on temperature at various food levels for Diaphanosoma excisum and Moina micrura. The regression coefficients were compared by the SS-STP test and the differences between elevations by the S-N-K test. Regression coefficients and means underlined are not significantly different at p = 0.05 level; group numbers are given in ascending order of magnitude.

Regression equation: $\ln Y = \ln a - b \ln X$
Y = duration of embryonic development in hours, X = temperature in °C.
df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

(a) Diaphanosoma excisum

Comparison of regression coefficients

Food conc. mg.C.L^{-1}	Group no	Regression coeff ± SE	df	F	P	SS-STP
1.0	1	-2.46712 ± 0.1488	3,154	0.132	0.9411	4 1 2 4
0.5	2	-2.39124 ± 0.1486				
0.1	3	-2.32659 ± 0.2197				
0.05	4	-2.49867 ± 0.3068				

Comparison of the elevation of the curves

Food conc. mg.C.L^{-1}	Group no	Adjusted mean ± SE	df	F	P	S-N-K
1.0	1	3.4317 ± 0.0836	3,154	2.012	0.1145	1 2 3 4
0.5	2	3.4604 ± 0.0836				
0.1	3	3.4932 ± 0.0836				
0.05	4	3.5321 ± 0.0843				

continued

Table 5.3 continued

(b) Moina micrura

Comparison of the regression coefficients.

Food conc $mg.C.L^{-1}$	Group no	Regression coeff ± SE	df	F	P	SS-STP
1.0	1	-2.56337 ± 0.1569	3.165	2.4	0.0698	4 3 1 2
0.5	2	-2.22809 ± 0.1480				
0.1	3	-2.27445 ± 0.1527				
0.05	4	-3.04477 ± 0.2293				

Comparison of the elevations of the curves.

Food conc $mg.C.L.^{-1}$	Group no	Adjusted mean ± SE	df	F	P	S N K
1.0	1	3.3906 ± 0.0777	3,169	9.868	<.0001	2 1 3 4
0.5	2	3.3846 ± 0.0777				
0.1	3	3.4492 ± 0.0777				
0.05	4	3.5618 ± 0.0780				

Table 5.4 Curvilinear regressions relating the duration of embryonic development to food concentration at different temperatures for Diaphanosoma excisum and Moina micrura.

Regression equation $\ln Y = \ln a - b \ln X$
Y = duration of embryonic development in hours, X = food concentration in $mgC.L^{-1}$
df = degress of freedom; F = variance ratio; P = level of significance.

Moina micrura

Temp °C	ln a	b	df	F	P
22	3.9503	-0.00458	1,46	0.0	0.7827
27	3.2429	-0.10032	1,59	27.5	<0.0001
32	3.0500	-0.03024	1,74	5.1	0.0257

Diaphanosoma excisum

Temp °C	ln a	b	df	F	P
22	3.9861	-0.00650	1,42	0.3	0.5818
27	3.4157	-0.07589	1,52	11.3	0.0014
32	3.0696	-0.01425	1,78	1.0	0.3104

Table 5.5 Duration of post-embryonic development to primipara (hours) and instar of primipara attained in paranthesis in various combinations of temperature-food concentrations in Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi
(Duration in hours as means ± standard deviation)

Temp °C	Food concentration $mgC.L^{-1}$				
	1.0	0.5	0.25	0.1	0.05
(a) Diaphanosoma excisum					
22	178.0 ± 22.9 (xii-xiii)	148.0 ± 0.0 (xii)	-	358.0 ± 71.6 (xi-xiii)	juveniles died
27	81.5 ± 14.5 (v-vi)	104.0 ± 24.0 (v-vii)	-	130.0 ± 12.0 (vi-viii)	174.0 ±31.5 (vi-vii)
32	60.0 ± 10.1 (v-vi)	68.0 ± 11.3 (v-vi)	69.0 ± 6.7 (v -vi)	140.0 ± 0.0 (vi)	168.0 ± 1.4 (vii-viii)
(b) Moina micrura					
22	66.5 ± 13.8 (iii-iv)	76.0 ± 19.6 (iii-v)	-	106.0 ± 28.6 (iv-v)	juveniles died
27	36.0 ± 9.2 (iii)	37.0 ± 8.2 (iii)	-	44.0 ± 0.0 (iii)	96.0 ± 50.8 (iv-vi)
32	28.1 ± 1.0 (iii)	28.0 ± 0.0 (iii)	32.0 ± 8.0 (iii)	46.0 ± 4.0 (iii-iv)	47.0 ± 3.8 (iii-iv)
(c) Ceriodaphnia cornuta					
	79.9 ± 13.8 (iv-vi)	67.9 ± 1.0 (iv)	100.0 ± 0.0 (v)		
(d) Daphnia lumholtzi					
	212.0 ± 29.8 (v)	142.0 ± 12.0 (vi)		266.0 ± 166.8 (v)	

Table 5.6 Curvilinear regressions relating the duration of post-embryonic development to temperature at various food concentrations for Diaphanosoma excisum and Moina micrura.

Regression equation $\ln Y = \ln a - b \ln X$
Y = post-embryonic in hours, X = temperature in °C
df = degrees of freedom; F = variance ratio; P = level of significance.

Diaphanosoma excisum

Food conc. $mg.C.L^{-1}$	ln a	b	df	F	P
1.0	13.428	-2.690	1,10	67.9	<0.0001
0.5	11.497	-2.097	1,10	38.9	<0.0001
0.1	13.600	-2.550	1,10	23.2	<0.0001
0.05	6.341	-0.362	1,6	0.2	0.6689

Moina micrura

Food conc. $mg.C.L.^{-1}$	ln a	b	df	F	P
1.0	10.702	-2.137	1,10	31.2	<0.0002
0.5	12.363	-2.645	1,10	42.0	<0.0001
0.1	11.420	-2.234	1,10	21.0	<0.001
0.05	16.731	-3.717	1,6	17.9	<0.005

Table 5.7 Curvilinear regressions relating the duration of post-embryonic development to food concentration at various temperatures for Diaphanosoma excisum and Moina micrura.

Regression equation $\ln Y = \ln a - b \ln X$
Y = post-embryonic duration in hours X = food concentration in $mg.C.L^{-1}$)
df = degrees of freedom; F = variance ratio; P = level of significance

Diaphanosoma excisum

Temp °C	ln a	b	df	F	P
22	5.004	-0.342	1,10	24.2	0.0006
27	4.432	-0.221	1,14	37.6	<0.0001
32	4.024	-0.341	1,18	71.5	<0.0001

Moina micrura

Temp °C	ln a	b	df	F	P
22	4.147	-0.214	1,10	9.1	0.0129
27	3.455	-0.265	1,14	14.7	0.0018
32	3.265	-0.199	1,18	51.1	<0.0001

Table 5.8 Covariance analysis comparing the regressions of duration of post-embryonic development on temperature at different food concentrations (given in Table 5) for Diaphanosoma excisum and Moina micrura. The regression coefficients were compared by the SS-STP test and the difference between elevations by the S-N-K test. Regression coefficients and means underlined are not significantly different at P = 0.05 level; group numbers are given in ascending order of magnitude.

Regression equation $\ln Y = \ln a - b \ln X$
Y = duration of post-embryonic development in hours. X = food concentration in $mgC.L^{-1}$
df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error

(a) Diaphanosoma excisum
Comparison of the regression coefficients

Food conc $mgC.L^{-1}$	Group no	Regression coeff ± SE	df	F	P	SS-STP test
1.0	1	-2.69004 ± 0.3264	2,28	0.576	0.568	1 3 2
0.5	2	-2.09732 ± 0.3359				
0.1	3	-2.55034 ± 0.5300				

Comparison of the elevation of the curves

Food conc $mgC.L^{-1}$	Group no	Adjusted mean ± SE	df	F	P	S-N-K test
1.0	1	4.5931 ± 0.12156	2,32	35,294	<0.0001	1 2 3
0.5	2	4.6084 ± 0.12156				
0.1	3	5.2241 ± 0.12156				

continued

Table 5.8 continued

(b) Moina micrura

Comparison of regression coefficients

Food conc $mgC.L^{-1}$	Group no	Regression coeff ± SE	df	F	P	SS-STP test
1.0	1	-2.13737 ± 0.3824	3,40	17.615	0.511	1 3 2 4
0.5	2	-2.62491 ± 0.4048				
0.1	3	-2.23420 ± 0.4871				
0.05	4	-3.71759 ± 1.3409				

Comparison of the elevation of the curve

Food conc $mgC.L^{-1}$	Group no	Adjusted mean ± SE	df	F	P	S-N-K test
1.0	1	3.6404 ± 0.12012	3,40	17.615	<0.0001	1 2 3 4
0.5	2	3.7002 ± 0.12012				
0.1	3	4.0401 ± 0.12013				
0.05	4	4.3548 ± 0.12169				

Table 5.9 Covariance analysis comparing the regressions of duration of post-embryonic development on food concentration at different temperatures (given in Table 5.7) for Diaphanosoma excisum and Moina micrura. The regression coefficients were compared by the SS-STP test and the difference between elevations by the S-N-K test. Regression coefficients and means underlined are not significantly different at P = 0.05 level; group numbers are given in ascending order of magnitude.

Regression equation $\ln Y = \ln a - b \ln X$
Y = duration of post-embryonic development, X = food concentration in $mgC.L^{-1}$
df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error

(a) Diaphanosoma excisum

Comparison of regression coefficients

Temperature °C	Group no	Regression coeff ± SE	df	F	P	SS-STP
22	1	-0.34245 ± 0.0696	2,42	2.568	0.0887	2 3 1
27	2	-0.22150 ± 0.0361				
32	3	-0.34192 ± 0.0404				

Comparision of the elevation of the curves

Temperature °C	Group no	Adjusted mean ± SE	df	F	P	S N K
22	1	5.4535 ± 0.11729	2,45	83.202	<0.0001	3 2 1
27	2	4.7246 ± 0.11694				
32	3	4.4965 ± 0.11693				

continued

Table 5.10 Linearized Michaelis - Monod regressions of the juvenile development rate on food concentration for *Diaphanosoma excisum* and *Moina micrura*

Equation $1/(1/Dj) = 1/(1/Djmax) + Ks/(1/Djmax) \cdot 1/S$

$$Dj = Djmax + (Ks \cdot Djmax).\frac{1}{S}$$

$$Y = a + b\,X$$

Dj = juvenile duration in hours, Djmax = maximal juvenile duration, Ks = food concentration at which half the maximal rate is obtained, S = food concentration in $mg.C.L^{-1}$

$\frac{1}{Djmax}$ was converted to d^{-1} for comparison purposes.

(a) *Diaphanosoma excisum*

Temp °C	a ± SD	b ± SD	df	F	P	Ks $mgC.L^{-1}$	1/Djmax day^{-1}
22	130.95 ± 20.130	22.397 ± 0.902	1,10	43.324	<0.0001	0.176	0.183
27	84.26 ± 7.671	4.352 ± 0.683	1,14	40.632	<0.0001	0.053	0.285
32	59.65 ± 5.180	5.586 ± 0.624	1,18	121.184	<0.0001	0.094	0.406

(b) *Moina micrura*

Temp °C	a ± SD	b ± SD	df	F	P	Ks $mgC.L^{-1}$	1/Djmax day^{-1}
22	63.301 ± 8.796	4.3150 ± 1.487	1,10	8.423	0.0158	0.068	0.379
*27	32.351 ± 2.708	1.7387 ± 0.261	1,14	44.504	<0.0001	0.054	0.743
32	28.235 ± 1.778	1.0898 ± 0.174	1,18	39.159	<0.0001	0.039	0.850
27	27.986 ± 9.528	3.0620 ± 0.848	1,14	13.041	0.0028	0.109	0.856

* excluding the result from the anomalous individual.

Table 5.11 Temperature coefficients and vant' Hoff's Q_{10} approximations for the maximal juvenile developmental rate of Diaphanosoma excisum and Moina micrura

Equation $\frac{1}{Djmax\ t_2} = \frac{1}{Djmax\ t_1} e^{b[t_2 - t_1]}$

Djmax = maximum juvenile development rate d^{-1}; t = temperature °C

Temp range °C	Diaphanosoma excisum		Moina micrura	
	b	Q_{10}	b	Q_{10}
22-27	0.088	2.43	0.135	3.84
27-32	0.071	2.03	0.027	1.30
22-32	0.077	2.22	0.081	2.24

Table 5.12 Parameters of the multiple regressions relating the effect of food concentration (S in $mg.C.L^{-1}$) and temperature (T in °C) on duration of post-embryonic duration (Dj in hours) of (a) Diaphanosoma excisum and (b) Moina micrura

Regression equation: $1/Dj = a - b.\frac{1}{T} - c.\frac{1}{S}$

(Dj = juvenile duration in hours, T = temperature in °C, S = food concentration in $mgC.L^{-1}$)
df = degrees of freedom; F = variance ratio; P = level of significance.

(a) Diaphanosoma excisum

a	b	c	df	F	P
0.0321	0.549	0.000408	2,45	79.6	<0.001

F due to food concentration: 83.5

F due to temperature: 75.7

(b) Moina micrura

a	b	c	df	F	P
0.0763	1.29	0.000807	2,45	91.8	<0.001

F due to food concentration: 77.8

F due to temperature: 105.7

CHAPTER 6

THE EFFECT OF TEMPERATURE AND FOOD CONCENTRATION ON REPRODUCTION

Although there are papers in the literature on the effects of various environmental factors, such as temperature, food and predation on reproduction in Cladocera, these deal with factors acting singly and there is little published concerning factors acting in combination, which is what happens in nature. The present study aims to show how the species being studied respond to various combinations of temperature and food concentration and in the absence of predation when other factors are kept reasonably constant (for example, food quality was kept constant - light was not).

Energy assimilated from food is used for metabolic activities and various forms of growth, whether body growth or reproductive growth. Body growth occurs mainly during the juvenile phase of the life cycle and to a lesser extent, in the Cladocera, after the onset of maturity when ovarian growth is a continuous and predominant growth process. In non-food limiting conditions, oocytes are passed into the brood pouch, after an ecdysis and release of neonates, and a new ovary starts growing. Any energy excess to the animal's requirements for metabolism and growth may be stored in the body as oil.

The onset of reproduction in the life cycle occurs in the primiparous female, which is the first developmental stage in the life cycle at which assimilated food energy goes into

reproduction. This stage is usually taken to be distinguished by the presence of developing eggs in the brood pouch rather than of a mature ovary (seen one instar earlier). The primiparous female can be defined by her age (from release from the brood pouch), by her size (usually carapace length) and/or by her developmental stage or instar. All these characteristics can vary under different conditions and can be used as measures for evaluating environmental effects on reproduction along with equally important measures of reproductive effort such as the number and size of eggs contained in a clutch or brood. The aim of this chapter is to assess how various combinations of temperature and food concentrations affect these reproductive characteristics so that insight can be gained into what is limiting reproduction in field populations despite the fact that some, but not all, of these reproductive characterstics can be determined. It should be noted that, as mentioned in Chapter 3, results for Ceriodaphnia cornuta and Daphnia lumholtzi can be discussed only at one temperature (at 32°C for the former and at 22°C for the latter).

6.1 FOOD THRESHOLD CONCENTRATIONS FOR REPRODUCTION

Estimates of fecundity (eggs in the first four broods per female) were possible only at the higher food concentrations in combination with the three temperatures. This was because an attempt was made to determine the threshold food concentration for reproduction under different temperature conditions. The stress of low food availability at the lower food concentrations

had a marked effect on juvenile mortality so that estimates of fecundity were not always possible. Thus, in both 0.01 and 0.03 $mgC.L^{-1}$ food concentrations, juvenile animals died at all three temperatures and in the case of Diaphanosoma excisum and Moina micrura lived for less than four days at 27°C and 32°C but lived longer, 4-6 days, at 22°C. They managed to attain their 3rd instar in food of 0.03 $mgC.L^{-1}$. None of these juveniles died suddenly; it was possible to observe a slow decrease in their heart beat over a period of one to two days prior to their death. In 0.05 $mgC.L^{-1}$ food and at 22°C, the juvenile animals of the two species also died but they did manage to attain maturity at 27°C in this food concentration. In Diaphanosoma excisum not all of these animals, however, managed to produce four successive broods. Two individuals died after their second brood and the other two replicate animals died after their third brood. In contrast, Moina micrura completed four successive broods after maturity and attained the 5th adult instar but one animal had an empty brood pouch in between reproductive instars. On the other hand, at 32°C, all individuals of both species completed four successive broods and attained their 5th adult instar in a food concentration of 0.05 $mgC.L^{-1}$. At 0.1 $mgC.L^{-1}$ food concentration and 22°C, animals were reared to their 5th adult instar but Diaphanosoma excisum did not always manage to produce eggs and empty brood pouches were recorded for some adult instars while although Moina micrura always produced eggs, one animal produced males. At the higher temperatures of 27°C and 32°C and in 0.1 $mgC.L^{-1}$ food, and at all other higher food concentrations, all the animals of these two species managed to complete four broods and the results are given in Table 6.2.

In Ceriodaphnia cornuta at 32°C, in all food concentrations below 0.1 $mgC.L^{-1}$, the animals survived for only 3-4 days in the neonate stage. Of the four replicates at 0.25 $mgC.L^{-1}$ one died in a juvenile instar after 5 days, one produced one brood of one neonate, the third managed two broods of one neonate each and the fourth produced three broods of one neonate each but died in the fourth adult instar with an empty brood pouch. Of the six replicates at 0.5 $mgC.L^{-1}$ the juveniles died after surviving 4-7 days in three, while the other three managed to reproduce. At 1.0 $mgC.L^{-1}$ all four replicates reached the 5th adult instar. Thus even at the highest temperature this species needed a relatively high food concentration to reproduce. For Daphnia lumholtzi, at 22°C, in 0.01, 0.03 and 0.05 $mgC.L^{-1}$, eight replicates were carried out but juveniles died after 3-5, 4-7 and 6-12 days respectively, without reaching reproductive size. Of eight replicates at 0.1 $mgC.L^{-1}$ only two managed to reproduce, one died after producing one brood of two neonates, and the other two broods of two neonates one of which in each case was male. One of the remaining three replicates survived up to 9-14 days, managed to develop one ovary, but did not produce eggs. A striking feature observed in Daphnia lumholtzi was that the ovary started to appear a number of instars prior to the primipara instar. It developed gradually through successive instars until it produced eggs. At 0.5 $mgC.L^{-1}$ in three replicates out of six, the animal survived 7-8 days: in the other three one animal produced two neonates in one brood, the second managed two broods of one neonate each, the third managed two broods of two, one of

the second pair being a male. Of four replicates at the highest food concentration (1mgC.L^{-1}) three managed to reach the 4th adult instar while the other gave four broods, with six males in the second brood, before it died.

The results in Table 6.2 indicate that there is a threshold food concentration for reproduction in both Diaphanosoma excisum and Moina micrura which varied with temperature. The threshold food concentration in Diaphanosoma excisum at 22°C was 0.1 mgC.L^{-1}, at 27°C it was 0.05 mgC.L^{-1} and at 32°C it lay between 0.05 mgC.L^{-1} and 0.03 mgC.L^{-1}. In Moina micrura it was similar to Diaphanosoma excisum except at 22°C in which it lay between 0.05 and 0.1 mgC.L^{-1}. That is, the threshold food concentration for reproduction became lower at higher temperatures or became higher at lower temperatures. The threshold level for reproduction in Daphnia lumholtzi at 22°C was very similar (0.1 mgC.L^{-1}) but that of Ceriodaphnia cornuta at 32°C was higher than that of all the other species, at about 0.25 mgC.L^{-1}.

6.2 FECUNDITY AND TEMPERATURE

At all food levels above the threshold food concentrations, the level of fecundity both in Diaphanosoma excisum and Moina micrura increased with temperature. This is illustrated in Fig. 6.1. It proved possible to fit regressions of fecundity (mean number of eggs from the first four broods per female) on temperature for each of three food concentrations (1.0, 0.5 and 0.1 mgC.L^{-1}) and Table 6.3 shows that all the regressions were statistically significant. The regression equations are fitted

to the data in Fig. 6.1 and show that the predicted intercept at 0°C is negative. These regressions predict for no reproduction at temperatures below about 15°C, an interesting result for tropical species.

Covariance analysis was used to test whether the fecundity-temperature relationships at different food levels were significantly different and these results are given in Table 6.4. This table shows that, in both Diaphanosoma excisum and Moina micrura, there is no significant difference between the slopes and elevations of the regressions for the two higher food levels of 1.0 and 0.5 $mgC.L^{-1}$ and that the regression coefficient (slope) for the 0.1 $mgC.L^{-1}$ food level is also not different from the other two in Diaphanosoma exicsum but about half the value in Moina micrura. However, the latter regression (0.1 $mgC.L^{-1}$) has an adjusted mean value which is statistically different from the other two and is about one third lower in Diaphanosoma excisum and about half in Moina micrura. That is, the incipient limiting food concentration for reproduction starts for both these species between 0.1 and 0.5 $mgC.L^{-1}$ at the temperature tested.

6.3 FECUNDITY AND FOOD CONCENTRATION

The relationship between fecundity (mean number of eggs from the first four broods per female) and food concentration at different temperatures (in Diaphanosoma excisum and Moina micrura is illustrated in Fig. 6.2 and is curvilinear. A logarithmic expression could be fitted to the empirical data and a series of statistically significant log-linear regressions were computed,

the results of which are given in Table 6.5. The results of covariance analysis of these, given in Table 6.6, indicates that in Diaphanosoma excisum the regressions for 22°C and 27°C are not significantly different but that the regression for 32°C has both a different slope and adjusted mean value. This regression lies above the other two (Fig. 6.2). In Moina micrura the slopes at 22°C and 27°C are not significantly different but the latter has a higher adjusted mean; the regression lies above the former. The regression at 32°C not only lies even higher than the other regressions but also has a higher slope, indicating a steeper increase in fecundity as food concentration increases at 32°C compared to the other two temperatures.

Table 6.2 presents some simple correlation coefficients between the total fecundity (sum of eggs in four successive broods per female) and the environmental influences of temperature and food concentration as well as the biological responses by the species in terms of size, age and instar stage of the primparous female, and the size of the neonates. It is clear that, for both species, total fecundity is strongly correlated with both temperature and food concentration, with food showing the stronger influence.

An attempt was made to examine the possibility of interactive effects of temperature plus food concentration upon fecundity by multiple regression analysis, the results of which are given in Table 6.7. Significant regressions were obtained for both species. By comparing the magnitude of the variance ratio F, when assessed for the influence of food concentration and

temperature separately and in combination, it was possible to show that the food effect on fecundity was about four times greater than that of temperature alone and that, when in combination, the interaction of these two environmental variables lowered the magnitude of the combined F value.

6.4 SIZE OF EGGS AND NEONATES

Under conditions of food limitation, the amount of assimilated food energy available for ovarian growth is constrained by the animal's need to cover its metabolic costs. It is therefore, ecologically interesting to determine how a species responds to these food constraints in terms of numbers and size of eggs. Does it produce a large number of small eggs or a smaller number of larger eggs with the same limited amount of food? The ecological implications of the choice made are very different and will be discussed later.

As mentioned earlier in Methods, it proved impossible to measure the diameter of the eggs whilst inside the brood pouch during the course of the experiments, without the risk of damaging the animals. But the length of all neonates, produced by females grown under the different experimental conditions, was measured and the results are given in Table 6.13. In neither Diaphanosoma excisum nor Moina micrura does the neonate length appear to change with temperature nor with food concentration from 1.0 down to 0.1 $mgC.L^{-1}$ but in both species the neonates appear to become smaller in the 0.05 $mgC.L^{-1}$ food concentration at most temperatures. These results for Diaphanosoma excisum and Moina micrura are illustrated three-dimensionally in Fig. 6.7.

The results are sparse for Ceriodaphnia cornuta and Daphnia lumholtzi (Table 6.13). In Ceriodaphnia cornuta there is no clear trend. The results for Daphnia lumholtzi at 22°C for 0.5 and 1.0 mgC.L^{-1} show similar sized neonates. The Student's t-test carried out on the size of neonates produced at 0.1 and 0.5 mgC.L^{-1} indicates a significant difference between the size of the neonates at these two food levels (t = -6.094; d.f. = 18; P (two-tailed) = 0.00001). The outstanding feature observed here, in contrast to the other species, was that bigger neonates were produced at the lower food concentrations than at the higher concentrations

Table 6.1 lists a series of simple correlation coefficients between neonate size and various other influences on biological properties. One strong, positive correlation which is present in both species is that with total fecundity, the larger neonates are associated with higher levels of fecundity which is measured as number of eggs in the first four broods. The higher levels of fecundity are associated with the higher temperatures and food levels above 0.1 mgC.L^{-1} and these presumably produce large-sized eggs from which larger neonates are born.(although there is no actual evidence for the larger-sized eggs). In Diaphanosoma excisum, there is a positive correlation between neonate size and food concentration but not with temperature, which supports the above neonate size-fecundity association. There is a difference between the two species here, as in Moina micrura, there is a correlative link between neonate size and neither food concentration or temperature, although one might have expected one with temperature.

On the other hand, there is little evidence for any correlation between neonate size and the properties of the primiparous female of either species, apart from the positive association with the size of the Diaphanosoma excisum primipara: here the neonates born from the larger-sized primiparous females tend to be bigger.

6.5 THE PRIMIPAROUS FEMALE: FECUNDITY, SIZE, AGE AND INSTAR STAGE

The primiparous female represents an important phase during the development of the cladoceran life cycle when first reproduction occurs. Generally, during the previous instar, the female has to produce an ovary from which the eggs will be laid in the brood pouch and this has to be done whilst her body is still growing at a juvenile rate. In Daphnia lumholtzi, and at low temperature and food concentration (22°C and 0.1 $mgC.L^{-1}$) in Diaphanosoma excisum, the ovary begins to mature several instars prior to the primipara stage and continues developing during successive instars. Competition for resources between these two growth processes must become severe under conditions of food limitation. It is therefore of interest to examine the level of fecundity that this stage can achieve when reared in different food concentrations and at various temperatures as well as to examine what variation occurs in her body size, age and instar stage under the different experimental conditions.

Table 6.10 provides information on the brood size (eggs per primiparous female) for each experimental combination of temperature and food concentration and Fig. 6.6 illustrates these results in a three dimensional diagram for Diaphanosoma excisum and Moina micrura. At each temperature, there is a tendency for the brood size to increase with food level but this is not very marked at 22°C, is rather more pronounced at 27°C and is greatest at 32°C. When one examines the influence of temperature upon fecundity at any one food level, it is clear that the influence of temperature is dampened down at the lower food levels compared with those which are above the incipient limiting levels for reproduction.

The mean size of primiparous females of Diaphanosoma excisum and Moina micrura obtained under experimental conditions are given in Table 6.11 (and illustrated as the three dimensional diagram in Fig. 6.5). These do not reveal any strong tendency for a change in body size with change in food concentration in either species, although Diaphanosoma excisum is smallest at the lowest food level (0.05 $mgC.L^{-1}$). There is, however, a trend towards smaller size with the higher temperatures in Moina micrura which does not occur in Diaphanosoma excisum. This trend permitted the calculation of a multiple regression equation predicting the size of the Moina micrura primipara in relation to temperature and food concentration, which is given in Table 6.12. An analysis of the components of the variance shows that most is due to temperature and not to food concentration. No similar multiple regression could be obtained for Diaphanosoma excusim. Food concentration did not have any significant influence on size of primipara of Daphnia lumholtzi at 22°C and Ceriodaphnia cornuta at 32°C (Table 6.11.).

Many of these interactions are usefully summarised in Table 6.1, which lists some simple correlation coefficients between total fecundity (number of eggs in four broods) and both external influences of temperature and food concentration and internal responses of the primiparous female in terms of body size, age and instar stage of development.

The Table 6.1 shows that temperature, which positively correlates with total fecundity, also has a strong, negative effect in both species on the primipara age and instar stage as well as, in Moina micrura alone, on primipara body size. Food concentration, which is also strongly and positively correlated with total fecundity, has a significant negative effect on the age and instar stage of the primipara of Diaphanosoma excisum but only one instar stage in Moina micrura. In neither species is there a correlative link between food concentration and primipara body size.

However, as can be seen in Table 6.1, there is a strong, positive correlation in Diaphanosoma excisum between total fecundity and primipara size and a strong but negative one with primipara age and instar stage. The response is similar in Moina micrura, apart from the absence of any correlation between fecundity and primipara size.

To sum up for both species (Fig 6.4). Food concentration and temperature both influence total fecundity in a positive direction, though food rather more than temperature (Table 6.1; Table 6.7). There is an increase in total fecundity with larger primipara sizes (in Diaphanosoma excisum only) and with younger

primipara of low instar stage. Temperature influences the age and instar stage of both species by reducing both at higher temperatures; in Moina micrura alone, it also reduces the primipara body size at higher temperatures. On the other hand, food concentration also reduces age and instar stage of Diaphanosoma excisum but only the instar stage of Moina micrura. In neither species does food concentration affect the body size of the primipara. The main differences between the two species are that temperature influences body size in Moina micrura but not in Diaphanosoma excisum, food concentration influences age in Diaphanosoma excisum but not in Moina micrura and that a strong link between total fecundity and primipara size exists only in Diaphanosoma excisum.

6.6 FECUNDITY AND BODY SIZE

That fecundity (eggs per brood) is related to body size in Cladocera is well known in the literature and also true in the tropical species studied. How this relationship changed under the experimental conditions imposed is illustrated in Fig. 6.3 for each particular food level at different temperatures. In general, in conditions where reproduction did take place, the number of eggs per brood was rather higher in the higher food levels (0.25 - 1.0 $mgC.L^{-1}$) than in the lower ones (0.1 and 0.05 $mgC.L^{-1}$), and at the higher than lower temperatures. The conditions under which no reproduction occurred were 0.05 $mgC.L^{-1}$ food concentration at 22°C for both species (Diaphanosoma excisum and Moina micrura).

It proved possible to quantify this relationship between fecundity and female length by means of simple linear regressions, which are given in Table 6.8. In Diaphanosoma excisum, there was no significant relationship at the higher temperature of 27°C and 32°C in food concentration of 0.05 $mgC.L^{-1}$. This was also true for Moina micrura but in addition no significant regression was obtainable for this species in any food level at 22°C nor in 0.1 $mgC.L^{-1}$ at 27°C. Otherwise, the relationships were significant at all other temperature-food concentration combinations, although with somewhat variable probability. In Daphnia lumholtzi a significant relationship was found at 1 $mgC.L^{-1}$ and 22°C. In Ceriodaphnia cornuta at 32°C and 0.5 $mgC.L^{-1}$, maternal body size was found to have no significant effect on fecundity but at 1.0 $mgC.1^{-1}$ there was a significant linear relationship as shown in Table 6.8. Covariance analysis was used to test the similarity or difference of the five or four significant regressions for each food level at 32°C for Diaphanosoma excisum and Moina micrura. The results are given in Table 6.9. In Diaphanosoma excisum, none of the regression coefficients were significantly different, so that the slopes of the relationships can be considered to lie parallel and it is possible to test whether the regression elevations differ. Table 6.9 shows that neither of the adjusted means of the regressions for the two higher food concentrations (1.0 and 0.5 $mgC.L^{-1}$) nor those for the two lower food levels (0.1 and 0.5 $mgC.L^{-1}$) differed significantly between themselves. However, when the regressions for the higher and lower food levels were compared, these were significantly different, those for the higher food levels lying much higher or exhibiting a greater fecundity.

In Moina micrura, only the two regressions for the higher food levels (1.0 and 0.5 mgC.L^{-1}) were not significantly different in slope and their adjusted means were statistically different. The slopes of the regressions for the two lower food levels (0.25 and 0.1 mgC.L^{-1}) differed between each other but both were much shallower, suggesting a reduced influence of body size on fecundity. This may be the result of the negative effect of temperature on the primipara body size mentioned earlier (Table 6.1 and Fig. 6.5).

6.7 INITIAL REPRODUCTIVE RATE

In order to examine the effect of temperature and food concentration on the rate of egg production, the initial reproductive rate, up to the end of the fourth adult instar, was considered. The calculations were done as described at the beginning of the chapter; the results are given in Table 6.14 and plotted three-dimensionally in Fig. 6.8. Both in Diaphanosoma excisum and Moina micrura the highest rate occurred at the highest temperature-food combination (32°C and 1.0 mgC.L^{-1}) and decreased towards both low food and low temperature. In Ceriodaphnia cornuta, the initial reproductive rate at 32°C (Table 6.14) does not show much difference between 1.0 and 0.5 mgC.L^{-1} but at 0.25 mgC.L^{-1} it is approximately one third of that at the higher food concentrations. The initial reproductive rate calculated for the animals reared at 22°C and 1.0 mgC.L^{-1} was 1.218 $\pm$ 0.434 and at 0.5 mgC.L^{-1}, 0.234 $\pm$ 0.112 (means $\pm$ s.d.) eggs per female per day. The result for 0.5 mgC.L^{-1} cannot be used because only two sets of data are available. There are no results at 27°C for these two species.

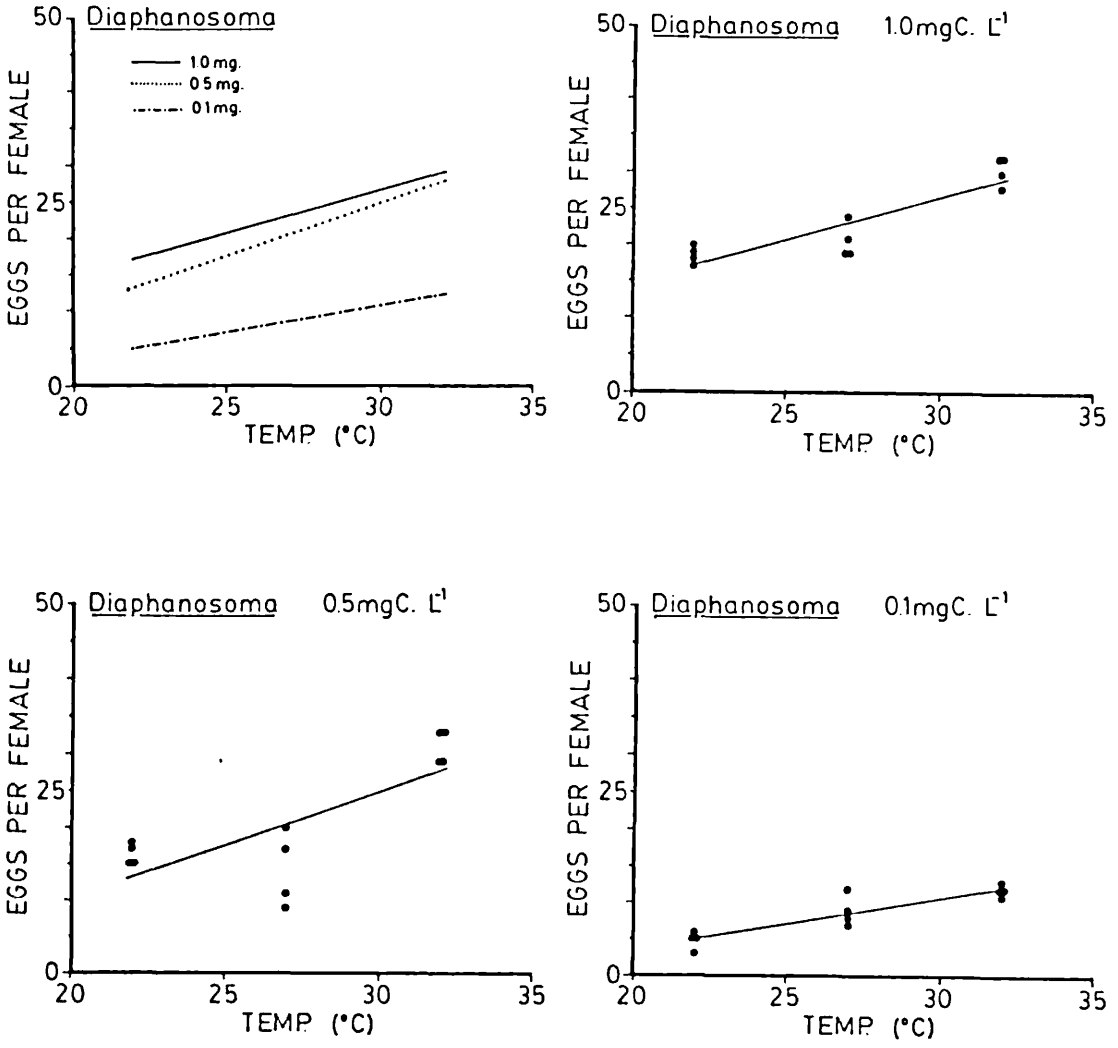


Figure 6.1 Linear relationships between average number of eggs produced in first four successive adult instars (averaged for four females) against temperature at different food concentrations. Regression statistics are given in Table 6.3.

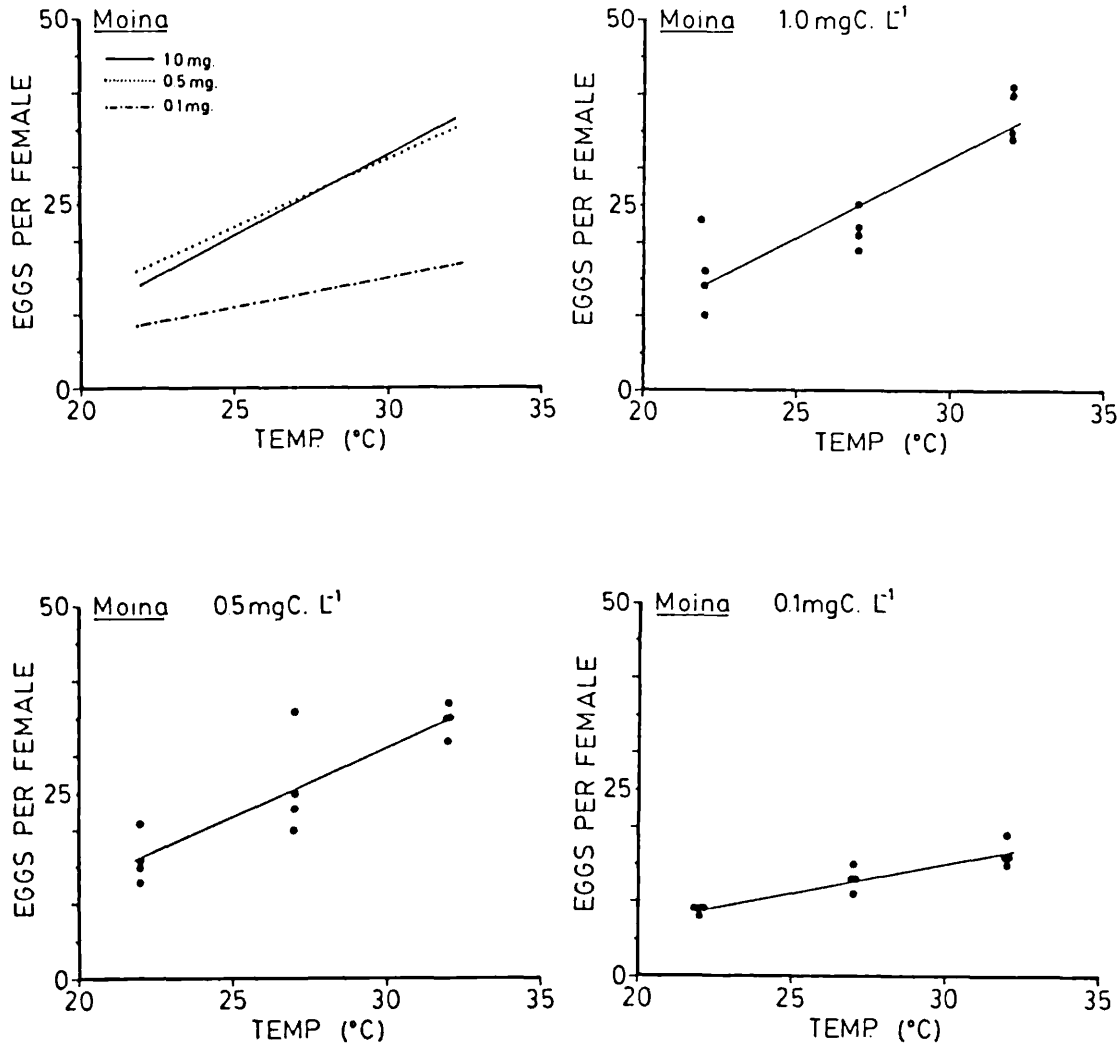


Figure 6.1 continued

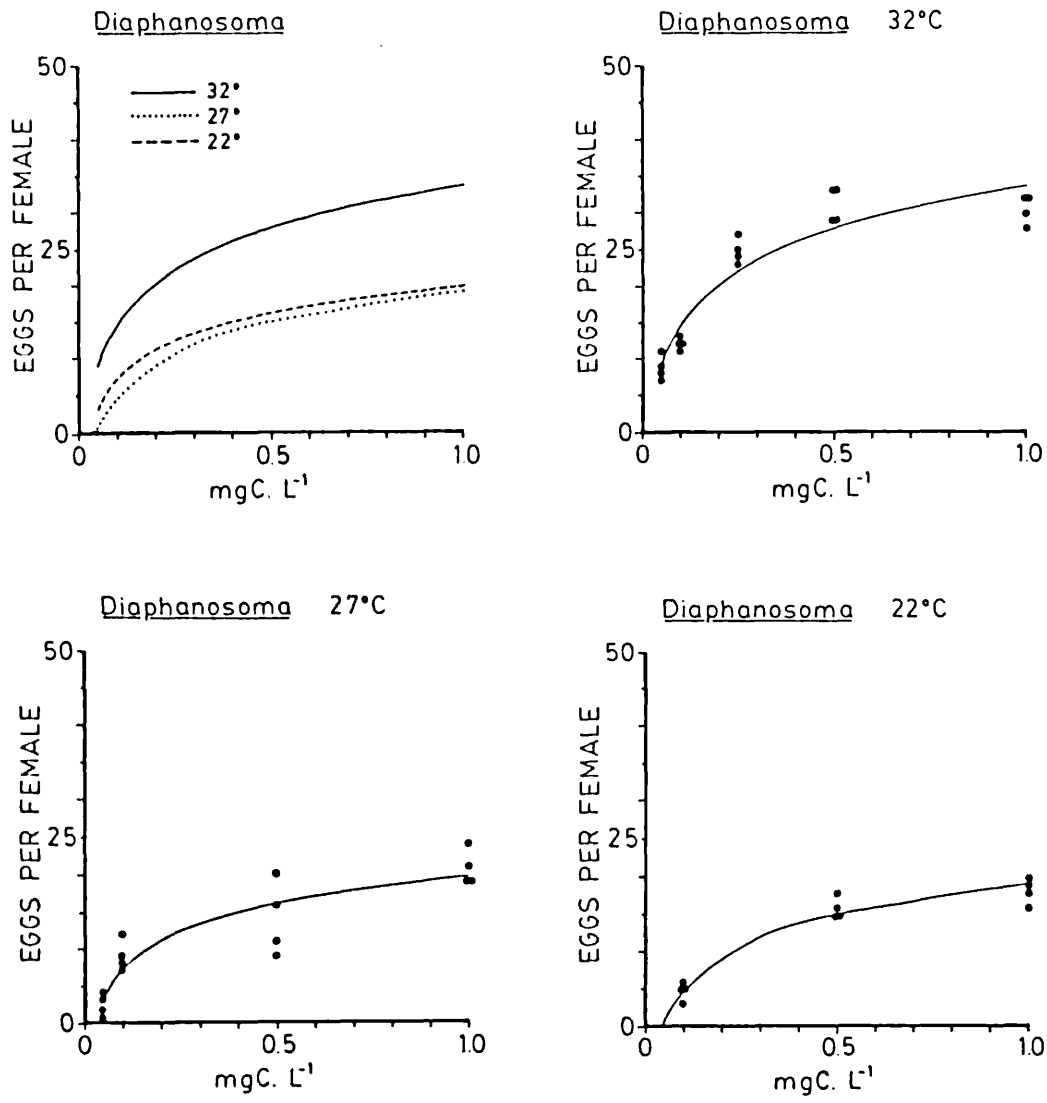


Figure 6.2 Logarithmic relationships between total number of eggs produced in the first four successive adult instars (mean of four replicates) against food concentration. Regression statistics are given in Table Table 6.5.

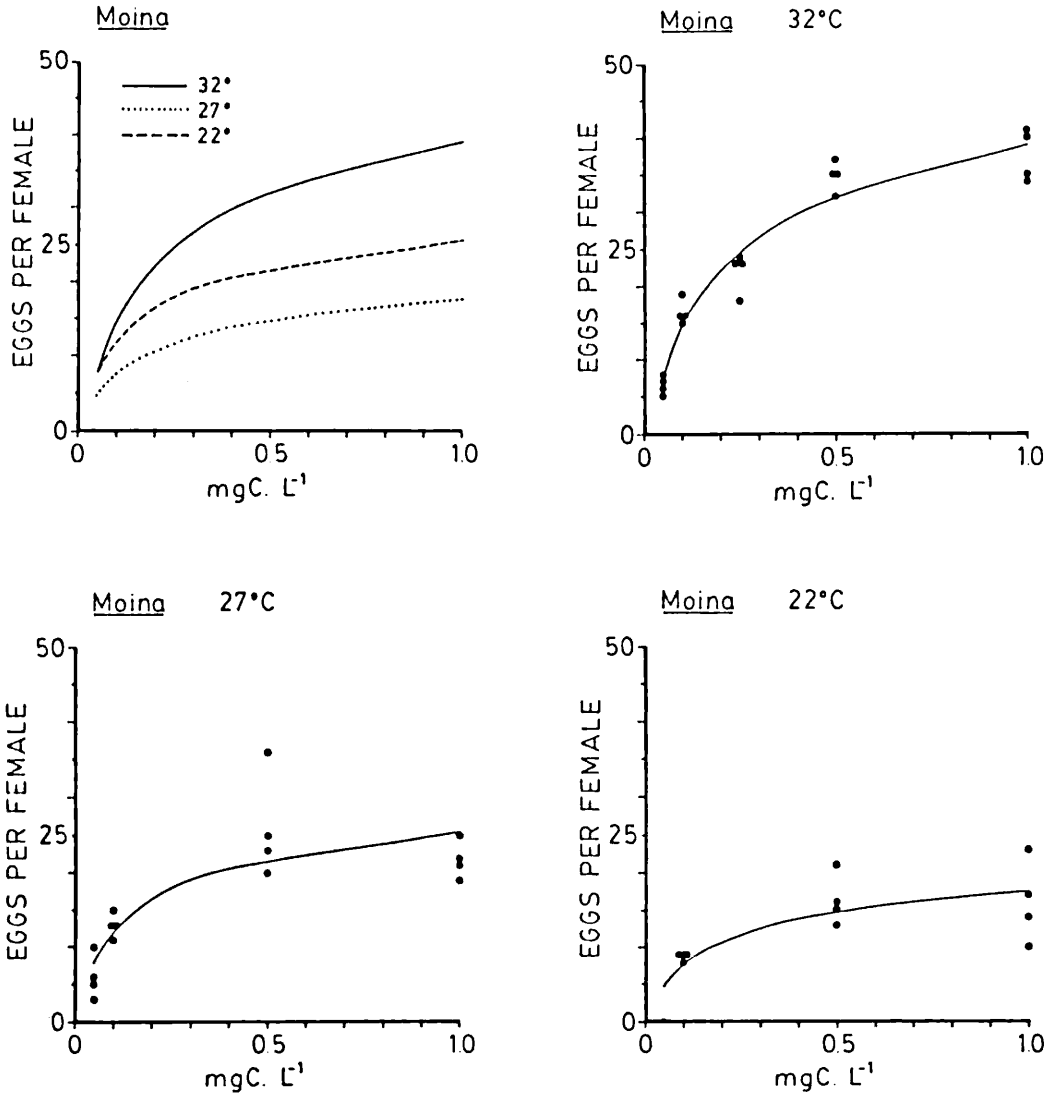


Figure 6.2 continued

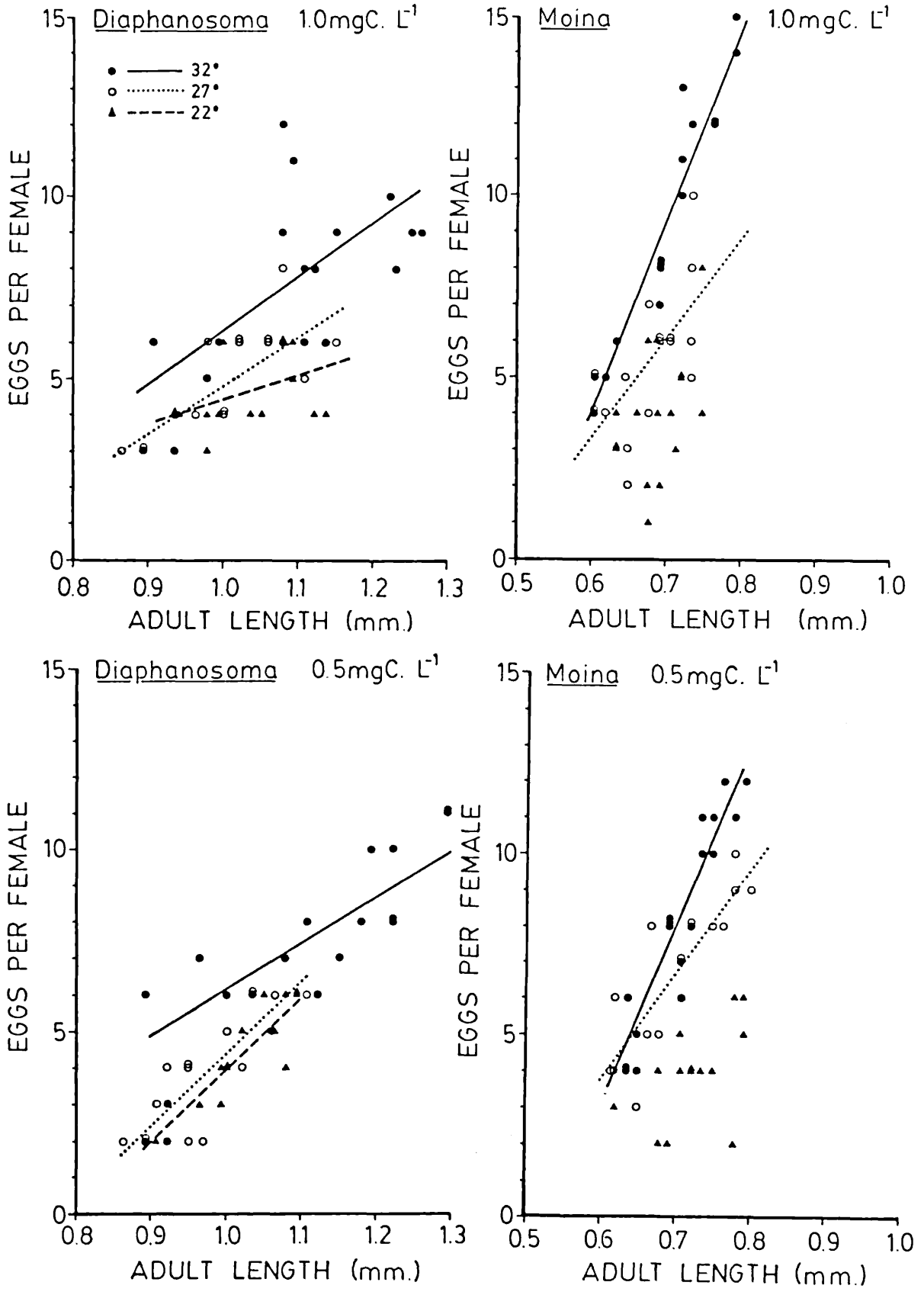


Figure 6.3 The linear relationships between the number of eggs carried by the females in relation to their body length. The regression statistics are given in Table 6.8; only those which are significant have been drawn in.

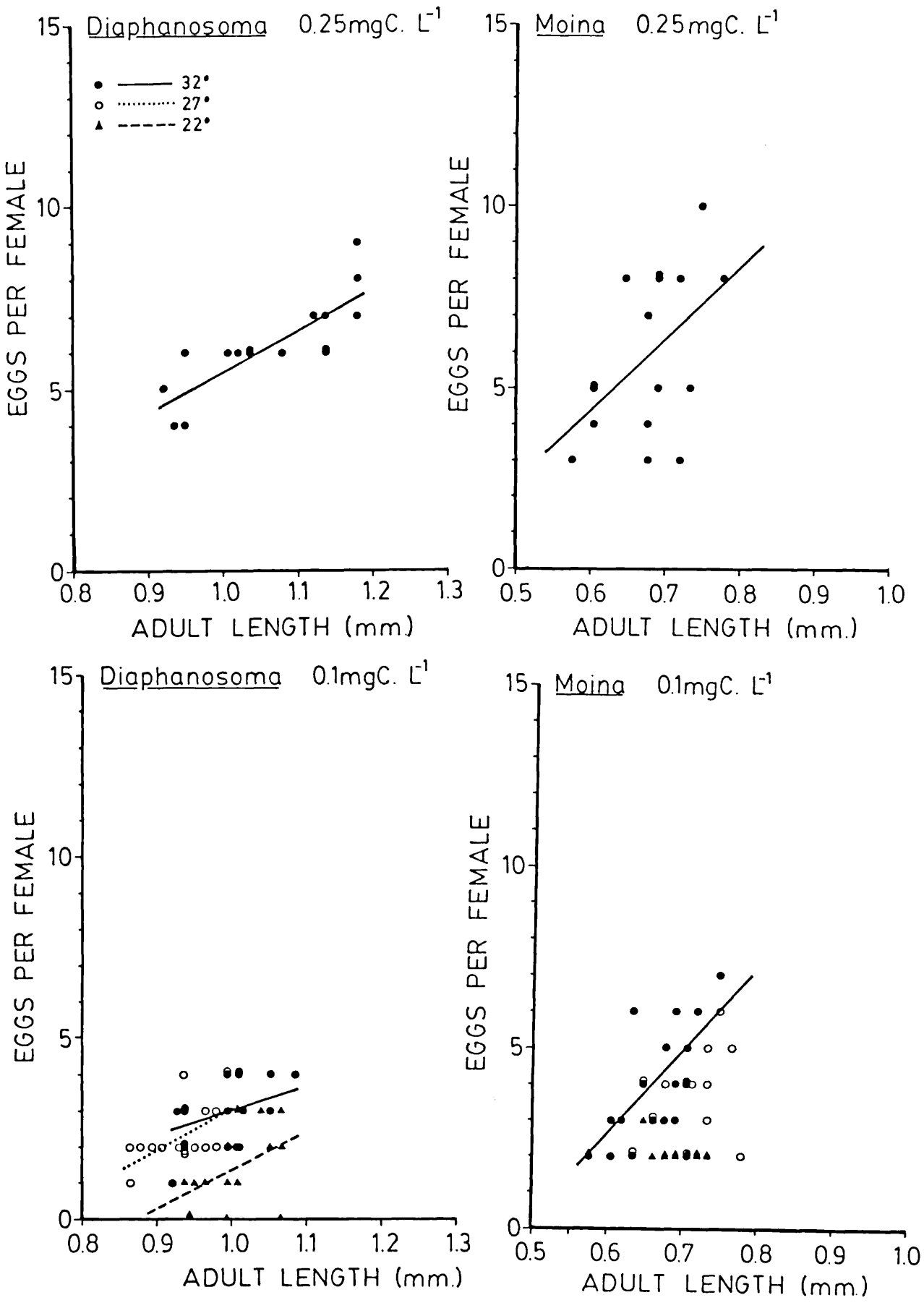


Figure 6.3 continued

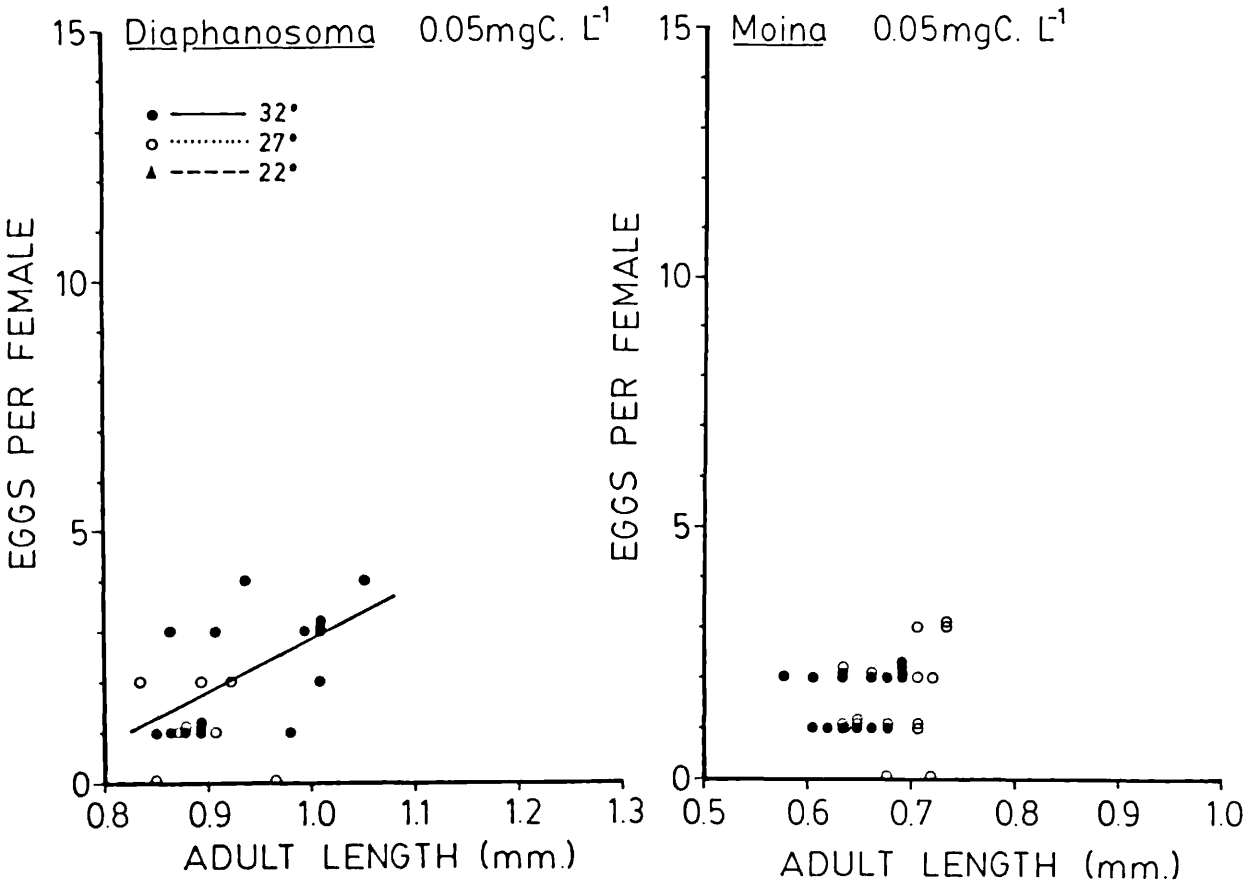


Figure 6.3 continued

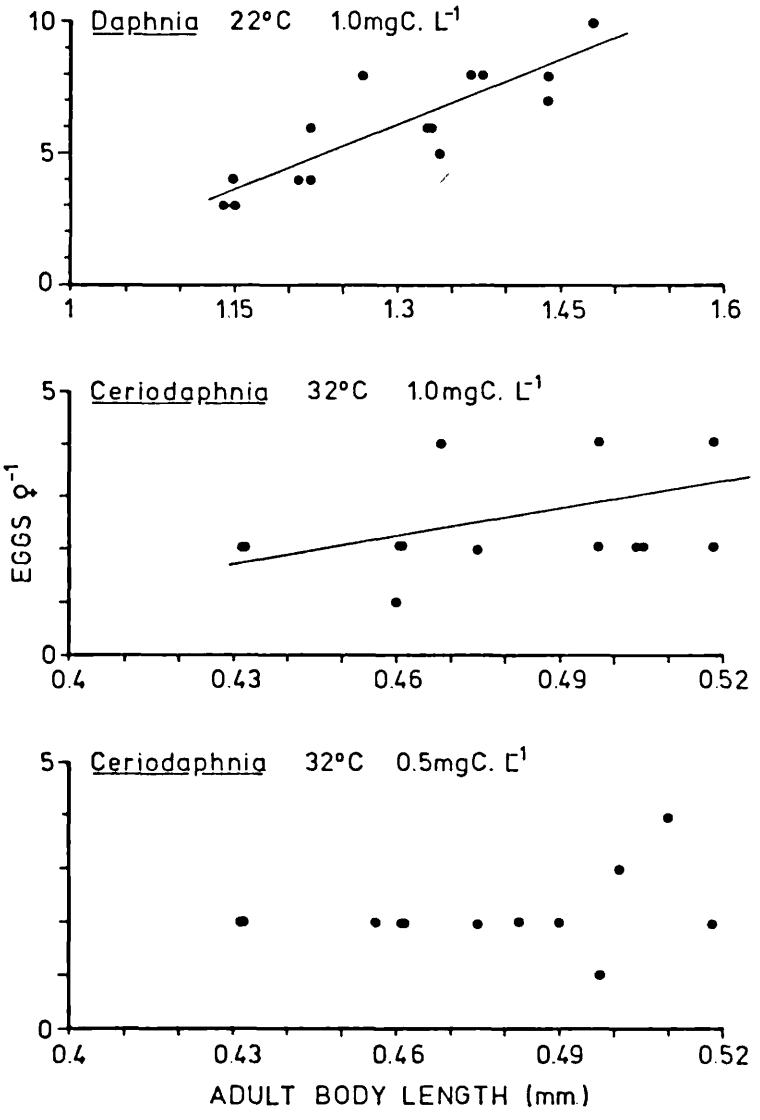


Figure 6.3 continued

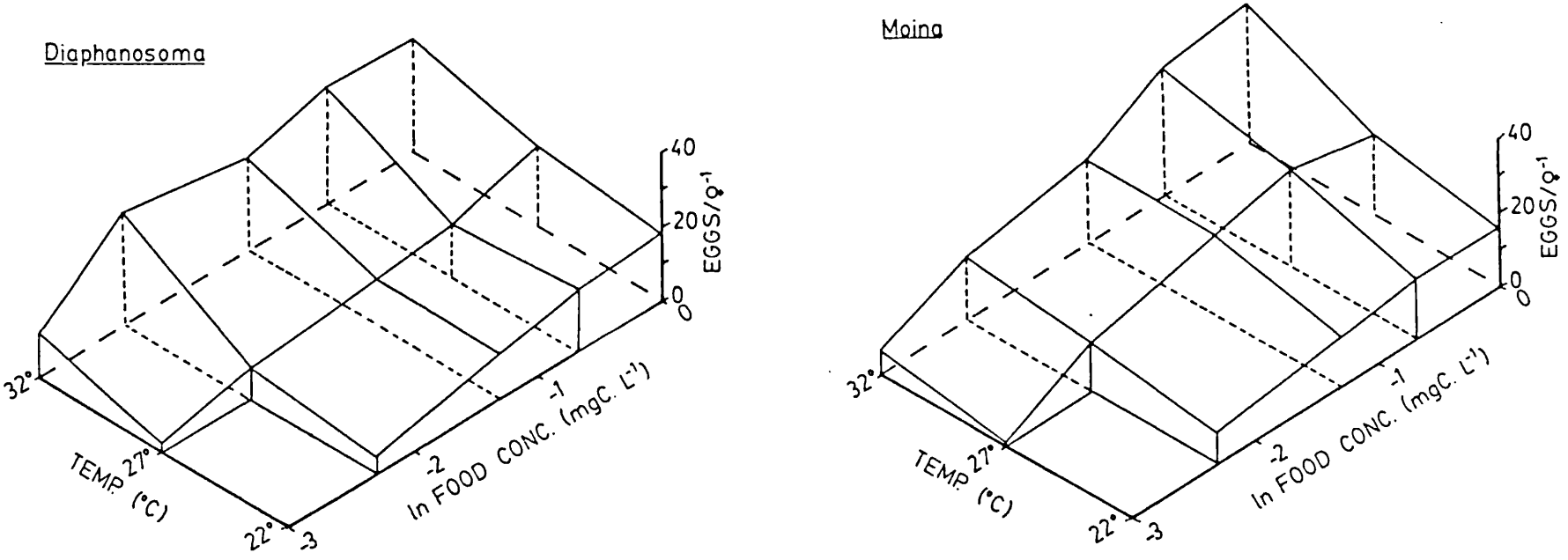


Figure 6.4 Three dimensional plots to show the total number of eggs produced in the first four successive broods of Diaphanosoma excisum and Moina micrura reared in different combinations of food concentration and temperature.

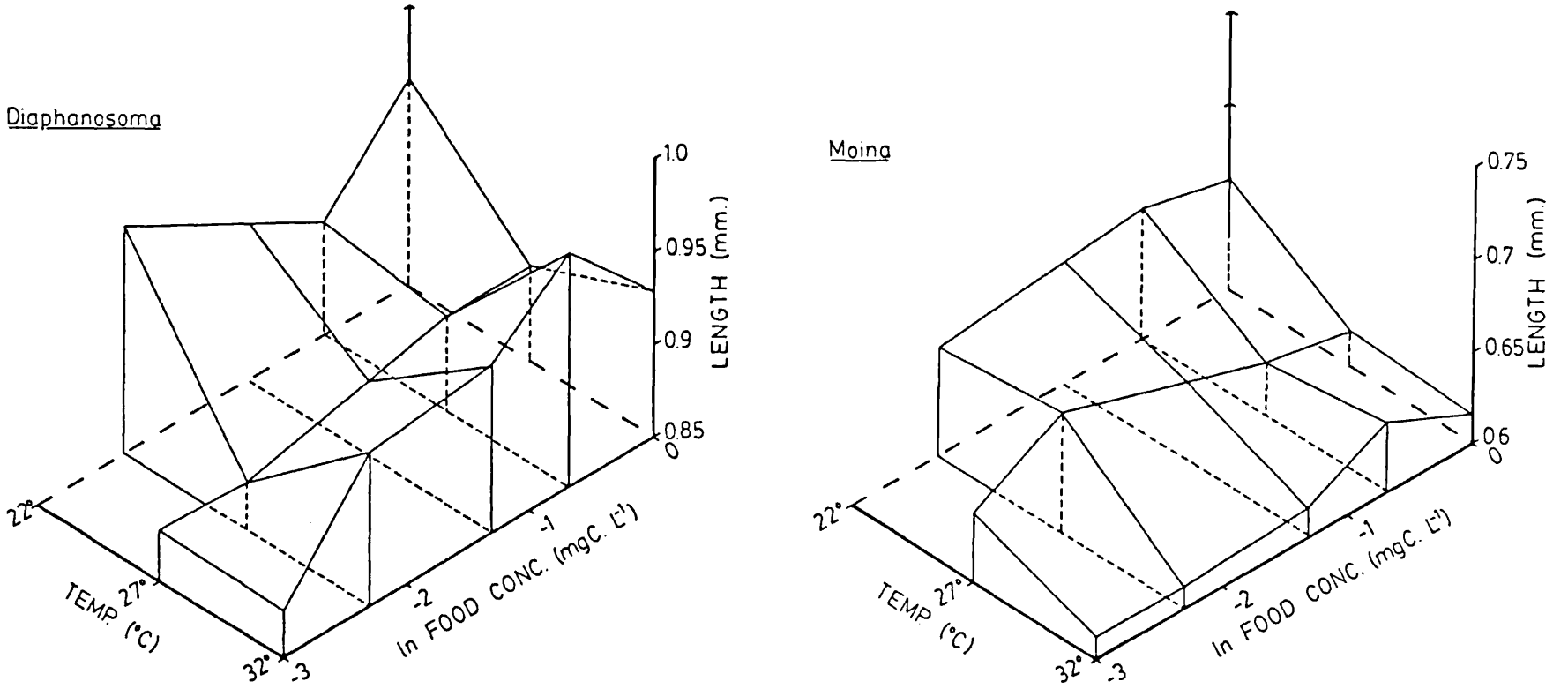


Figure 6.5 Three dimensional plots to show the body length of the primiparous female of Diaphanosoma excisum and Moina micrura reared in different combinations of food concentration and temperature.

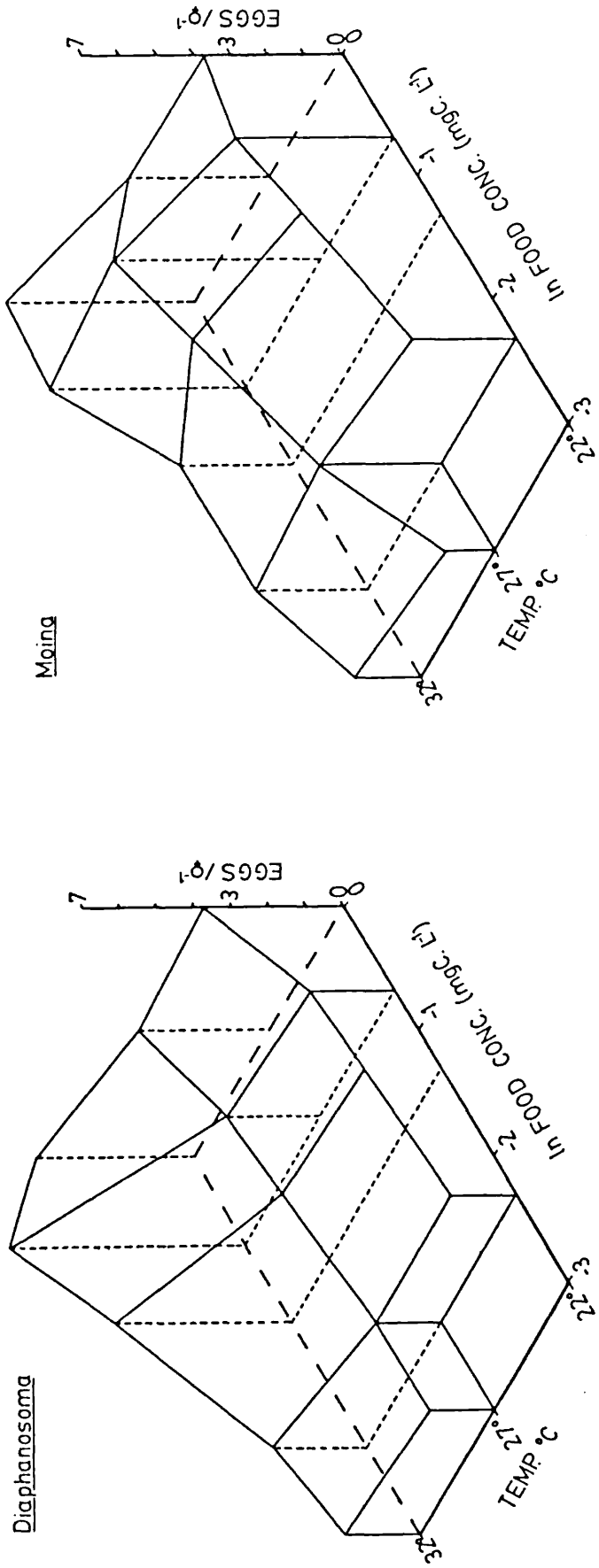


Figure 6.6 Three dimensional plots to show the brood size (no. eggs per female) of the primiparous female of Diaphanosoma excisum and Moina micrura reared in different combinations of food concentration and temperature.

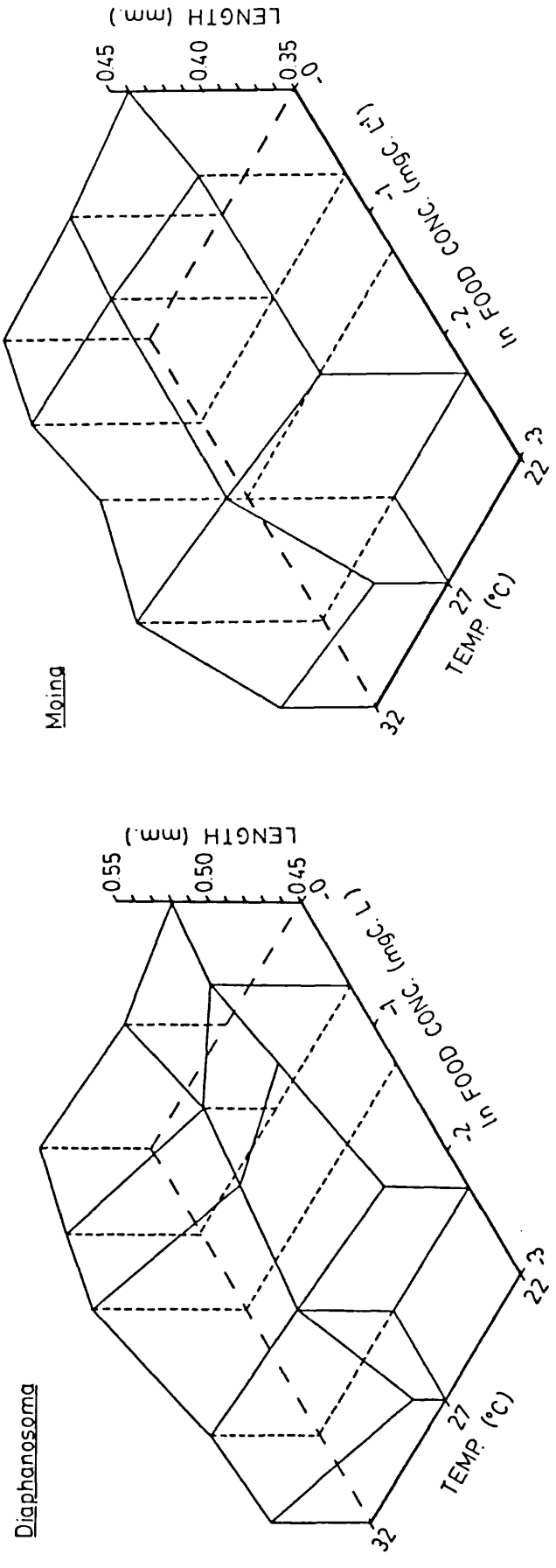


Figure 6.7 Three dimensional plots to show the body length of the neonates of Diaphanosoma excisum and Moina micrura reared in different combinations of food concentration and temperature.

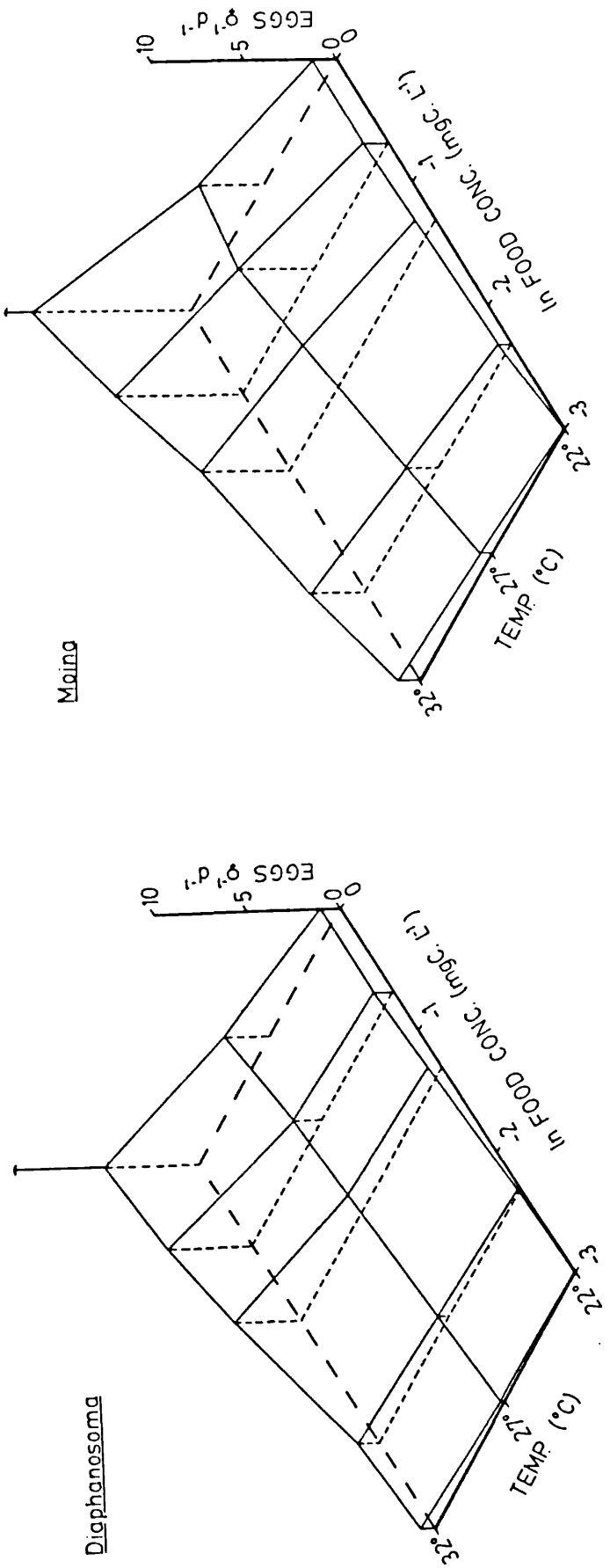


Fig. 6.8 Three dimensional plots to show the initial reproductive rate (the rate of egg production for the first four adult instars) in different combinations of food concentration and temperature.

Table 6.1 Simple correlation coefficients between reproductive parameters.

Diaphanosoma excisum

	Temp.	Food	TE	NS	PS	PA
Food	0.158					
TE	0.401**	0.662***				
NS	0.019	0.353*	0.550***			
PS	-0.083	0.230	0.393**	0.391**		
PA	-0.595***	-0.390**	-0.658***	-0.241	0.223	
PI	-0.577***	-0.365*	-0.593***	-0.207	0.225	0.949***

Moina micrura

	Temp	Food	te	NS	PS	PA
Food	-0.158					
TE	0.400**	0.597***				
NS	0.004	0.222	0.342*			
PS	-0.547***	-0.014	-0.061	0.112		
PA	-0.601***	-0.272	-0.508***	-0.184	0.435***	
PI	-0.296*	-0.407**	-0.515***	-0.182	0.342*	0.866***

Significance levels: * 0.05 ** 0.01 *** 0.001

Temp. - Temperature
Food - Food concentration
TE - Total eggs female^{-1} in 4 successive broods
NS - Size of neonate
PS - Size of primipara
PA - Age of primipara
PI - Instar number of primipara

Table 6.2 Total number of eggs per female in four successive broods (mean ±SD per female; n = 4) at different food and temperature combinations. (Food concentration in $mgC.L^{-1}$ and temperature in °C).

*jd = juveniles died

Temp °C	Food concentration ($mgC.L^{-1}$) 1.0	0.5	0.25	0.1	0.05	0.03	0.01
(a) Diaphanosoma excisum							
22	18.5 ± 1.29	16.25 ± 1.5		4.75 ± 1.26	* jd	* jd	* jd
27	20.75 ± 2.36	14.25 ± 5.12		9.02 ± 2.16	2.5 ± 1.29*	* jd	* jd
32	30.5 ± 1.91	31.0 ± 2.3	24.75 ± 1.71	12.0 ± 0.82	8.75 ± 1.71	* jd	* jd
(b) Moina micrura							
22	16.0 ± 5.47	16.25 ± 3.40		8.75 ± 0.5	* j d	* jd	* jd
27	21.75 ± 2.25	26.0 ± 6.97		13.0 ± 1.63	6.0 ± 9.4	* jd	* jd
32	37.5 ± 3.51	34.75 ± 2.06	22.5 ± 3.1	15.5 ± 1.73	6.5 ± 1.29	* jd	* jd

*died before producing four broods.

Table 6.3 Linear regression relationships between fecundity and temperature at different food concentrations ($mgC.L^{-1}$).

Regression equation $Y = a + bx$ where Y = total number of eggs per female in the first four successive broods. x = temperature.

df = degrees of freedom; F = variance ratio; P = level of significance; r = correlation coefficient.

Food conc.	a	b	df	F	P	r
(a) *Diaphanosoma excisum*						
1.0	- 9.150	1.200	1,10	41.0	<0.001	0.897
0.5	-19.325	1.475	1,10	13.0	<0.001	0.751
0.1	-10.992	0.725	1,10	48.2	<0.001	0.910
(b) *Moina micrura*						
1.0	-32.967	2.150	1,10	43.5	<0.001	0.904
0.5	-24.283	1.850	1,10	35.3	<0.001	0.883
0.1	- 8.175	0.775	1,10	66.3	<0.001	0.932

Table 6.4 Covariance analysis comparing the regressions of the number of eggs per female in the first four adult instars on temperature at different food concentrations. The regression coefficients were compared by the SS-STP test and the difference between the elevations by the S-N-K test. Regression coefficients or means underlined are not significantly different at P = 0.05 level. Group numbers are given in ascending order of magnitude.
df = degrees of freedom; f = variance ratio; P = level of significance.

a) Diaphanasoma excisum

Food conc	Group no	Regression coeff: ± SE	df	F	P	SS STP
1.0	1	1.20 ± 0.1874	2,30	2.019	0.1505	3 2 1
0.5	2	1.475 ± 0.4098				
0.1	3	0.725 ± 0.1044				

Food conc.	Group no	Adjusted mean ± SE	df	F	P	SNK
1.0	1	23.25 ± 2.2124	2,33	49.58	.0001	3 2 1
0.5	2	20.50 ± 2.2145				
0.1	3	8.58 ± 2.2145				

b) Moina micrura

Food conc.	Group no	Regression coeff. ± SE	df	F	P	SS STP
1.0	1	2.15 ± 0.33	2,30	7.384	0.0025	3 2 1
0.5	2	1.85 ± 0.31				
0.1	3	0.78 ± 0.09				

Food conc.	Group no	Adjusted mean ± SE	df	F	P	SNK
1.0	1	25.08 ± 2.53	2,33	33.25	0.0001	3 1 2
0.5	2	25.67 ± 2.53				
0.1	3	12.75 ± 2.53				

Table 6.5 Log-linear relationships between fecundity (total number of eggs per female in the first four successive broods) against food concentration ($mgC.L^{-1}$)

Regression equation: $Y = a + b \ln x$
Y = fecundity x = food concentration
df = degrees of freedom; F = variance ratio; P = level of significance; r = correlation coefficient.

(a) Diaphanosoma excisum

Temp °C	a	b	df	F	P	r
22	19.391	6.281	1,11	247.6	<0.001	0.978
27	19.801	5.458	1,14	65.8	<0.001	0.908
32	33.689	8.329	1,18	162.9	<0.001	0.949

Moina micrura

Temp °C	a	b	df	F	P	r
22	17.530	4.265	1,11	17.2	<0.001	0.780
27	25.509	5.889	1,14	31.4	<0.001	0.823
32	38.979	10.524	1,18	270.0	<0.001	0.969

Table 6.6 Covariance analysis comparing the regressions of the number of eggs per female in the first four adult instars on food concentration. The regression coefficients were compared by the SS-STP test and the differences between elevations by the S-N-K test. Regression coefficients and means underlined are not significantly different at P = 0.05 level; group numbers are given in ascending order of magnitude.

df = degrees of freedom; F = variance ratio; P = level of significance.

(a) Diaphanosoma excisum

Temperature	Group no	Regression coeff. ± SE	df	F	P	SS-STP
22	1	6.2810 ± 0.399	2,43	6.113	0.0046	2 1 3
27	2	5.4581 ± 0.673				
32	3	8.3286 ± 0.653				

Temperature	Group no	Adjusted mean ± SE	df	F	P	SNK
22	1	10.5023 ± 1.808	2,46	66.69	<.0001	1 2 3
27	2	12.3051 ± 1.806				
32	3	21.9295 ± 1.806				

Moina micrura

Temperature	Group no	Regression coeff. ± SE	df	F	P	SS-STP
22	1	4.265 ± 1.029	2,43	12.985	<0.0001	1 2 3
27	2	5.889 ± 1.050				
32	3	10.524 ± 1.641				

Temperature	Group no	Adjusted mean ± SE	dF	F	P	SNK
22	1	10.841 ± 2.859	2,46	28.102	<0.0001	1 2 3
27	2	17.419 ± 2.855				
32	3	24.019 ± 2.855				

Table 6.7 Multiple regression analysis of the effect of temperature and food concentration on fecundity

Regression coefficient $Y = -a + bT + c \ln S$
Y = fecundity, total number of eggs per female in first four broods;
T = temperature; S = food concentration ($mgC.L^{-1}$)
df = degrees of freedom; F = variance ratio; P = level of significance:
correlation coefficient = r.

(a) Diaphanosoma excisum

a	b	c	df	F	P	r
8.44	1.22	6.94	2,45	128	0.0001	0.922

F due to temp.	F due to food conc.
48.25	207.4

(a) Moina micrura

a	b	c	df	F	P	r
8.46	1.34	7.28	2,45	77.8	0.0001	0.881

F due to temp.	F due to food conc.
32.016	123.57

Table 6.8 Parameters of linear regressions relating fecundity to the size of the mother at different temperature and food concentrations for Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi.

Regression equation $Y = a + bx$
(Y = number of eggs per female; x = adult length in mm)
df = degrees of freedom; F = variance ratio; P = level of significance; r = correlation coefficient; NS = not significant

(a) Diaphanosoma excisum

Food conc $mgC.L^{-1}$	a	b	df	F	P	r
Temp 32°C						
1.0	- 8.337	14.645	1,14	12.0	<0.005	0.685
0.5	- 6.806	12.887	1,14	24.5	<0.001	0.797
0.25	- 5.760	11.230	1,14	28.6	<0.001	0.820
0.1	- 3.652	6.660	1,14	4.4	<0.05	0.491
0.05	- 7.623	10.441	1,14	7.7	<0.025	0.596
Temp 27°C						
1.0	- 8.194	12.986	1,14	22.3	0.001	0.784
0.5	-15.213	19.605	1,14	52.4	0.001	0.888
0.1	- 8.596	11.649	1,14	6.3	0.025	0.558
0.05	+ 5.103	-4.468	1,6	0.3	NS	0.024
Temp 22°C						
1.0	- 2.557	6.920	1,14	4.3	0.05	0.485
0.5	-15.710	19.650	1,14	69.4	0.001	0.912
0.1	- 9.444	10.794	1,14	4.6	0.05	0 499

Table 6.8 continued

(b) Moina micrura

Temp 32°C

Food conc mgC.L^{-1}	a	b	df	F	P	r
1.0	-28.387	53.797	1,14	144.6	<0.001	0.912
0.5	-26.831	49.718	1,14	139.4	<0.001	0.909
0.25	- 7.357	19.523	1,14	4.6	<0.05	0.249
0.1	-11.161	22.948	1,14	14.7	<0.005	0.512
0.05	- 0.150	2.731	1,14	0.6	NS	0.034
Temp 27°C						
1.0	-13.191	27.460	1,14	10.3	<0.01	0.424
0.5	-14.040	29.483	1,14	37.2	<0.001	0.727
0.1	- 3.222	9.483	1,14	1.6	NS	0.112
0.05	- 5.307	9.948	1,14	2.3	NS	0.141
Temp 22°C						
1.0	-10.439	20.954	1,14	3.4	NS	0.193
0.5	- 2.960	9.839	1,14	2.6	NS	0.115
0.1	+ 4.372	-3.177	1,14	1.5	NS	0.099

Table 6.8 continued

(c) Ceriodaphnia cornuta

Temp 32°C Food conc	a	b	df	F	P	r
1.0	- 5.164	16.124	1,14	4.774	0.046	0.5042
0.5	- 1.9052	8.53204	1,10	1.399	NS	0.3503

(d) Daphnia lumholtzi

Temp 22°C Food conc	a	b	df	F	P	r
1.0	-15.092	16.247	1,13	36.676	0.0001	0.8592

Table 6.9 Parameters of covariance analysis comparing the regressions of fecundity against adult length at different food concentrations at 32°C. The regression coefficients are compared by the SS-STP test and the differences between the elevations by the S-N-K test. Regression coefficients, or adjusted means underlined are not significantly different at P = 0.05. Group numbers are given in ascending order of magnitude.

df = degrees of freedom; F = variance ratio; P = level of significance.

a) Diaphanasoma excisum

Comparison of the regression coefficient.

Food conc $mgC.L.^{-1}$	Group no	Regression coeff. ± SE	df	F	P	SS-STP
1.0	1	15.541 ± 4.44	4,70	0.674	0.6120	4 5 3 2 1
0.5	2	12.639 ± 2.08				
0.25	3	11.761 ± 2.23				
0.1	4	6.772 ± 3.23				
0.05	5	9.824 ± 3.55				

Comparison of the elevation of the curves.

Food conc $mgC.L^{-1}$	Group no	Adjusted mean ± SE	df	F	P	S-N-K
1.0	1	7.146	4,75	25.05	0.0001	5 4 3 2 1
0.5	2	6.802				
0.25	3	5.894				
0.1	4	3.472				
0.05	5	3.437				

continued

Table 6.9 continued

b) Moina micrura

Comparision of the regression coefficient.

Food conc $mgC.L.^{-1}$	Group no	Regression coeff ± SE	df	F	P	SS-STP
1.0	1	53.798 ± 4.42	3,60	8.273	0.0001	3 4 2 1
0.5	2	49.718 ± 4.21				
0.25	3	19.523 ± 9.06				
0.1	4	22.949 ± 5.98				

Comparision of the elevation of the curves.

Food conc $mgC.L.^{-1}$	Group no	Adjusted mean ± SE	df	F	P	SNK
1.0	1	8.883 ± 0.78	3,60	17.35	0.0001	4 3 2 1
0.5	2	7.667 ± 0.78				
0.25	3	6.289 ± 0.78				
0.1	4	4.974 ± 0.78				

Table 6.10 Mean number of eggs carried by the primiparous female (n= 4 ± SD) at different temperature-food combinations.

Temp. °C	1.0	0.5	0.25	0.1	0.05
(a) *Diaphanosoma excisum*					
22	3.75 ± 0.5	2.25 ± 0.5	-	1.75 ± 1.0	juveniles died
27	3.50 ± 0.6	2.50 ± 1.0	-	1.75 ± 0.5	1.75 ± 0.5
32	4.25 ± 1.5	6.25 ± 0.5	4.75 ± 1.0	2.50 ± 0.6	2.00 ± 1.15
(b) *Moina micrura*					
22	3.75 ± 1.7	4.25 ± 1.3	-	2.75 ± 0.5	juveniles died
27	3.75 ± 1.3	5.50 ± 1.9	-	3.25 ± 1.0	1.25 ± 0.5
32	5.00 ± 0.8	5.25 ± 1.0	3.0 ± 0.8	3.00 ± 0.8	1.75 ± 0.5
(c) *Ceriodaphnia cornuta*					
32	2.50 ± 1.0	*2.00 ± 0.0	*1.0 ± 0.0	juveniles dies	juveniles died
(d) *Daphnia lumholtzi*					
22	4.00 ± 1.4	2.67 ± 1.15	-	2.00 ± 0.00	juveniles died

* n = <4 as some animals did not attain maturity.

Table 6.11 Mean sizes (mm) of the primiparous female (± SD) at different temperature and food combinations.

Temp °C	Food concentration ($mgC.L.^{-1}$) 1.0	0.5	0.25	0.1	0.05
(a) *Diaphanosoma excisum*					
22	0.961 ± 0.03	0.911 ± 0.01		0.972 ± 0.05	juvenile death
27	0.907 ± 0.04	0.904 ± 0.04		0.875 ± 0.01	0.878 ± 0.03
32	0.929 ± 0.04	0.976 ± 0.06	0.940 ± 0.018	0.933 ± 0.01	0.875 ± 0.02
(b) *Moina micrura*					
22	0.659 ± 0.03	0.670 ± 0.04		0.658 ± 0.02	juvenile death
27	0.619 ± 0.02	0.628 ± 0.03		0.664 ± 0.02	0.638 ± 0.01
32	0.616 ± 0.01	0.638 ± 0.007	0.616 ± 0.04	0.612 ± 0.03	0.612 ± 0.03
(c) *Ceriodaphnia cornuta*					
32	0.441 ± 0.02	0.451 ± 0.02	0.446 ± 0.02		
(d) *Daphnia lumholtzi*					
22	1.159 ± 0.11	1.114 ± 0.05		1.163 ± 0.03	

Table 6.12 Parameters of the multiple regression of the size of primiparous female in relation to temperature and food concentration.

Regression equation $Y = a - bT - cF$ where y = size of primipara (mm)
T = temperature (°C), F = food concentration (mg. $C.L^{-1}$).
df = degrees of freedom; F = variance ratio; P = level of significance; r = correlation coefficient.

Moina micrura

a	b	c	df	F	P	r
0.760	0.00434	0.0085	2,45	10.05	0.005	0.556

F value due to Temperature 19.449
F value due to food concentration 0.6629

Simple linear regression relating primipara size with temperature in Moina micrura
$Y = 0.753 - 0.00421\ T$ P value = 19.594 r = 0.547

Table 6.13 Mean sizes (mm.) of the neonates ± SD (mean value of total neonates produced at all temperature- food combinations in 4 replicates) at different temperature and food concentrations.

Temp °C	Food Concentration ($mgC.L^{-1}$) 1.0	0.5	0.25	0.1	0.05
(a) *Diaphanosoma excisum*					
22	0.521 ± 0.006	0.526 ± 0.005		0.497 ± 0.006	juveniles died
27	0.505 ± 0.007	0.489 ± 0.017		0.503 ± 0.014	0.468 ± 0.008
32	0.510 ± 0.016	0.523 ± 0.011	0.533 ± 0.004	0.509 ± 0.023	0.503 ± 0.012
(b) *Moina micrura*					
22	0.440 ± 0.004	0.430 ± 0.014		0.430 ± 0.013	juveniles died
27	0.433 ± 0.009	0.437 ± 0.020		0.441 ± 0.006	0.390 ± 0.06
32	0.428 ± 0.004	0.441 ± 0.005	0.429 ± 0.021	0.450 ± 0.023	0.403 ± 0.02
(c) *Ceriodaphnia cornuta*					
32	0.260 ± 0.005	0.262 ± 0.010	0.261 ± 0.004	juveniles died	juveniles died
(d) *Daphnia lumholtzi*					
32	0.533 ± 0.007	0.532 ± 0.012		0.580 ± 0.22	juveniles died

Table 6.14 Initial reproductive rate (eggs per female per day ± SD) up to the end of the fourth brood of *Diaphanosoma excisum* and *Moina micrura* at different temperature-food combinations and for *Ceriodaphnia cornuta* at 32°C and three different food concentrations.

Temp °C	Food Concentration ($mgC.L^{-1}$)				
	1.0	0.5	0.25	0.1	0.05
Diaphanosoma excisum					
22	1.145 ± 0.037	1.055 ± 0.120		0.206 ± 0.061	juveniles died
27	2.446 ± 0.309	1.587 ± 0.683		0.837 ± 0.194	0.209 ± 0.090
32	5.011 ± 0.786	4.651 ± 0.278	3.599 ± 0.159	1.267 ± 0.127	0.813 ± 0.139
Moina micrura					
22	1.315 ± 0.511	1.377 ± 0.354		0.734 ± 0.182	juveniles died
27	3.527 ± 0.405	4.217 ± 1.132		1.813 ± 0.546	0.556 ± 0.189
32	8.846 ± 0.806	6.983 ± 0.310	4.803 ± 0.674	2.972 ± 0.320	1.029 ± 0.171
Ceriodaphnia cornuta					
32	1.573 ± 0.096	1.231 ± 0.407	0.486 ± 0.12	juveniles died	juveniles died

CHAPTER 7

MORPHOLOGY OF THE FILTERSTRUCTURE

Although the morphology of the filtering limbs of the four species of Cladocera studied is not of direct relevance to the main subject matter of this thesis, some of the differences which appeared in the life-cycle experiments might arise from differences in the capabilities of some of the species to filter off the alga, Scenedesmus acutus which was used as food. It was therefore decided to check this by investigating the morphology of the filtering limbs.

During the experiments conducted at different combinations of temperature and food concentration, it was found that Ceriodaphnia cornuta and Daphnia lumholtzi were unable to complete their life cycle to maturity in low food concentrations such as 0.1 $mgC.L^{-1}$, although Moina micrura and Daphanosoma excisum were able to do so. On the other hand, Ceriodaphnia cornuta did manage to attain maturity when reared at 22°C on a food medium of bacteria, though numbers of animals in the culture were low, (page 94) but not when reared at the same temperature in high algal densities (page 94) These differences might be caused by structural differences (filtering area and intersetular distance) in their filtering combs.

There were other reasons for investigating filter limb morphology of these species. This has not been studied at all for tropical species apart from Daphnia carinata from tropical

Australia (Ganf and Shiel, 1985a,b). Tropical zooplankton are known to be smaller in size than temperate forms (Fernando, 1980a) and this is also the case with planktonic cladocerans from Sri Lanka (Fernando, 1980b). The smallest temperate cladoceran whose filter limb structure has been investigated is Ceriodaphnia quadrangula, the rest being larger in size. The small size of tropical species may be an adaptation to the greatly reduced viscosity of water at high tropical temperatures (2.5 times less at 35°C than at 5°C) as it could offset the enhanced sinking rate in low viscosity water (Hutchinson, 1967; Vogel, 1981). Viscosity will also affect the functioning of the filtering limbs since viscosity and density are important components of Reynolds Number and there may be changes in length characteristic (eg. intersetal and intersetular distances) to counteract viscosity changes. This is a more fundamental point concerning the physical constraints imposed upon tropical filter-feeders.

Other reasons for studying filter-structures is that so far there is no information in the literature about the Moinidae in relation to their filter structure. Nothing is known about whether Moina micrura has filtering limbs similar in ultra-structure or filtering area to species of the Daphnidae, the other Anamopoda cladoceran family studied. There is also no information available in the literature about the relationship between filtering area and body size for the ctenopod cladocerans and this was established for Diaphanosoma excisum (Sididae).

The limbs investigated were chosen because of the presence of what are called filtering combs (Fig. 3.3a). These were on limbs III and IV in Daphnia lumholtzi, Ceriodaphnia cornuta and Moina micrura and limbs I to V of Diaphanosoma excisum. Two characteristics were measured: (1) filtering area of the actual comb (Fig. 3.3a) and inter-setular distance (Fig. 3.3b). After dissecting out both right and left limbs of fresh animals (preserved in the case of Moina micrura), the area of the comb of a mounted limb was drawn to scale using a Leitz drawing tube, and later the drawn area was measured on a Graf pad. The inter-setular distance was measured on enlargements of micrographs of known magnification which were produced by a Cambridge S-400 scanning electron microscope. Full details of each procedure are given in Chapter 3.

7.1 AREA OF FILTERING COMBS

As previous workers (Egloff and Palmer, 1971; Kořínek and Machacek, 1980; Arunda, 1983; Brendelburger and Geller, 1985; Ganf and Shiel ; 1985a) had found that the area of the filter combs increased with body size in (mainly) daphnid species, the appropriate limbs were dissected out from as wide a range of body sizes as possible in order to determine whether a similar relationship existed for the four tropical cladoceran species in this study. As can be seen from Fig. 7.1, about 6-14 sizes were investigated using 26-32 specimens and therefore approximately 300 limbs of Diaphanosoma excisum and 150 limbs from each of the other three species were measured. Sizes were replicated where possible. The mean values and standard deviations of the comb area, summed for both (right and left) limbs, are plotted in Fig. 7.1.

Several mathematical equations were fitted to the experimental data but the best fitting relationship between comb area (Y) and body length (X) was the power equation calculated as a linear regression with ln transformed values. Table 7.1 presents the linear regressions, ln transformed data for comb area, and body length data both for separate pairs of limbs and for all the limbs. As can be seen from the regression statistics in Table 7.1, all regressions were statistically significant and Fig 7.1 illustrates how the regression curve fits the empirical data.

The relationships between total comb area and body length, in these four species of cladocerans, were compared by covariance analysis the results of which are presented in Table 7.2. A comparision of regression coefficients shows that the coefficient for Daphnia lumholtzi is significantly smaller than Ceriodaphnia cornuta and the rest are not significantly different from each other (Table 7.2). Comparison of elevations gives evidence that Diaphanosoma excisum of a particular length have the largest area, followed by Daphnia lumholtzi, Moina micrura and Ceriodaphnia cornuta respectively and they differ from each other significantly.

A comparison was made of the filtering area of both limbs of each thoracic appendage, of a 0.7 mm animal belonging to each of the four species. The results are given in Table 7.3a. The values for Ceriodaphnia cornuta have to be considered with caution as this species never attained 0.7 mm in the field or in the experiments but to choose an even smaller size would be

unrealistically small for the other species. The comb area per limb varies in the four species, being largest in Daphnia lumholtzi, then Ceriodaphnia cornuta, Moina micrura and smallest in Diaphanosoma excisum. However, the picture is different when the total comb area is considered. Diaphanosoma excisum with its comb structure on five thoracic limbs has 1.5 times greater filtering area than Daphnia lumholtzi and almost double the area of the other two species.

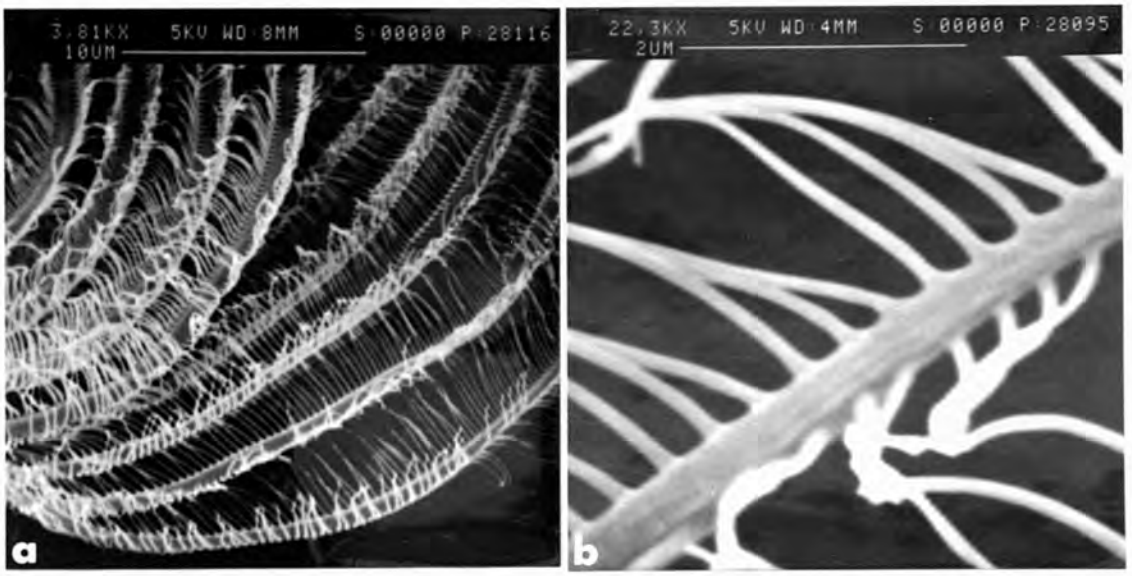
Table 7.3b, which shows the contribution of each pair of limbs to the total area, indicates that in anomopods the third limb contributes two-thirds of the total area. In the ctenopod (Diaphanosoma excisum) each pair of the first four limbs contributes 20-30% of total area while the fifth pair contributes a comparatively small (6%) proportion on the total.

7.2 INTER-SETULAR DISTANCE

Electron-micrographs of one comb from each species under two magnifications are given in Plate 4. The inter-setular distance measured from the micrographs are plotted against body size in Fig 7.3 and mean values given in Table 7.4. These show that the distance increases with increased body size in all species except Ceriodaphnia cornuta. Ceriodaphnia cornuta is also somewhat exceptional in the reduced variance about the mean values. A significant allometric relationship can be obtained to fit the data for the other three species as is shown in Table 7.5. No significant relationship was found in Ceriodaphnia cornuta ($df = 1,6$; $F = 0.8$; $P = NS$). Although Daphnia lumholtzi,

Plate 4. Electron micrographs of a) a portion of the filter comb and b) a seta of the comb of each species studied.

Diaphanosoma excisum



Moina micrura

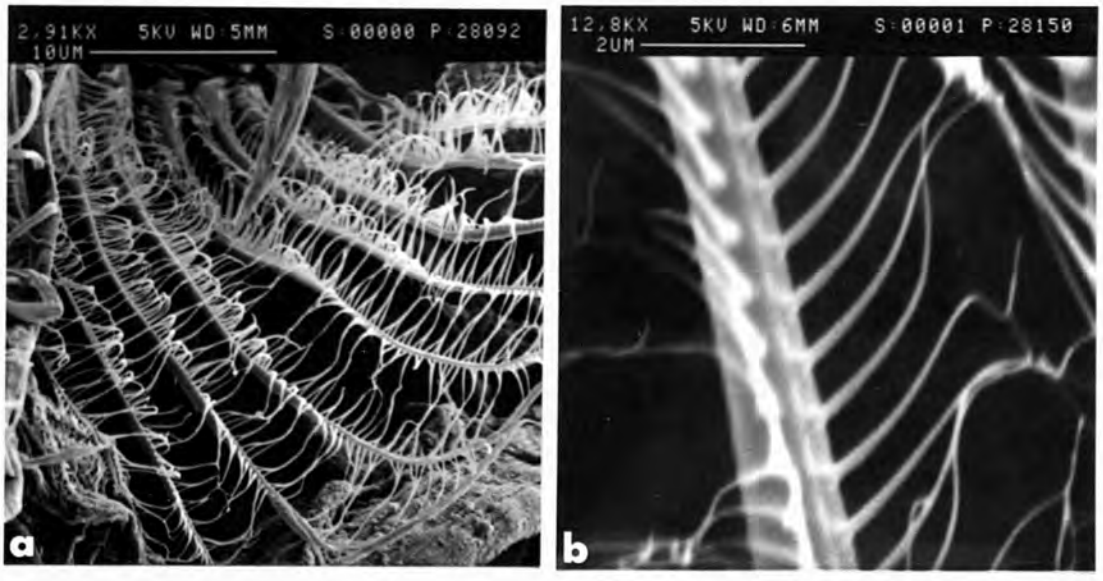
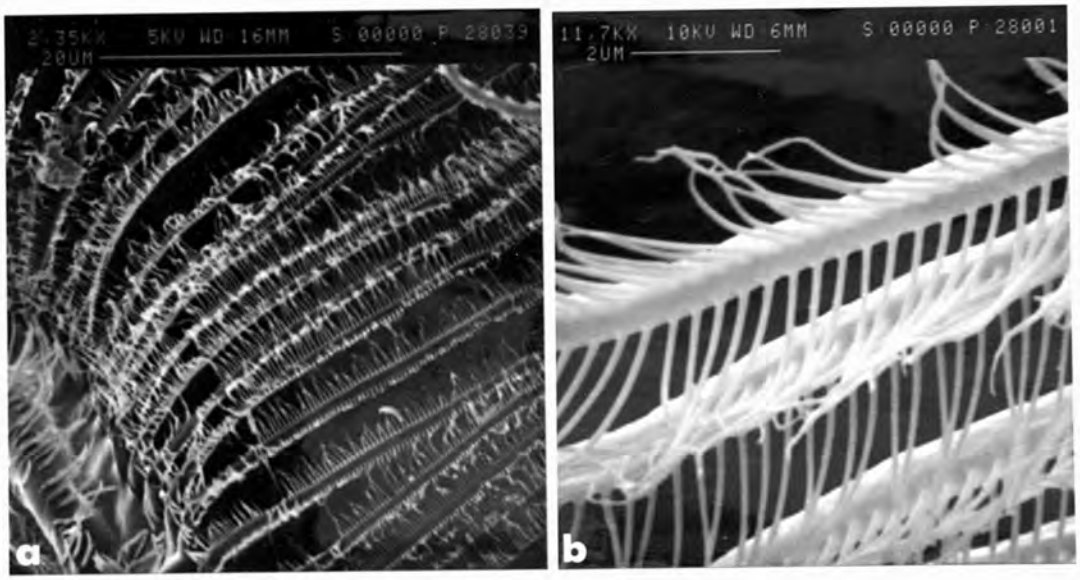
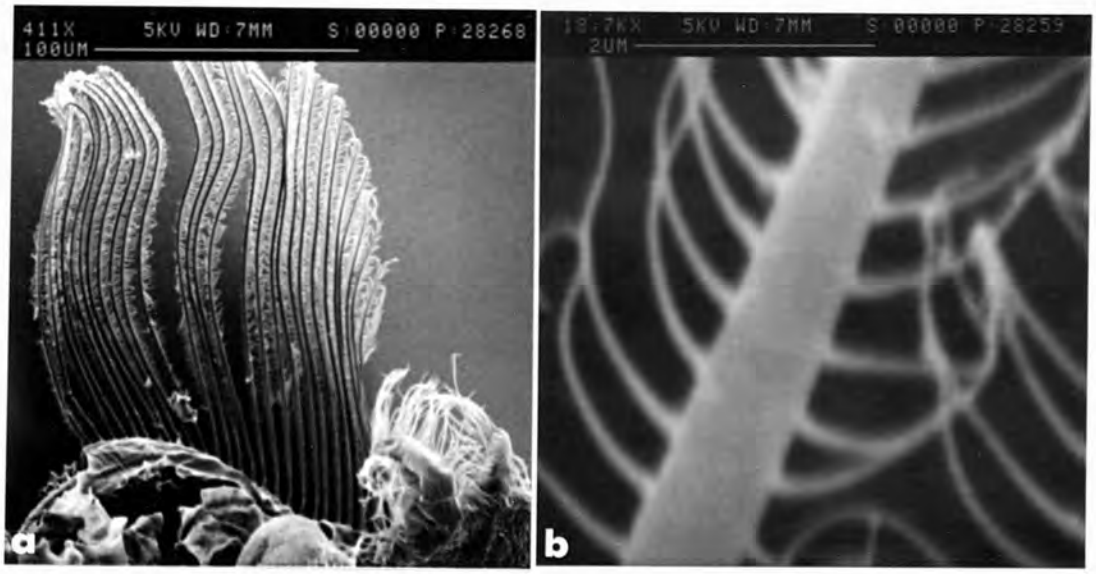


Plate 4 continued.

Daphnia lumholtzi



Ceriodaphnia cornuta

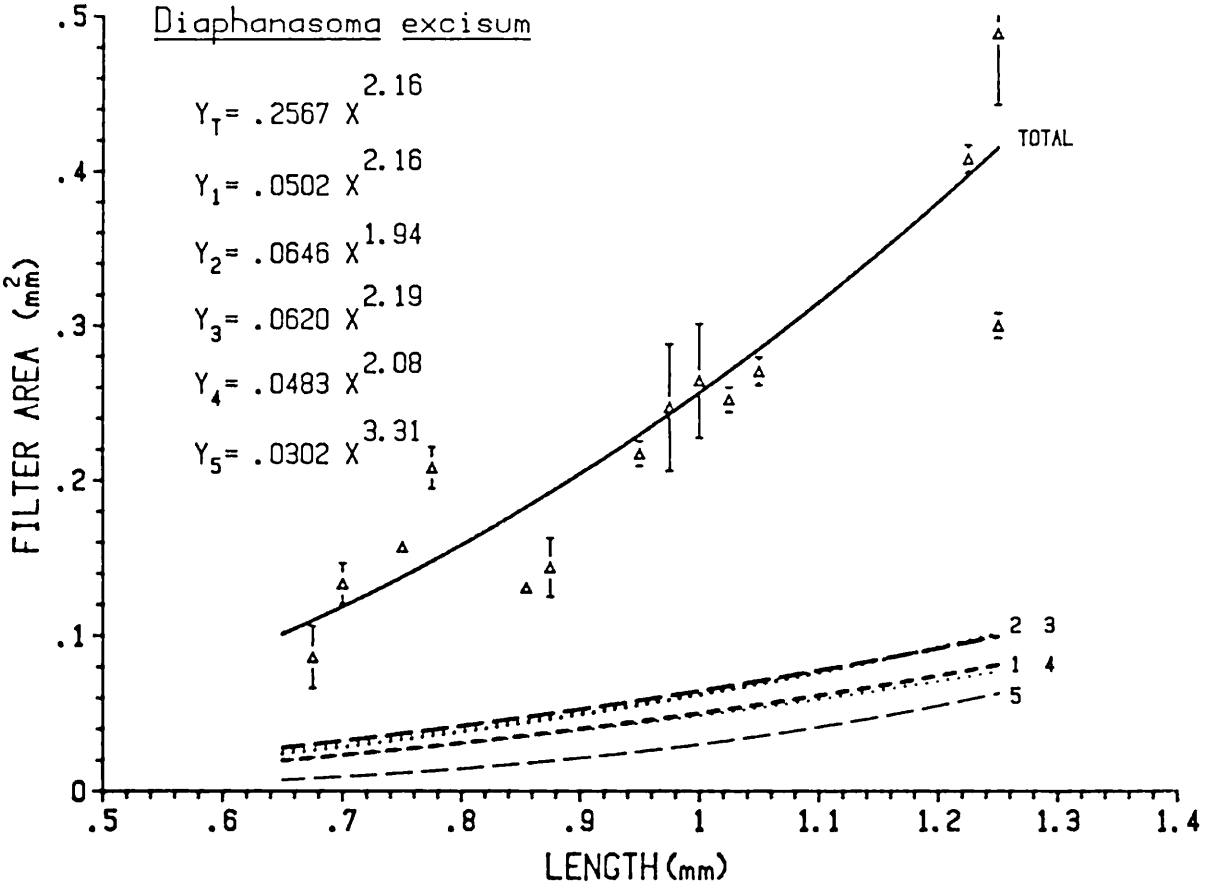


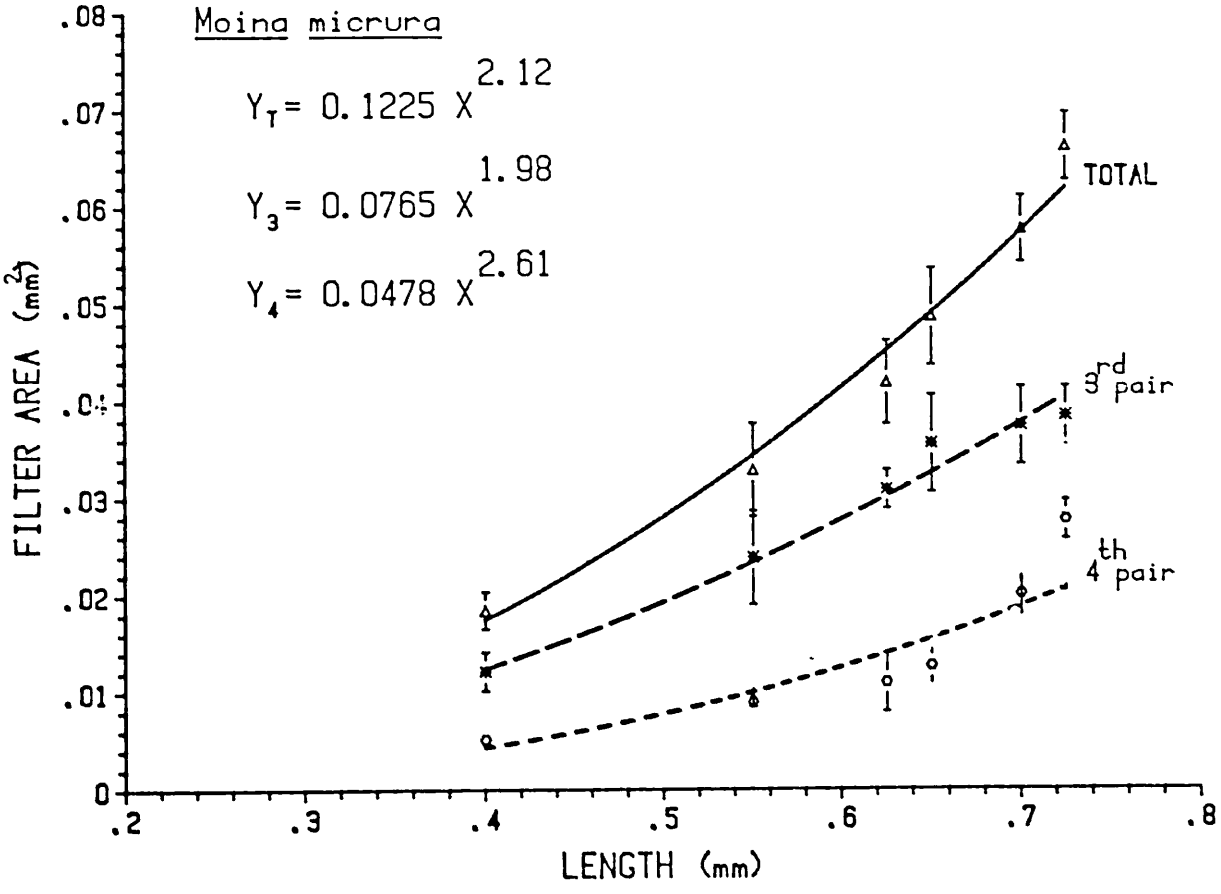
Moina micrura and Diaphanosoma excisum did show an increase in spacing with body length, it should be noted that this increase is within a very limited range, from 0.170 ± 0.008 - 0.416 ± 0.047 μm (Table 7.4.). But even within this small range Moina micrura shows a sharp increase related to the square of the body length.

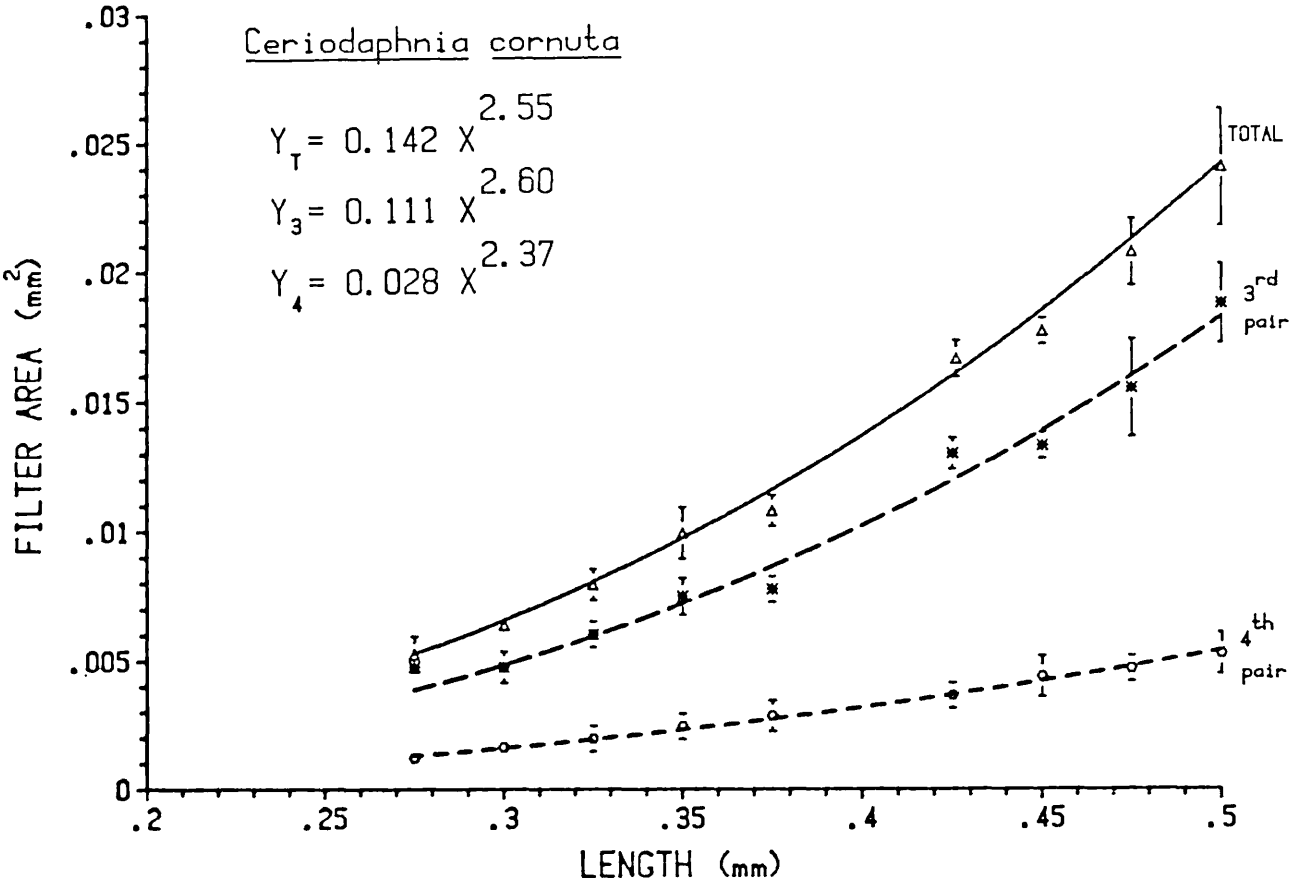
No significant relationship was found between inter-setular distance and filtering area in Ceriodaphnia cornuta (df 1,6; F = 0.5; P = NS) while the significant relationships which were found in the other three species are given in Table 7.6. Hence in these three species inter-setular distance increases as the comb increases with increase in body size while in Ceriodaphnia cornuta the inter-setular distance does not increase as comb area area increases with increase in body size but is the same in all body sizes.

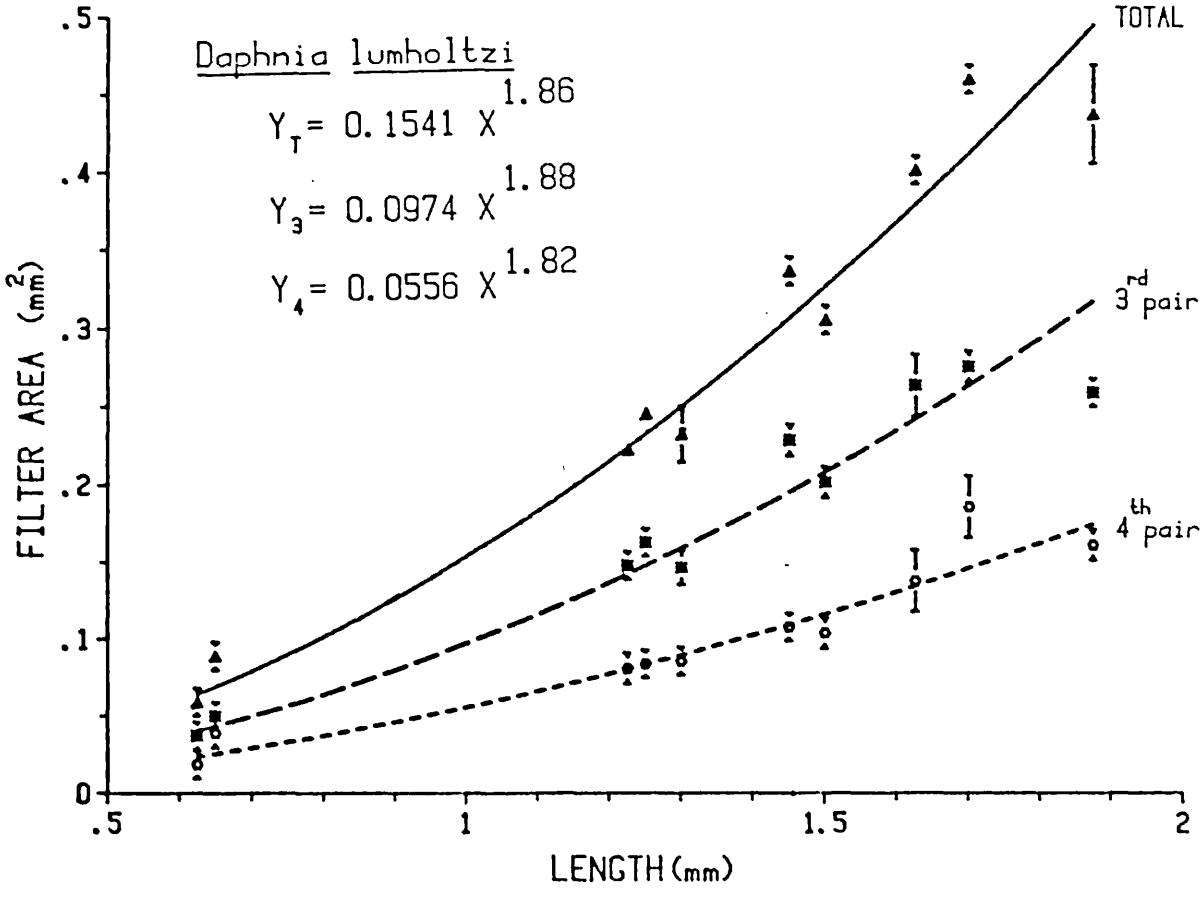
According to these present findings it seems that in Ceriodaphnia cornuta the length of the seta which carry setules does not grow. Perhaps growth takes place at the tips and new setules are added, thus keeping intra-setular distance more or less the same irrespective of increase in filtering area. In contrast, as filtering area increases in the other three species the already existing setal length, which carries the setules, seems to grow and they thus become more widely spaced with age.

Fig. 7.1 Mean values and standard deviations of the comb area summed for both (right and left) limbs and total filtering area in relation to body length of Diaphanosoma excisum, Moina micrura, Daphnia lumholtzi and Ceriodaphnia cornuta.
The line indicates the best fitting line, given in Table 7.1.


Diaphanasoma excisum
$Y_T = .2567\ X^{2.16}$
$Y_1 = .0502\ X^{2.16}$
$Y_2 = .0646\ X^{1.94}$
$Y_3 = .0620\ X^{2.19}$
$Y_4 = .0483\ X^{2.08}$
$Y_5 = .0302\ X^{3.31}$
TOTAL
2 3
1 4
5
FILTER AREA (mm^2)
LENGTH (mm)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
1.1
1.2
1.3
1.4


Moina micrura
$Y_T = 0.1225\ X^{2.12}$
$Y_3 = 0.0765\ X^{1.98}$
$Y_4 = 0.0478\ X^{2.61}$
TOTAL
3rd pair
4th pair
FILTER AREA (mm^2)
LENGTH (mm)
0
.01
.02
.03
.04
.05
.06
.07
.08
.2
.3
.4
.5
.6
.7
.8


Ceriodaphnia cornuta
$Y_T = 0.142\ X^{2.55}$
$Y_3 = 0.111\ X^{2.60}$
$Y_4 = 0.028\ X^{2.37}$
TOTAL
3rd pair
4th pair
FILTER AREA (mm²)
LENGTH (mm)
.03
.025
.02
.015
.01
.005
0
.2
.25
.3
.35
.4
.45
.5


Daphnia lumholtzi
$Y_T = 0.1541\ X^{1.86}$
$Y_3 = 0.0974\ X^{1.88}$
$Y_4 = 0.0556\ X^{1.82}$
TOTAL
3rd pair
4th pair
FILTER AREA (mm²)
LENGTH (mm)
.5
.4
.3
.2
.1
0
.5
1
1.5
2

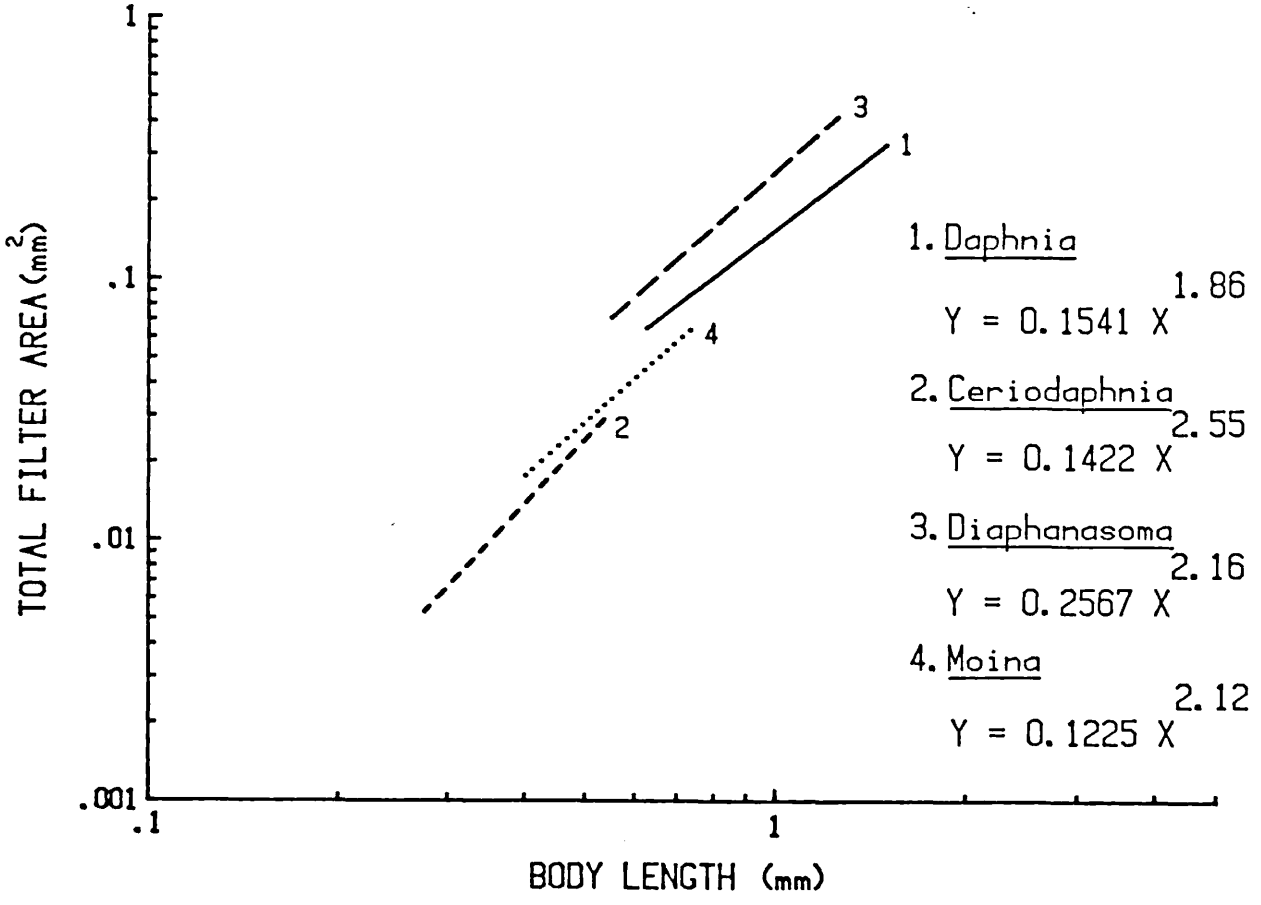


Fig. 7.2 Relationship between total comb area to body length, plotted on logarithmic axes for Diaphanosoma excisum, Moina micrura, Daphnia lumholtzi and Ceriodaphnia cornuta.

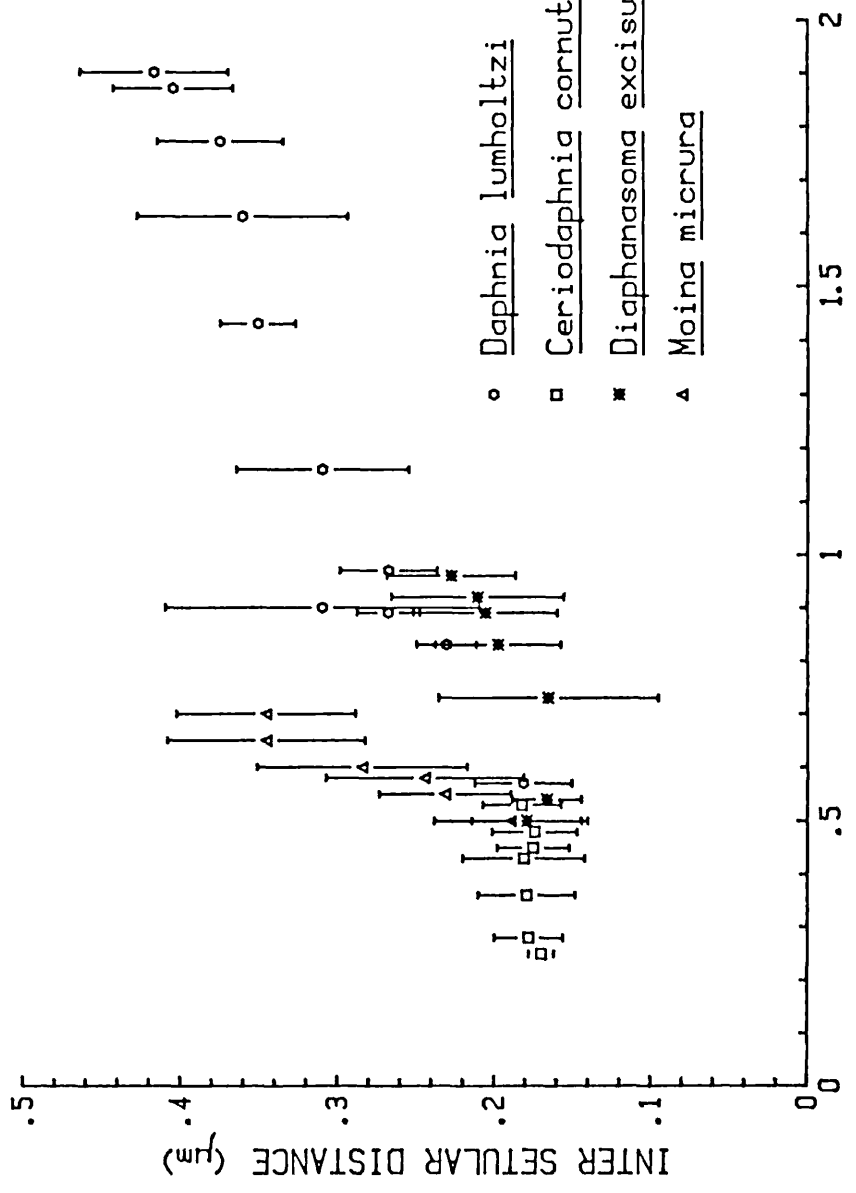


Fig. 7.3 Mean inter-setular distance and standard deviation in relation to body length of Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi.

Table 7.1 Curvilinear regressions relating the total filter area and filter area of each pair of limbs to body length in Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi.

Regression equation $\ln Y = \ln a + b \ln X$
Y = filter area in mm, X = body length in mm
df = degrees of freedom; F = variance ratio;
r = correlation coefficient; P = level of significance.

Limb	lna	b	df	F	r	P
(a) Diaphanosoma excisum						
1st pair	-2.9917	2.16	1,30	44.7	0.774	<0.0001
2nd pair	-2.7395	1.94	1,30	71.7	0.840	<0.0001
3rd pair	-2.7806	2.19	1,30	64.4	0.856	<0.0001
4th pair	-3.0303	2.08	1,30	38.0	0.748	<0.0001
5th pair	-3.4999	3.31	1,30	85.1	0.861	<0.0001
TOTAL	-1.3598	2.16	1,30	106.1	0.883	<0.0001
(b) Moina micrura						
3rd pair	-2.5705	1.98	1,24	231.0	0.952	<0.0001
4th pair	-3.0407	2.61	1,24	94.7	0.889	<0.0001
TOTAL	-2.0996	2.12	1,24	427.0	0.972	<0.0001
(c) Ceriodaphnia cornuta						
3rd pair	-2.1982	2.60	1,32	937.0	0.990	<0.0001
4th pair	-3.5756	2.37	1,32	952.0	0.983	<0.0001
TOTAL	-1.9519	2.55	1,32	729.0	0.979	<0.0001
(d) Daphnia lumholtzi						
3rd pair	-2.3289	1.88	1,28	906.0	0.906	<0.0001
4th pair	-2.8896	1.82	1,28	333.0	0.960	<0.0001
TOTAL	-1.8702	1.86	1,28	753.0	0.982	<0.0001

Table 7.2 Covariance analysis of the regressions comparing the filtering area with the body length in Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi. The difference between regression coefficients were tested by the SS-STP test and the difference between the elevations of the curves by the S-N-K test. The regression coefficients and means underlined are not significantly different at P = 0.05 level. The group numbers are given in ascending order of magnitude.

Regression equation $\ln Y = \ln a + b \ln X$
Y = total filter area in mm^2; X = body length in mm
df = degrees of freedom; F = variance ratio; P = level of significance

(a) Comparison of regression coefficient

Species	Group no.	Regression coeff ± SE	df	F	P	SS-STP Test
Daphnia	1	1.86 ± 0.07	3,114	8.455	<0.0001	1 4 3 2
Ceriodaphnia	2	2.55 ± 0.06				
Diaphanosoma	3	2.16 ± 0.07				
Moina	4	2.12 ± 0.11				

(b) Compariaon of elevation

Species	Group no	Adjusted mean ± SE	df	F	P	S-N-K Test
Daphnia	1	-2.64 ± 0.08	3,118	155.4	<0.0001	2 4 1 3
Ceriodaphnia	2	-3.19 ± 0.08				
Diaphanosoma	3	-2.11 ± 0.07				
Moina	4	-2.88 ± 0.08				

Table 7.3a The filtering or comb area of each pair of limbs predicted for a 0.7 mm animal of the four species calculated from the regressions given in Table 7.1. The percentages in parenthesis are given in relation to that of Daphnia lumholtzi which is assumed to be of 100%. Filtering area is given in mm^2.

Limb	Ceriodaphnia	Moina	Diaphanosoma	Daphnia
1st pair			0.0232	
2nd pair			0.0323	
3rd pair	0.0439 (88%)	0.0378 (76%)	0.0284 (58%)	0.0498 (100%)
4th pair	0.0120 (41%)	0.0188 (65%)	0.0230 (79%)	0.0291 (100%)
5th pair			0.0093	
TOTAL	0.0559 (77%)	0.0566 (72%)	0.1160 (147%)	0.0789 (100%)

Table 7.3b Percentage contribution of each pair of limbs to the total filtering area of a 0.7 mm_2animal. The percentages are given in parenthesis. Filtering area is given in mm^2.

Limb	Ceriodaphnia	Moina	Diaphanosoma	Daphnia
1st pair			0.0232 (20%)	
2nd pair			0.0323 (28%)	
3rd pair	0.0439 (71%)	0.0378 (67%)	0.0284 (24%)	0.0498 (63%)
4th pair	0.0120 (29%)	0.0188 (33%)	0.0230 (20%)	0.0291 (37%)
5th pair			0.0093 (6%)	
TOTAL	0.0559 (100%)	0.0566 (100%)	0.1160 (100%)	0.0789 (100%)

Table 7.4 Measurements of the inter-setular distance of the four species.
L = body length in mm. ISD = inter-setular distance in µm, n = number of measurements, N = number of animals investigated. X = mean.

L	ISD (mean ± SD)	n	N	SD/X%
Ceriodaphnia cornuta				
0.25	0.170 ± 0.008	10	2	4.7
0.28	0.178 ± 0.022	12	2	12.4
0.36	0.179 ± 0.031	30	4	17.3
0.43	0.181 ± 0.039	34	5	21.6
0.45	0.175 ± 0.023	28	5	13.1
0.48	0.174 ± 0.027	42	6	15.5
0.53	0.183 ± 0.025	40	5	13.7
Moina micrura				
0.50	0.189 ± 0.059	20	2	31.2
0.55	0.231 ± 0.042	77	6	18.2
0.58	0.244 ± 0.063	73	7	25.8
0.60	0.284 ± 0.067	46	4	23.6
0.65	0.345 ± 0.063	94	8	18.3
0.70	0.345 ± 0.057	57	5	16.5
Diaphanosoma excisum				
0.50	0.179 ± 0.035	150	6	19.6
0.54	0.116 ± 0.022	25	2	19.0
0.73	0.165 ± 0.070	51	4	42.4
0.83	0.197 ± 0.040	109	8	20.3
0.89	0.205 ± 0.046	88	5	22.4
0.92	0.210 ± 0.055	93	5	26.2
0.96	0.227 ± 0.041	70	4	18.1
Daphnia lumholtzi				
0.57	0.181 ± 0.031	157	7	17.1
0.83	0.230 ± 0.019	49	5	8.3
0.89	0.267 ± 0.020	53	7	7.5
0.97	0.267 ± 0.031	38	3	11.6
0.90	0.319 ± 0.111	40	5	34.8
1.16	0.309 ± 0.055	40	4	17.8
1.63	0.360 ± 0.067	137	6	18.6
1.43	0.370 ± 0.024	40	4	6.5
1.77	0.374 ± 0.040	40	5	10.7
1.87	0.344 ± 0.038	40	4	11.0
1.90	0.416 ± 0.047	40	2	11.3

Table 7.5 Curvilinear regressions relating the mean inter-setular distance to the body length of Daphnia lumholtzi, Diaphanosoma excisum and Moina micrura.

Regression equation lnY = lna + blnX
Y = inter-setular distance in μm, X = body lenght in mm.
df = degrees of freedom; F = variance ratio; r = correlation coefficient;
P = level of significance.

Species	lna	b	df	F	r	P
Daphnia	-1.2765	0.617	1,9	84.0	0.951	<0.0001
Diaphanosoma	-1.5465	0.370	1,5	8.5	0.795	0.03
Moina	-0.3065	1.940	1,4	75.0	0.974	<0.001

Table 7.6 Curvilinear regressions relating mean inter-setular distance to the mean filtering area of Daphnia lumholtzi, Diaphanosoma excisum and Moina micrura.

Regression equation lnY = lna + blnX
Y = inter-setular distance in μm, X = filtering area in mm^2.
df = degrees of freedom; F = variance ratio; r = correlation coefficient
P = level of significance.

Species	lna	b	df	F	r	P
Daphnia	-0.5763	0.368	1,9	50.0	0.92	<0.0001
Diaphanosoma	-1.3020	0.175	1,5	8.3	0.79	0.03
Moina	-1.6195	0.918	1,4	74.0	0.97	<0.001

CHAPTER 8

APPLICATION OF THE EXPERIMENTAL RESULTS TO FIELD CONDITIONS

The results of the experiments reported here (Chapter 4-6) clearly indicate that both temperature and food concentration do have an influence on life-cycle parameters. Thus, before applying the experimental results to field conditions in order to make estimates of production, information on the water temperature and the amount of edible food available to the animal in the reservoir water must be ascertained. Measuring the water temperature is simple, but how to make direct measurements of the quantity of edible food is not yet resolved. The trophic status of a water body is frequently expressed in terms of chlorophyll "a" concentration, number of algal cells or particulate organic matter per unit volume, but this does not indicate the quantity of edible food available to a particular herbivorous species at a particular time.

Duncan (1985) critically reviewed various ways that have been adopted for measuring the amount of edible algal or sestonic biomass available to herbivores in natural waters. One technique is size-fractioning the particles in water by filtration through different meshes which are chosen, more or less appropriately, from knowledge of what sizes and shapes of particles the feeding mechanism of the studied herbivore is capable of capturing; the particles in the filtrate are then measured. Another method involves selecting from phytoplankton counts those algal species

known to be edible from previously analysed gut contents of the herbivore or from food selection experiments; the biomass of these can be calculated either from cell volumes and densities or from cell carbon-cell volume relationships which are available for freshwater algae. She suggests that neither method is wholly satisfactory for estimating the quantity of edible food for a particular species. The mechanical seiving of the former method may collect sizes and shapes of particles which a particular species cannot accept, thus over-estimating the field food level. The latter technique will miss out the non-living detrital particles and the bacteria which may be abundantly present in some waters and which are taken by some herbivores. Nevertheless, these seem to be the best methods available at present for direct measurement of field food levels.

Duncan (1985) goes on to suggest an indirect method which could indicate the food levels at which field populations are operating and which could be done simultaneously with the above techniques. From long-term life cycle growth experiments with Daphnia species conducted under defined conditions of food and temperature, she found that length-carbon weight relationships changed in elevation according to the amount of food available. The decrease in regression elevation is associated with food limiting concentrations, which are temperature dependent. A comparison of field-derived length-carbon weight regressions with these experimental ones can indicate indirectly the field food level available to the species. In addition, she suggested the possibility of using the same relationship to distinguish between

the effects of predation and food limitation due to low food levels and presence of interfering algae or less nutritive non-algal food. It is known that the size structure of a population may be dependent on predator pressure in addition to the effects of factors such as food.

Information is available in recent literature concerning length-carbon weight relationships of both experimental (Lampert, 1977b) and field (Butorina, 1973) populations of temperate cladoceran species (Daphnia pulex and Polyphemus pediculus, respectively). These studies do not give any comparison between field and experimental relationships. Rocha (1983) found significant seasonal variation in length-carbon weight relationships in Daphnia magna, Daphnia pulicaria and Daphnia hyalina co-existing in a London reservoir (Wraysbury reservoir) and Duncan et.al. (1985) established a relationship between length and carbon weight of animals grown at very low food levels. Duncan (1985) made the first attempt to compare these relationships for animals grown under defined food and temperature conditions with those of the naturally occurring animals in Lake Washington (USA) and, in doing so, established when the animals are food limited in the lake. An attempt was made to apply this approach in the present study to evaluate whether the cladoceran species co-existing in Kalawewa reservoir are food limited. Four visits were made to the reservoir during which chlorophyll a, sestonic carbon and the length:carbon-weight relationships of the cladocera were measured (Chapter 3).

8.1 CHLOROPHYLL A AND THE CARBON CONTENT OF THE KALAWEWA RESERVOIR

The chlorophyll a and sestonic carbon content of the water were measured at the time of the field collections (Table 8.1) (a) as the total amount in the water and (b) the quantity due to particles less than 33μm. This mesh size was chosen as 33μm is the maximum particle size that the four species studied could ingest in relation to their body size, according to the relationship established by Burns (1969) for temperate Daphnid species. Using the relationship between chlorophyll a concentration and carbon given by Talling (1984), the concentration of chlorophyll a obtained was converted to carbon by multiplying by a factor of 30. Non-algal carbon was computed by subtracting this calculated algal carbon from the total sestonic carbon measured. During May and August 1984, the measurements were made using water from about half a metre depth and the results are presented in Table 8.1, each value being the mean of five measurements. In November 1984 and February 1985, a series of depths were sampled at 0.5m intervals with two replicates at each depth. The mean value for the water column is also given in Table 8.1.

The results in Table 8.1 show changes in the amounts of sestonic carbon present in Kalawewa Reservoir during these four months and are particularly interesting for the edible (less than 33μm) fraction. As algal carbon, this fraction exceeded $0.5 mgC.L^{-1}$ for all months except May, when it was rather low. The same is true for the carbon levels of the non-algal fraction,

that it exceeded 0.5 $mgC.L^{-1}$ except in May. In May, therefore, there is a likelihood of food limitation not only because the summed algal and non-algal carbons only just attain 0.5 $mgC.L^{-1}$ in the edible (less than 33μm) fraction but also 73% of the edible seston consists of less nutritive detrital particles. During the other months, there is three to four times more edible carbon present and it exceeds 1.0 $mgC.L^{-1}$. However, during August 1984 and February 1985, as in May 1984, most of the edible biomass consisted of non-algal carbon, thus indicating a less nutritive situation than in November 1984.

The percentage of non-algal carbon in relation to sestonic carbon, given in parenthesis in Table 8.1, shows that in May and February the non-algal component of the sestonic carbon was greater than on the other two dates. It is also evident that the non-algal contribution to the total carbon content of the particles <33μm is about 40-75.%

8.2 LENGTH:CARBON-WEIGHT RELATIONSHIPS OF FIELD ANIMALS

The animals were collected from the field on a number of occasions (Table 3.4) and sorted out into different size classes. Their body carbon content was then measured using the dichromate wet-oxidation method. The details of the sampling times are given in Table 3.4 and the number of animals used in each sample as well as their carbon content is given in Appendix 4

During all sampling occasions it was noted that all the field animals, except Ceriodaphnia cornuta, were internally parasitized. In Ceriodaphnia cornuta, external growths were

found on the carapace but it did not seem to interfere with reproduction as these animals carried eggs. These infected animals were not used for carbon determination. The other three species had a unicellular internal parasite common to all three species. These parasites were found exactly in the position of the ovary and gradually extending to other parts of the body. A photograph of a parasitized Moina micrura is given in Plate 2 (page 63). No eggs were found in these parasitized animals and they were not used for carbon determination. Another parasite was found in Daphnia lumholtzi circulating in the body. Very few of these parasitized animals carried eggs but they were also rejected for carbon determination. Diaphanosoma excisum collected in May 1984 were subjected to the wet oxidation (modified micro) method and most of the samples were less than 10μgC sample^{-1} (Appendix 4). As this is the minimum carbon content required for this method, it was not possible to take this set of results into consideration especially since the lack of proper results in the lower size category would affect the regression relationship. This set of results did, however, serve to provide a proper judgement regarding the number of field animals required for the carbon analysis in each size category.

The length:carbon-weight relationships computed for sampling occasions are given in Table 8.2* and all were found to be highly significant. An attempt was made to compare the field regressions for these four species with those obtained experimentally, as given in Chapter 4, by analysis of covariance. Comparison of the November and February regressions with each

* and in Fig. 8.1

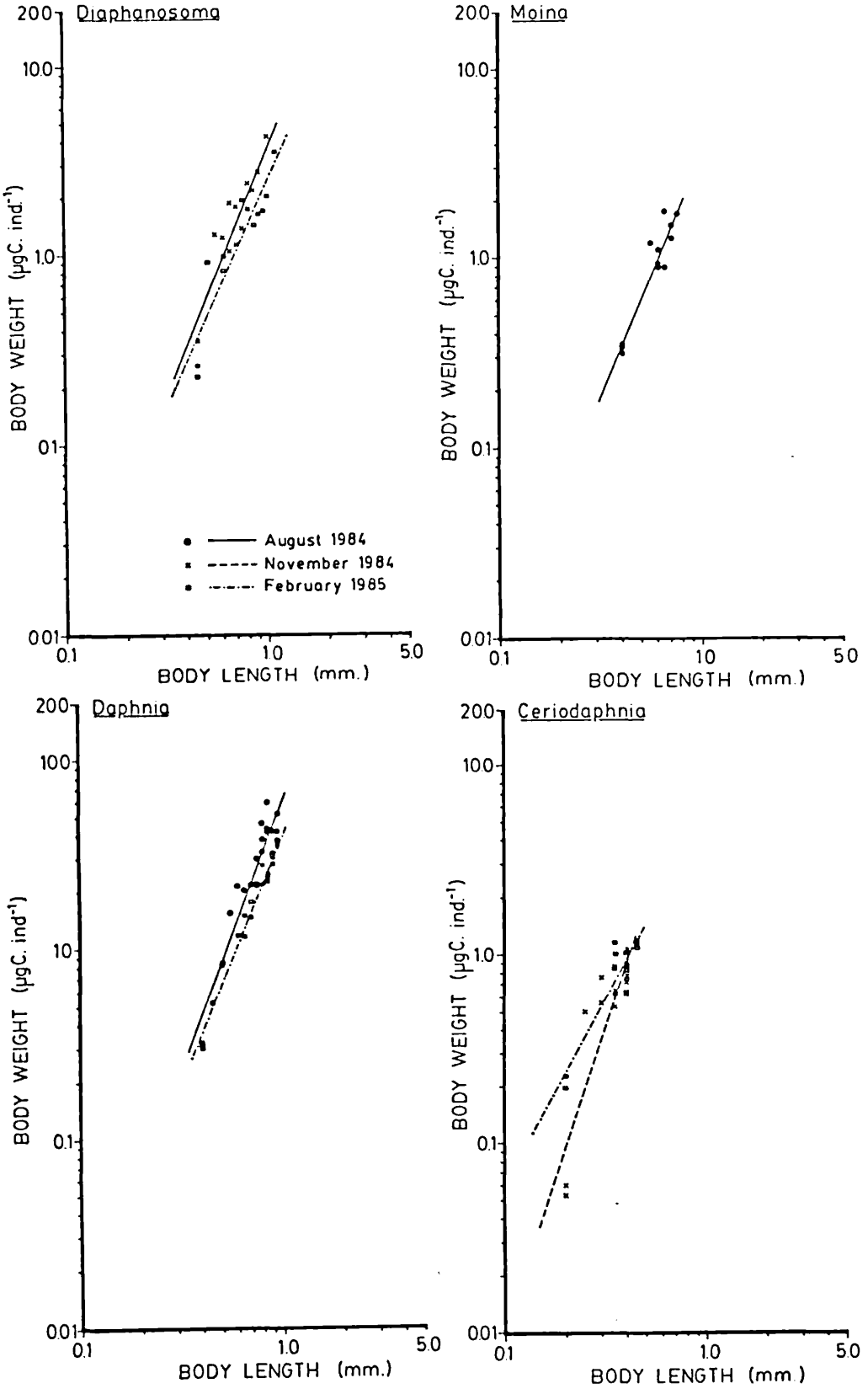


Figure 8.1 Body weight (µgC. ind^{-1}) plotted against body length (mm) on logarithmic axes for animals collected from the Kalawewa reservoir. The statistics for the regression lines are given in Table 8.1.

other and with the experimental regressions for Diaphanosoma excisum obtained when grown at in food concentrations of 1.0 $mgC.L^{-1}$ and 0.25 $mgC.L^{-1}$ is given in Table 8.3. From this table it is evident that there are no significant differences between the slopes and elevations of the November field regressions and the 1.0 $mgC.L^{-1}$ experimental regressions. This suggests that in November the animals in the reservoir were not food limited as it has been shown in previous chapters that 1.0 $mgC.L^{-1}$ is above the limiting food level for growth and reproduction in this species. In contrast the absence of significant differences between the February field and 0.25 $mgC.L^{-1}$ experimental regressions suggests that Diaphanosoma excisum is food limited in the reservoir during this period.

Although Moina micrura was present in the reservoir in November and February, their numbers were very low and mostly parasitized so it was possible to obtain a length:carbon-weight relationship only in August 1984 (Table 8.2). This relationship is compared with the regressions for experimental animals grown at 32°C and in 1.0 $mgCL^{-1}$ and 0.25 $mgC.L^{-1}$ in Table 8.4. The lack of significant difference between the field relationship and that of animals grown in 0.25 $mgC.L^{-1}$ and these two being at a lower elevation than that of those grown in 1.0 $mgC.L^{-1}$, suggest that Moina micrura in the reservoir were food limited in August 1984. It has been shown in previous chapters that 1.0 $mgC.L^{-1}$ is a non-limiting food concentration for growth and reproduction in this species also.

Daphnia lumholtzi was sparse in November 1984 and, after five days of sampling, it was possible to find only one animal of this species. Sufficient animals were collected in August 1984 and February 1985 and the significant relationship computed between length and carbon content is given in Table 8.2. These relationships were compared with experimentally obtained regression at 32°C and 1.0 $mgC.L^{-1}$ in Table 8.5. This reveals that the August population was similar to that of the animals grown at 1.0 $mgC.L^{-1}$ but the February population had a significantly lower elevation. It is evident therefore that in February the animals were food limited without a doubt, but it is not possible to say positively whether the August population was food limited or not because it was not possible in the present study to determine the incipient limiting food concentration for this species.

The significant relationship between length and carbon weight of the Ceriodaphnia cornuta collected in November 1984 and February 1985 is given in Table 8.2 and compared with the experimentally obtained relationship at 32°C and in 1.0 and 0.25 $mgC.L^{-1}$ in Table 8.6. This species did not show any significant difference between the November and February populations, and the November population was not significantly different from the animals grown at 1.0 $mgC.L^{-1}$ (both slope and elevation). The February population had a flatter slope than that for the experimental animals in 1.0 $mgC.L^{-1}$. As in the case of Daphnia lumholtzi it was not possible to experimentally establish the incipient limiting food concentration for Ceriodaphnia cornuta

and hence it is not possible to conclude whether the reservoir animals were food limited or not in November 1984. The fact that the elevation of the line obtained in February was not significantly different from that obtained in 1.0 $mgC.L^{-1}$ (as well as from that of the November population) but had a flatter slope than any of the others indicates that the increase in weight per unit length was less in the reservoir populations, at that time. But it is also evident from Table 8.6 that since the regression for animals grown at 0.25 $mgC.L^{-1}$ is a significantly lower elevation to the rest, the animals of this February population did have a higher body carbon content than those grown at 0.25 $mgC.L^{-1}$.

To sum up (Table 8.8): there are signs of food limitation in Diaphanosoma excisum during February 1985 but not in November 1984 and in Moina micrura during August 1984. Earlier, in Chapter 4, the incipient limiting food levels for growth in both these species were defined as starting between 0.5 - 0.1 $mgC.L^{-1}$ at the temperatures existing in the reservoir (27-32°C). The measured values for edible algal carbon (less than 33 μm) (summarised in Table 8.8) are a little higher than this in August and February and much higher when the non-algal carbons are included. The main difference in the November food situation, when Diaphanosoma excisum was not food limited, is the low proportion of non-algal food or, conversely, the high proportion of nutritive algal carbon. Following on from this, it seems likely that May was a month with severe food limitation for both species because of both low carbon levels and high proportions of

detrital carbon. Unfortunately, a field trip of one day gave insufficient time to collect adequate samples. Less can be said about the other two species. Daphnia lumholtzi showed signs of food limitation in February 1985 and possibly in August 1984 and Ceriodaphnia cornuta in November and February. The incipient limiting food concentrations is much higher in these two species (more than 1 $mgC.L^{-1}$) so that these are likely results, despite the higher values for the sestonic edible fractions. The edible fraction never fell to food threshold levels for growth, which are 0.03-0.05 $mgC.L^{-1}$ for Diaphanosoma excisum and Moina micrura but may be a little higher for Daphnia lumholtzi and Ceriodaphnia cornuta at the reservoir temperature.

8.3 SIZE OF NEONATE AND PRIMIPARA OF THE FIELD ANIMALS

Comparison of neonate and primipara size in the reservoir populations of Diaphanosoma excisum and Moina micrura (Table 8.7) with those of the experimental animals (Table 6.11; 6.13), grown under defined food and temperature conditions, indicates that the sizes of the reservoir neonates of Diaphanosoma excisum are within the size range of the experimental animals (0.468-0.526mm) but the size of the primipara is smaller (0.875-0.976mm). It is also evident that the primipara of the November population is smaller than that of the February population. It seems unlikely that the November population is food limited whereas there is evidence that the February population is food limited. Therefore, in relation to food availability, a smaller body size would not be expected in November as it was shown in Chapter 6

that there is no direct relationship between primipara size and food quantity in this species. There may be some other factor reducing the size of the primipara and the one possibility is the effect of size-selective predation taking the larger sizes. In the February population also, the fact that the primipara is smaller than in the experimental animals (0.875-0.976mm) also may be an effect due to predation.

The August population of Moina micrura (Table 8.7) had neonates within the size range of the experimental population but the size of the primipara was slightly smaller than that found in the experiments (0.670-0.61 mm). Since the difference between the experimental and field population is less well marked than in Diaphanosoma excisum (the highest field population size being 0.60 mm, and the lowest experimental size 0.61 mm), this difference of 0.01 mm may be due to an error in measuring. Hence it is not possible to speculate whether the August population of Moina micrura is subjected to predation or not.

In contrast to these two species, the size of both neonate and primipara in Daphnia lumholtzi (Table 8.7) is much smaller than that of the experimental animals (neonate 0.53 mm - 0.58mm; primipara 1.08 mm - 1.23 mm). It was shown above that the length-carbon weight relationship of the August population was similar to that of animals reared in 1.0 $mgC.L^{-1}$. Therefore the small neonate and primipara could be due to some other cause, most probably predation on the August population. As the length-carbon weight relationship of the February population is lower than that of the 1.0 $mgC.L^{-1}$ reared animals, it is evident

that during this period less food (<1.0 mgC.L^{-1}) was available to the reservoir animals. It was shown in Chapter 6 that there is a tendency for this species to produce larger neonates at lower food levels (below 0.5 mgC.L^{-1}). Thus one would expect larger neonates in February, when less food is available, than those produced by animals grown in 1.0 mgC.L^{-1}; as well as in the August population. It is therefore evident that the shift toward a smaller size of Daphnia lumholtzi is not a food quantity effect but may be due to size selective predation or some other factor.

Comparing the size of neonates and primipara of the reservoir populations of Ceriodaphnia cornuta it is evident that there is no difference in the size of neonates (Table 8.7) in relation to experimentally reared animals (0.25 - 0.26mm) but primipara of the November population (Table 8.7) tend to be smaller than experimentally reared animals (0.44 - 0.45 mm). Here again there may be some effect, other than the availability of edible food, which shifted the November primipara to a smaller size.

From these data it can be suggested that there might be predation effects on five out of seven sets of observation. Only Ceriodaphnia cornuta in February, and probably Moina micrura in August, show no evidence of decreased primipara and neonate size when compared with the experimental animals. The scattered nature of the data on the reservoir populations does not justify any further speculation.

Table 8.1. Chlorophyll a, algal carbon, total sestonic carbon and non-algal carbon content of Kalawewa reservoir water during the days of sampling. Results are expressed in terms of mg.L^{-1} ± SD. The percentage of non-algal carbon in relation to total algal carbon is given in parenthesis.

Date	Chlorophyll a concentration mg .L^{-1}		Algal carbon content mgC.L^{-1}		Total sestonic carbon content mgC.L^{-1}		Non-algal carbon content mgC.L^{-1}	
	Total	<33µm	Total	<33µm	Total	<33µm	Total	<33µm
21st May 1984	0.02329 ±0.0064	0.00468 ±0.00045	0.69881 ±0.01921	0.14036 ±0.01337	1.38806 ±0.03319	0.51279 ±0.00620	0.68925 (50%)	0.37243 (73%)
23rd August 1984	0.04083 ±0.00460	0.018534 ±0.00527	1.22496 ±0.13791	0.55604 ±0.15798	1.55555 ±0.0580	1.32227 ±0.14946	0.33059 (21%)	0.76623 (58%)
8th November 1984	0.0387 ±0.0113	0.0251 ±0.0093	1.16095 ±0.3396	0.75295 ±0.2795	1.8291 ±0.3261	1.2672 ±0.2590	0.66815 (37%)	0.5143 (41%)
16th February 1985	0.0292 ±0.0066	0.0225 ±0.0088	0.8973 ±0.1807	0.6736 ±0.2638	1.9913 ±0.1688	1.8016 ±0.2231	1.0945 (55%)	1.12802 (63%)

Table 8.2 Parameters of the linear regressions relating organic carbon content ($\mu gC.ind^{-1}$) to length (mm) of Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi collected from the Kalawewa reservoir.

Regression equation $\ln Y = \ln a + b \ln X$
Y = organic carbon content ($\mu gC.ind^{-1}$), X = length (mm)
df = degrees of freedom; F = variance ratio; P = level of significance;
r = correlation coefficient

Date	lna	b	df	F	P	r
Diaphanosoma excisum						
11-16 February 1985	0.9019	2.3381	1,10	47.728	<0.0001	0.9093
3-8 November 1984	1.2909	2.5664	1,10	56.980	<0.0001	0.9223
Moina micrura						
19-23 August 1984	1.295	2.5454	1,11	106.101	<0.0001	0.9519
Ceriodaphnia cornuta						
3-8 November 1984	2.9447	3.2848	1,12	42.433	<0.0001	0.8829
11-16 Februrary 1985	1.7730	2.0161	1,12	88.694	<0.0001	0.9385
Daphnia lumholtzi						
19-23 August 1984	1.8416	2.8576	1,18	157.552	<0.0001	0.9473
11-16 February 1985	1.4144	2.6340	1,16	416.731	<0.0001	0.9813

Table 8.3 Results of the covariance analysis comparing the carbon-weight: length regressions of Diaphanosoma excisum obtained from the reservoir (Table 8.2) and those reared in the defined food concentration of 1 $mgC.L^{-1}$ and 0.25 $mgC.L^{-1}$ (Table 4.1) at 32°C. Differences between the regression coefficients were tested by the SS-STP test and those between the elevations of the curves by the S-N-K test. Lines connect group numbers which are not significantly different at P = 0.05 level. The group number is given in ascending order of the magnitude. Sampling dates are given in Table 8.2.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error

a) Comparision between regression coefficients

Regression	Group no	Regression coeff. ± SE	df	F	P	SS-STP Test
February	1	2.3381 ± 0.3384	3,52	3.632	0.0187	1 2 4 3
November	2	2.5664 ± 0.3401				
1.0 $mgC.L^{-1}$	3	3.5176 ± 0.2245				
0.25 $mgC.L^{-1}$	4	2.7814 ± 0.2548				

b) Comparision between the elevation of the curve

Regression	Group no	Adjusted mean ± SE	df	F	P	S-N-K Test
February	1	0.3878 ± 0.1488	3,56	5.507	0.0022	1 4 2 3
November	2	0.6982 ± 0.1487				
1.0 $mgC.L^{-1}$	3	0.7186 ± 0.1482				
0.25 $mgC.L^{-1}$	4	0.3954 ± 0.1496				

Table 8.4 Parameters of the covariance analysis comparing the carbon-weight: length regressions of Moina micrura obtained from the reservoir (Table 8.2) and those reared in defined food concentrations of 1.0 $mgC.L^{-1}$ and 0.25 $mgC.L^{-1}$ (Table 4.1) at 32°C. Differences between the regression coefficients were tested by the SS-STP test and those between the elevations of the curves by the S-N-K test. Lines connect group numbers which are not significantly different at P = 0.05 level. The group numbers are given in ascending order of magnitude. Sampling dates are given in Table 8.2.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

a) Comparison between regression coefficients

Regression	Group no	Regression coeff. ± SE	df	F	P	SS-STP test
August	1	2.5454 ± 0.2471	2,46	3.862	0.028	1 3 2
1.0 $mgC.L^{-1}$	2	4.1906 ± 0.5818				
0.25 $mgC.L^{-1}$	3	3.0271 ± 0.3031				

b) Comparison between the elevations of the curve

Regression	Group no	Adjusted mean ± SE	df	F	P	S-N-K test
August	1	0.0786 ± 0.2166	2,49	14.995	<0.0001	1 3 2
1.0 $mgC.L^{-1}$	2	0.8046 ± 0.2163				
0.25 $mgC.L^{-1}$	3	0.2589 ± 0.2167				

Table 8.5 Results of the covariance analysis comparing carbon-weight: length regressions of Daphnia lumholtzi obtained from the reservoir (Table) and those reared under defined food concentrations of 1 mgC.L^{-1} at 32°C (Table). Differences between the regression coefficients were tested by the SS-STP test and those between the elevations of the curves by the S-N-K test. Lines connect group numbers which are not significantly different at P = 0.05 level. The group number is given in ascending order of magnitude. Sampling dates are given in Table 8.2.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

a) Comparison between regression coefficients

Regression	Group no	Regression coeff. ± SE	df	F	P	SS-STP test
1 mgC.L^{-1}	1	3.2494 ± 0.4068	2,48	1.289	0.285	3 2 1
August	2	2.8576 ± 0.2277				
February	3	2.6340 ± 0.1290				

b) Comparison between the elevation of the curves

Regression	Group no	Adjusted mean ± SE	df	F	P	S-N-K test
1 mgC.L^{-1}	1	1.1842 ± 0.1829	2,51	10.44	0.0002	3 2 1
August	2					
February	3					

Table 8.6 Results of the covariance analysis comparing the carbon-weight: length regressions of Ceriodaphnia cornuta obtained from the reservoir (Table 8.2) and that reared in a defined food concentration of 1 mgC.L^{-1} (Table) at 32°C. Differences between regression coefficients were tested by S-S-STP test and those between the elevations of the curves by the S-N-K test. Lines connect group numbers which are not significantly different at P = 0.05 level. The group numbera are given in ascending order of magnitude. Sampling dates are given in Table 8.2.

df = degrees of freedom; F = variance ratio; P = level of significance; SE = standard error.

a) Comparision between regression coefficients.

Regression	Group no	Regression coeff. ± SE	df	F	P	SS-STP test
November	1	3.2848 ± 0.5043	3,51	4.379	0.0081	2 1 4 3
February$_1$	2	2.0161 ± 0.2141				
1 mgC.L^{-1}	3	3.6822 ± 0.3710				
0.25 mgC.L^{-1}	4	3.4384 ± 0.2895				

b) Comparision between the elevations of the curves

Regression	Group no	Adjusted mean ± SE	df	F	P	S-N-K test
November	1	-0.3243 ± 0.1830	3,55	16.009	<0.0001	4 3 1 2
February$_1$	2	-0.1752 ± 0.1814				
1 mgC.L^{-1}	3	-0.3251 ± 0.1833				
0.25 mgC.L^{-1}	4	-1.0003 ± 0.1813				

Table 8.7 The size of the neonate, primipara and maximum adult size of Diaphanosoma excisum, Moina micrura, Ceriodaphnia cornuta and Daphnia lumholtzi in reservoir samples.

Date	Size of neonate (mm)	$\mu gC.ind^{-1}$	Size of primipara (mm)	$\mu gC.ind^{-1}$	Max adult (mm)	$\mu gC.ind^{-1}$
Diaphanosoma excisum						
11-16 February 1985	0.45 - 0.50	0.261	0.80 - 0.85	1.723	1.10	3.54
3-8 November 1984	0.45 - 0.50	0.354	0.70 - 0.75	1.125	1.00	4.28
Moina micrura						
19-23 August 1984	0.40 - 0.45	0.3406	0.55 - 0.60	1.613	0.75 - 0.80	1.693
Ceriodaphnia cornuta						
3-8 November 1984	0.20 - 0.25	0.0600	0.35 - 0.40	0.6716	0.45 - 0.50	only 1 animal
11-16 February 1985	0.20 - 0.25	0.2099	0.40 - 0.45	0.8528	0.45 - 5.0	1.161
Daphnia lumholtzi						
19-23 August 1984	0.40 - 0.45	0.3142	0.75 - 0.80	2.6186	1.00	5.165
11-16 February 1985	0.40 - 0.45	0.3002	0.75 - 0.80	2.2117	1.07	1 animal

Table 8.8 Summary of conclusions about field food levels at Kalawewa Reservoir (27-32°C) during 1984-85.

(1) RESERVOIR SESTONIC BIOMASSES (< 33μm Fraction)	May 21 1984	August 19-23 1984	November 3-8 1984	February 11-16 1985
Algal carbon mgC.L^{-1}	0.140	0.556	0.753	0.673
Non-algal carbon mgC.L^{-1}	0.372	0.766	0.514	1.128
Sestonic carbon mgC.L^{-1}	0.512	1.322	1.267	1.802
Percentage non-algal carbon	73	58	41	63

(2) ELEVATIONS OF FIELD L/C REGRESSIONS (27-32°C) COMPARED WITH EXPERIMENTAL ONES (32°C)				
Diaphanosoma excisum[1]	(samples less than 10 μgC)	Parasitised animals	Same as for 1.0 mgC.L^{-1} NO FOOD LIMITATION	Lower than for 0.25 mgC.L^{-1}; same as for 1.0 mgC.L^{-1} FOOD LIMITATION
Moina micrura[1]	-	Lower than for 1.0 mgC.L^{-1}; same as 0.25 mgC.L^{-1} FOOD LIMITATION	Parasitised animals & densities too low	Parasitised animals & densities too low
Daphnia lumholtzi[2]	-	Same as for 1.0 mgC.L^{-1} possible food limitation	Only one individual found	Lower than for 1 mgC.L^{-1} FOOD LIMITATION
Ceriodaphnia cornuta[2]	-	Parasitised animals	Same as for 1.0 mgC.L^{-1} Possible food limitation	Same as for November but with flatter slope than for 1.0 mgC.L^{-1} Possible food limitation.

(1) Incipient limiting food concentrations - 0.5 to 0.1 mgC.L^{-1}; food threshold levels - 0.03 to 0.05 mgC.L^{-1}

(2) Incipient limiting food concentrations - more than 1.0 mgC.L^{-1}; food threshold levels may be higher than in (1)

CHAPTER 9

DISCUSSION

9.1 THRESHOLD FOOD CONCENTRATIONS FOR GROWTH AND REPRODUCTION

With exponentially dividing Scenedesmus acutus as food, the threshold food concentration for growth and reproduction of all the species, except Ceriodaphnia cornuta, is lower (0.03 - 0.05 $mgC.L^{-1}$) at higher temperatures (27°C and 32°C) than at the lower temperature of 22°C where it lies between 0.05 and 0.1 $mgC.L^{-1}$. Comparing these results with those of Rocha (1983), it is evident that temperate daphnids, Daphnia magna, Daphnia pulicaria and Daphnia hyalina, reared at 5°C - 20°C start growing at an even lower threshold food concentration of 0.01 $mgC.L^{-1}$. This is lower than any of the threshold levels for the tropical cladoceran species studied here. In both temperate and tropical species the threshold food level for reproduction decreases with increase in temperature so that in temperatures around 5°C, the three temperate Daphnia species must be able to reproduce at much lower food concentrations than any of the tropical species. Therefore it appears that there is a physiological adaptation which enables temperate species to live in their colder waters compared with tropical species in their warmer waters.

It is well known that both filtering rate (Burns and Rigler, 1967; Burns, 1969; Schindler, 1968) and metabolic rate (Blazka, 1966) of cladocera increases with increasing temperature. The

present results seem to demonstrate that, at a temperature as high as 32°C, a food concentration of 0.05 $mgC.L^{-1}$ is enough for the animals to be able to filter out sufficient food to cover both the increased metabolic costs at high temperature and to still have some energy available for growth and development. At low temperatures, such as 22°C, on the other hand, the rates of filtering and metabolism will be reduced but nevertheless a higher food concentration is required to cover both metabolic costs and to start growth and development. Duncan (1984) suggested that this could be due to the cumulated respiratory cost resulting from prolongation of the juvenile phase at low food levels, which will be greatly increased at low temperatures. This is supported by the fact that tropical species respond, like temperate species, to low food stress by also reducing their body carbon content.

It is difficult to consider the results for Ceriodaphnia cornuta in a similar way because this species did not grow in pure Scenedesmus acutus at 22°C, although it did grow in cultures of algae containing bacteria. It is possible that Scenedesmus acutus is not a good food for Ceriodaphnia cornuta. A similar effect is recorded by Smyly and Collins (1975) with Ceriodaphnia quadrangula which did not thrive in a bacteria-free, unialgal medium but managed to survive in a medium of algae with bacteria. Cowgill et al. (1985) claim that neonates of Ceriodaphnia affinis were able to survive for eight days and reproduce in the absence of food and suggests that they did this on the lipids produced by the mother. However, in the absence of

any attempt to estimate bacterial densities, this is probably not a justified conclusion and it is very likely that the medium contained bacteria. This question of the suitability of Scenedesmus acutus as food for Ceriodaphnia cornuta will be further discussed later.

Piyasiri (1985) found the threshold food concentration for growth and development of the tropical calanoid Phyllodiaptomus annae, which originated from Sri-Lanka (personal communication), to fall between 0.005 and 0.05 $mgC.L^{-1}$ within the temperature range of 22°C - 32°C while that for reproduction was 0.05 $mgC.L^{-1}$. Therefore Phyllodiaptomus annae, which was found to be co-existing in Kalawewa reservoir with the four cladoceran species of the present study (Jayatunga, 1982), has a competitive advantage over the four cladocerans at low food levels at the lower end of the temperature range. A similar threshold level for reproduction 0.05 $mgC.L^{-1}$ to those of these freshwater calanoids was found for a temperate marine calanoid Calanus helgolandius by Paffenhofer (1976).

It is evident from the literature that it is not possible to apply the experimentally defined threshold levels directly to the field conditions since food quality also matters in the determination of food thresholds. This is evident from the results of Lampert (1978) in which he found the minimum food concentration for reproduction in Daphnia longispina to be 0.1 $mgC.L^{-1}$ under laboratory conditions, with unialgal food, but the threshold shifted to 0.2 $mgC.L^{-1}$ for field populations. He suggested that the higher value in the field might be due to a

number of reasons: the size of particles (<60μm) that he considered to be food might have been greater than the preferred size range; the field food might also have included non-consumable particles; and the food quality of the natural seston may not have been as good as that provided in the experiments. Thus it is important to evaluate the quantity of edible food available in the field for each species. Duncan (1985) put forward an indirect method for evaluating this by means of length-carbon regressions which is explained in detail in Chapter 8.

9.2 LENGTH-CARBON RELATIONSHIPS

The results obtained for the length-carbon weight regressions for Diaphanosoma excisum, Moina micrura and Ceriodaphnia cornuta reared at 32°C in different food concentrations indicate that, in all three species, the carbon weight is reduced at food levels below 1.0 $mgC.L^{-1}$. It is not possible to say whether Daphnia lumholtzi follows this pattern of response to reduced food because no relationships between length and weight are available at food concentrations below 1.0 $mgC.L^{-1}$. In all three species there were no significant differences between the regressions at the limiting food levels of 0.25 and 0.1 $mgC.L^{-1}$ and the regressions were also significantly lower than that for the non-limiting food concentration of 1.0 $mgC.L^{-1}$. No regressions were obtained for 0.05 $mgC.L^{-1}$ at 32°C due to lack of time.

It was shown that the regressions for 32°C and 1.0 $mgC.L^{-1}$ in experimental conditions were significantly different in elevation for the four species of Cladocera. Each species had its own

characteristic length-weight regressions under optimal conditions of high temperature and non-limiting food (for most of the species). The heaviest (per unit length) animal was Ceriodaphnia cornuta which is a small species ranging from 0.25 - 0.6 mm in length. The lightest animal was Diaphanosoma excisum which is a comparatively large species ranging from 0.47 - 1.3 mm in length. Moina micrura (0.45 - 0.8 mm) and Daphnia lumholtzi (0.5 - 1.3 mm) fell between these two with intermediate body weights. Considering the length and width (Fig. 3.2) of these species taken from Sri-Lanka given in Rajapaksa (1981), the length:width ratio for Diaphanosoma excisum, Moina micrura and Ceriodaphnia cornuta and Daphnia lumholtzi follows 1:0.457, 1:0.61; 1:0.675 and 1:0.794 respectively. As the length of the first three species were measured in a similar manner (as total carapace length) it is clear from the length:width ratio that Diaphanosoma excisum is the most slender and Ceriodaphnia cornuta is the most rounded animal. This characteristic could be reflected in the differences in length-carbon weight regressions between genera. In addition, differences in carapace thickness will also affect the relationship. However, it is evident that it would be inaccurate to use a common length-carbon weight relationship for all the species.

Bottrell et al. (1976) reviewed length-dry weight regressions for cladocerans from the literature. These were all derived from field animals and indicated that carapace thickness affects the relationship since the bosminid regression lies above their pooled daphnid line.

From the present study and Duncan et.al. (1985) it is evident that, even within a species, it is not possible to have a common length-carbon weight relationship because the elevation of the line is affected by the nutritive status of the animal.

9.3 FOOD AND TEMPERATURE EFFECTS ON GROWTH

(a) Growth curves and growth rates

The pattern of growth under well defined food and temperature conditions is similar in both copepods and cladocerans irrespective of whether they are tropical or temperate. This is shown by examination of the growth curves obtained by Vidal (1978) for the temperate marine copepods Calanus pacificus and Pseudocalanus sp, by Piyasiri (1984) for the tropical freshwater copepod Phyllodiaptomus annae and a temperate freshwater copepod Arctodiaptomus spinosus, by Rocha (1983) for freshwater temperate daphnids, Daphnia magna, Daphnia hyalina and Daphnia pulicaria, and in the present study of tropical cladocerans. All these authors have analysed the growth of their animals using their weights and their precise ages and have used the Chapman-Richards function which is very similar to the Richards function (Chapter 4) used in this study. With the sole exception of Moina micrura in this study (at low temperatures and at low food-high temperature), all these examples showed an initial period of exponential growth. In the cladocera this was followed by an inflextion at or just after maturity in non-limiting food concentrations, but rather earlier in limiting food concentrations. Finally, they reach an asymptotic adult size.

It is evident from the growth rates obtained in the present study that whether they are expressed as instantaneous, relative or absolute growth rates they are dependent on both temperature and food and that the effects of food limitation on growth rates are more marked at higher temperatures than at lower temperatures (Table 4.5). There is, however, sparse and contradictory information available on the effect of environmental factors and body size on weight-specific growth rates (= relative growth rate). According to Vidal (1978) the percentage maximum weight-specific growth rate ($G\%_{max}$) of the small bodied Calanus pacificus is more affected by temperature than that of larger bodied animals in which, as the body size of the animal increases, the temperature effect on $G\%_{max}$ decreases. Q_{10} values calculated using the $G\%_{max}$ values given by Vidal (1978) are given in Table 9.1 and confirm his conclusion, since the value of Q_{10} decreases with increase in body size. This relationship is not at all clear for the tropical copepod, Phyllodiaptomus annae, whose Q_{10} was calculated using $G\%_{max}$ values given by Piyasiri (1984) (Table 9.1). No firm conclusion can be drawn in this case because the body weights for this species cover a much smaller range than those of the temperate species studied by Vidal (1978). Rocha (1983) graphically compared the relationship between $G\%_{max}$ and temperature and, on this visual evidence, concluded that temperate daphnids respond in the same way as Calanus pacificus. Q_{10} values were calculated using values of $G\%_{max}$ read back from her graphs and are also given in Table 9.1. From these it is evident that her predictions do not accord

Table 9.1. Q_{10} values computed for different sized animals. Raw data obtained from Rocha (1983), Piyasiri (1983) and Vidal (1978).
Q_{10} for $G\%_{max}$ = weight specific growth rate

Rocha (1983)

Size of the animal µgC	Q_{10} 5 - 15°C	Q_{10} 10-20°C
Daphnia magna		
12	2.33	3.27
40	6.00	3.20
74	5.50	6.25
Daphnia pulicaria		
14	4.67	2.89
80	7.33	1.00
200	6.40	4.00
Daphnia magna		
30	2.33	3.27
240	6.00	3.20
720	5.50	6.25

Vidal (1978)

Size of the animal µgC	Q_{10} 8-15.5°C
Calanus pacificus	
	3.03
50	2.11
100	1.61
150	1.28

Piyasiri (1984)

Size of the anima µg dry weight	Q_{10} 22-27°C	Q_{10} 27-32°C	Q_{10} 22-32°C
Phyllodiaptomus annae			
2.0	1.38	1.99	1.66
3.0	1.35	1.00	1.16
4.0	1.60	2.01	1.79

with the evidence of the Q_{10} values; in fact, her larger animals had higher Q_{10} values for $G\%_{max}$ than her smaller animals of the same species. It is clear that, at any one temperature, the $G\%_{max}$ of a smaller bodied animal is higher than that of the larger animals and also that the former shows a steeper slope in the relationship of $G\%_{max}$ to temperature than the latter, but the steeper slope does not in itself predict the relative effect of temperature since the Q_{10} values of the larger animals, in all three species, are greater than those of the smaller ones (Table 9.1.). This conclusion is further supported when percentage growth rates at different stages in the life-cycle were plotted against temperature for both temperate (Rocha, 1983) and tropical species (this study) grown under both non-limiting (1.0 $mgC.L^{-1}$) and limiting (0.1 $mgC.L^{-1}$) food concentrations as in Fig 9.1 . It is clear from this that in both temperate and tropical species the smaller bodied juveniles have the steepest slopes and highest elevations, followed by the primipara, and then the adult, which has the lowest slope and elevation. Nevertheless, the Q_{10} values given in Table 9.2, for all food concentrations for both temperate and tropical species, are greater for the adults than for the primipara and juvenile instars in the majority of cases. It is thus difficult to evaluate the effect of temperature on relative growth rate only by looking at the steepness of the slope and also impossible to conclude that in general the relative growth rates of smaller bodied animals are more affected by temperature than those of larger forms.

Table 9.2. Q_{10} values computed for relative growth rates under different food regimes from the present study and that of Rocha (1983)

Rocha 1983

Food conc mgC.L^{-1}	3rd Juvenile 5-15°C	3rd Juvenile 10-20°C	Primipara 5-15°C	Primipara 10-20°C	4th Adult 5-15°C	4th Adult 10-20°C
Daphnia magna						
5.0	5.16	3.74	4.28	3.33	8.78	2.61
1.0	6.19	4.36	3.76	3.31	5.92	2.31
0.1	3.40	2.58	3.36	7.61	0.83	17.78
0.01	4.17	3.01	-	2.83	-	3.03
Daphnia pulicaria						
5.0	4.30	3.02	7.45	6.60	1.52	3.73
1.0	4.21	1.82	10.37	7.70	3.50	8.29
0.1	3.12	1.56	4.21	6.95	8.79	4.50
0.01	1.87	1.99	-	1.53	-	2.64
Daphnia hyalina						
5.0	2.27	3.19	6.38	3.86	1.09	3.39
1.0	2.56	3.68	5.16	2.45	7.52	1.99
0.1	2.32	2.23	4.99	1.70	6.81	2.95
0.01	1.98	2.10	6.95	1.72	23.11	17.64

Present study

Food conc mgC.L^{-1}	2nd Juvenile 22-27°C	2nd Juvenile 27-32°C	2nd Juvenile 22-32°C	Primipara 22-27°C	Primipara 27-32°C	Primipara 22-32°C	5th Adult 22-27°C	5th Adult 27-32°C	5th Adult 22-32°C
Diaphanosoma excisum									
1.0	2.30	1.86	2.07	6.81	1.58	3.28	2.99	6.95	4.56
0.5	0.97	2.09	1.42	2.23	3.78	2.90	0.53	9.35	2.23
0.1	6.99	0.56	1.97	4.89	2.89	2.59	2.53	7.71	4.42
0.05	-	0.92	-	-	2.24	-	-	-	-
Moina micrura									
1.0	1.08	1.44	1.25	5.18	1.61	2.89	92.92	3.53	18.11
0.5	1.30	1.41	1.35	3.43	2.93	3.17	23.24	1.18	5.23
0.1	2.68	0.66	1.34	2.77	1.08	2.68	4.93	0.73	1.90
0.05	-	0.94	-	-	1.67	-	-	1.78	-

Fig. 9.1 also shows how well the relationship between relative growth rate and temperature for tropical species fits on to that of temperate species. The figure further illustrates the lowering of relative growth rate under food limiting conditions (comparing that of 1.0 $mgC.L^{-1}$ with 0.1 $mgC.L^{-1}$).

The intraspecific and interspecific variations in the effects of food on growth can also be compared by looking at values of K_s; the food concentrations required to attain 50% of maximum growth rate (Chapter 4). K_s values for relative growth rate, from the literature and from the present study, are compared in Table 9. 3. K_s values for both Diaphanosoma excisum and Moina micrura remained somewhat similar at 32°C and 27°C but increased with further decrease in temperature (22°C). This relationship is the opposite of that found by Rocha (1983) for temperate daphnids (Table 9.3) in which the K_s values increased with increasing temperature between 5° and 20°C for Daphnia magna and Daphnia pulicaria, while those of Daphnia hyalina remained constant. In addition, Daphnia magna required less food at the two higher temperatures than Daphnia hyalina (about 1/2-1/3) although the maximum relative growth rate of the former was greater than the latter. Therefore, as she indicates, it is evident that (a) there are intraspecific differences and (b) Daphnia hyalina can out-compete the other two species under conditions of food limitation at higher temperatures.

A lower food requirement at lower temperatures can be explained by the direct relationship of decreasing physiological rate processes with decline in temperature in poikilotherms. It

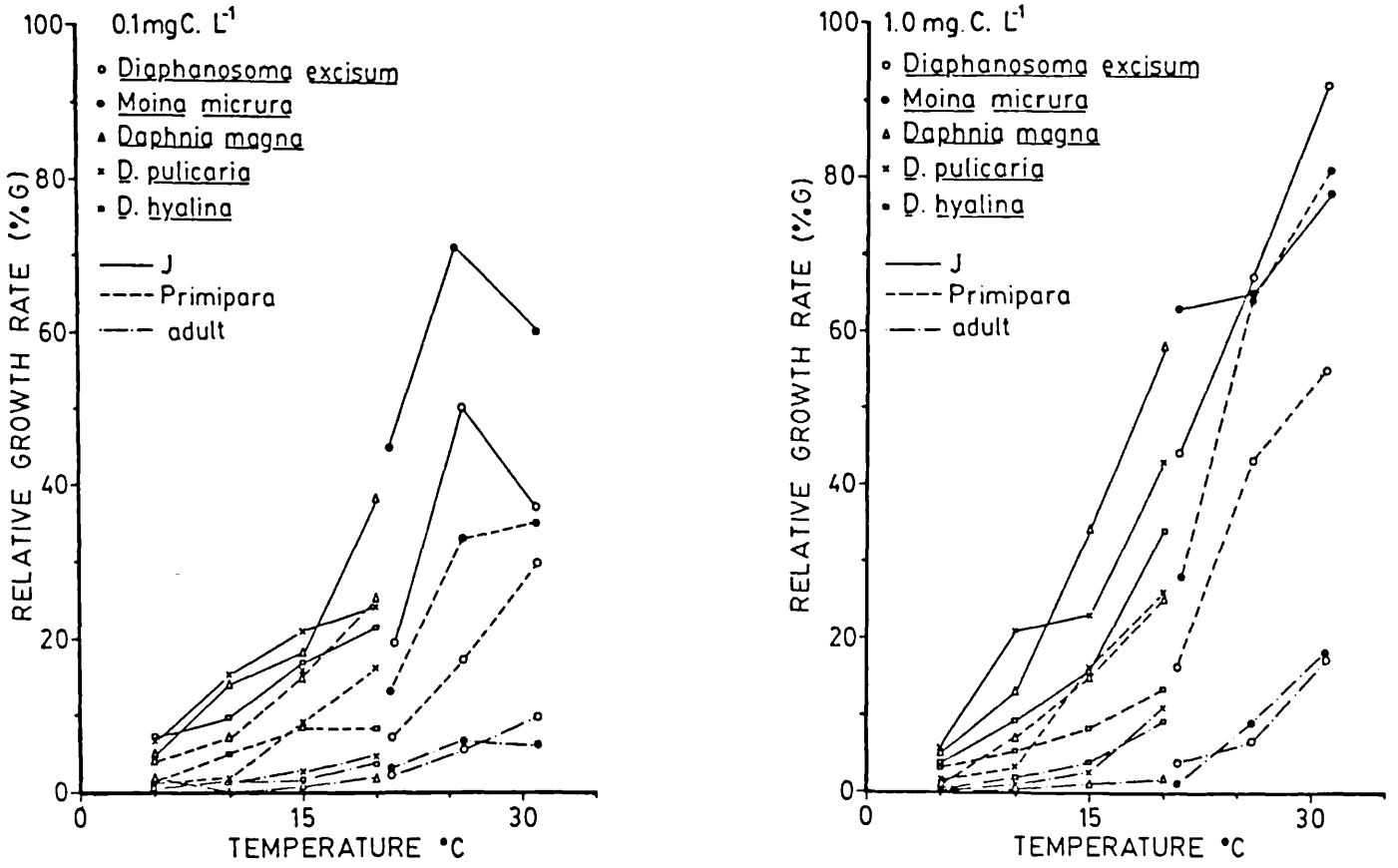


Fig. 9.1 A comparison of relative growth rates at different temperatures in temperate and tropical species of Cladocera.

The comparison is made for limiting (0.1 mgC.L^{-1}) and non-limiting (1.0 mgC.L^{-1}) food levels and for three stages in the life cycle: juvenile (J), primipara and adult. Data for the temperate species taken from Rocha (1983).

Table 9.3 A comparison of the maximum relative growth rates and K_s values from the present study with those from the literature: Daphnia magna Daphnia pulicaria and Daphnia hyalina from Rocha, (1983) and Phyllodiaptomus annae from Piyasiri, (1984).

Temp °C	Daphnia magna		Daphnia pulicaria		Daphnia hyalina	
	$G\%_{max}$	K_s ($mgC.L^{-1}$)	$G\%_{max}$	K_s ($mgC.L^{-1}$)	$G\%_{max}$	K_s ($mgC.L^{-1}$)
20	38.46	0.12	20.91	0.23	4.39	0.02
15	21.28	0.17	14.08	0.24	3.13	0.02
10	6.71	0.02	3.79	0.05	2.35	0.02
5	5.32	0.05	0.86	0.03	0.81	0.02

Temp °C	Phyllodiaptomus annae		Diaphanosoma excisum		Moina micrura	
	$G\%_{max}$	K_s ($mgC.L^{-1}$)	$G\%_{max}$	K_s ($mgC.l^{-1}$)	$G\%_{max}$	K_s ($mgC.L^{-1}$)
22	32.16	0.037	22.99	0.214	32.00	0.163
27	41.05	0.060	35.59	0.091	70.90	0.113
32	44.09	0.045	56.50	0.087	86.21	0.114

is known that the energy of food assimilated is equal to respiratory cost plus production. Decrease in temperature will decrease both the rate of respiration as well as the rate of production, which here is equivalent to the rate of growth up to the primipara. At present there is no data on the respiration of these species but in this study growth has been shown to be lower at lower temperatures. In the lowest food concentrations used by Rocha (1983) her daphnids were apparently able to assimilate sufficient food at low temperatures to cover their lower respiratory cost plus growth. In contrast, the tropical cladoceran species studied here were not able to assimilate sufficient food from the same low food concentrations at temperatures which, although much higher than the lowest used by Rocha, are the lowest experienced by these species under natural circumstances. This suggests that these species have higher respiratory costs per unit weight at their lowest environmental temperatures (c. 22°C) than the temperate species at their lowest environmental temperatures (c. 5°C) and that there is no evidence of acclimation. However, this conclusion is confused by the fact that the tropical cladocera for which we have data are much smaller than the temperate species studied by Rocha (1983) and for this reason also are likely to have higher weight-specific respiration rates. Until comparable data are available for some equally small temperate species it will not be possible to make direct comparisons of the lowest food concentrations at which different species can grow and reproduce in their respective environments.

A further complication is the fact that the area of the filtering limbs varies between species and with body size and their rate of beat will vary with temperature. Both these factors affect the quantity of food which can be collected per unit time. In the tropical species it seems likely that the combination of small body size, leading to a small absolute filter area (Chapter 7) in comparison to that of the temperate daphnids, plus a lowering of the beat rate at 22°C, does not enable them to collect sufficient food to cover their respiratory costs plus production in low food concentrations. At higher food concentrations these disadvantages are offset by the fact that each beat is able to collect more food. To reach a conclusion it would be necessary to investigate the effect of temperature on appendage beat rate. In temperate species, in spite of the low appendage beat rate at low temperature, the amount of food that can be gathered seems to be sufficient. The higher food requirement for growth at low temperatures (22°C) in tropical species of the present study was also evident from the threshold food concentrations discussed earlier.

Comparing the K_s values for the two tropical cladocerans (Moina micrura and Diaphanosoma excisum) with that of Phyllodiaptomus annae (Table 9.3; Piyasiri, 1984), which co-exists with them in Kalawewa reservoir (Jayatunga, 1982), it is evident that the calanoid copepod requires less food to achieve 50% of its maximum growth rate and thus has a competitive advantage over the two cladocerans under conditions in which food supply is limited.

(b) Body growth versus reproductive growth

It was shown in the present study that after maturity the energy channelled toward reproductive growth is greater than that channelled toward body growth in both Diaphanosoma excisum and Moina micrura, both under limiting and non-limiting food conditions and irrespective of temperature. Lynch (1980a), analysing his own results and those from the literature, categorized well-fed animals into two groups: (a) larger species which mature close to their maximum body size and in which, after maturation, most of the energy consumed is channelled into reproduction, (b) small sized species which mature early, continue to grow after the onset of maturity and in which a large proportion of the energy consumed goes to body growth and a small proportion, via progressively increasing clutch size, to reproduction. This is contradictory to the results of the present study which was carried out under well-defined food and temperature conditions. The results he used from the literature cannot always be considered to apply to well-fed animals because they were either grown in natural filtered water or on algae added to natural filtered water. In those experiments neither food quality nor quantity were defined. Therefore, there is no consistency in the data he was comparing because there was no indication of whether the animals were food limited or not. Since there is now evidence that life cycle characteristics change under limiting and non-limiting food conditions his categorisation cannot be accepted. Another point is that his comparisons were made with the results of experiments within which the temperature fluctuated by 2°-3°C. The results presented here confirm that significant differences are found in life cycle characteristics within a 5°C difference in temperature.

It is also now known that both food concentration and temperature play a role in controlling life cycle characteristics and a shift in either of these factors will alter the measurements of these characteristics. It is therefore very important only to make comparisions between animals grown under well-defined conditions of constant temperature and food supply. Under these circumstances it can be shown that Lynch's categorization between large and small cladocerans is not an acceptable generalisation.

According to Rocha (1983) the large daphnid species she investigated continued to grow after maturity at non-limiting food levels and in the present study relatively small species put the greatest proportion of their growth after maturity into reproduction. Neither of these examples fit into Lynch's categorisation.

9.4 FOOD AND TEMPERATURE EFFECTS ON FECUNDITY

Among the tropical species examined during the present study both food concentration and temperature were found to significantly affect the number of eggs produced by the mother the former having a more marked effect than the latter. Increase in fecundity with increased food concentration in cladocerans has been experimentally demonstrated by Hall (1964), Kerfoot (1974), Rocha (1983) and Orcutt and Porter (1984). The temperate Daphnia species studied by Rocha (1983) did not show a significant relationship between fecundity and temperature between 5°C - 20°C though the general trend was for fecundity to decrease with temperature. Orcutt and Porter (1984) found the number of eggs

per brood to increase from 10°C to 15°C but it decreased from 15°C to 20°C at all three food levels they investigated (0.02, 0.2 and 2.0 $mgC.L^{-1}$). This suggests that this species has its optimal temperature for reproduction around 15°C and decreasing in fecundity at 20°C is probably not due to food limitation.

Green (1966) made an attempt to relate the reduced fecundity of the field population of Simocephalus vetulus to temperature and concluded that the spring and summer variations in fecundity that he observed in Simocephalus vetulus were controlled by temperature. He found a summer population with fewer eggs than spring populations, although both seasons showed peaks in phytoplankton measured as chlorophyll a concentrations. Analysis of the relationship between fecundity and temperature showed that fecundity remained constant between 2°C - 7°C and increased between 12°C - 17°C and 18°C - 24°C. The first increase was steeper than the second. He showed that the lower number of eggs per female in this second phase was caused by a combination of factors, the dominant being the small size of adults at temperatures beyond 18°C as shown by relating mean size of adults against temperature. This author considered that at higher temperatures the animal matured at a smaller size which reduced the egg carrying capacity. The present study shows that the relationship between the temperature and size at maturity differs between the two genera studied as Diaphanosoma excisum does not show any variation in the size of the primipara with temperature but does respond to low temperature and low food concentration by delaying maturity. On the other hand Moina micrura showed a

negative relationship between the size of primipara and temperature, as Green suggested for Simocephalus vetulus. It is thus necessary to know the relationship between primipara size of a species and temperature, when other environmental conditions are kept constant, in order to analyse the responses of natural populations to changes in environmental temperature.

The present study provides evidence that fecundity is influenced not only by the nutritive level and temperature but also by maternal body size. Both in Diaphanosoma excisum and Moina micrura an increased fecundity with maternal body size was demonstrated. This is seen at all temperatures studied but is particularly clear at 32°C. There is an interesting difference in response by the two speceis. For example, at 22°C in Moina micrura, a significant increase in fecundity with maternal body size was obtained only at the highest food level (1.0 $mgC.L^{-1}$). At the two lower food levels (0.5 and 0.1 $mgC.L^{-1}$), where reproduction did occur, the egg numbers were low and constant at all adult sizes. On the other hand, of all the food temperature combinations where reproduction in Diaphanosoma excisum was possible, only one (0.05 $mgC.L^{-1}$ - 27°C) did not produce a significant fecundity-size regression (Table 6.8). In Daphnia lumholtzi there was a significant relationship at 22°C and 1.0 $mgC.L^{-1}$, while Ceriodaphnia cornuta at 32°C showed a significant effect at the highest food level of 1.0 $mgC.L^{-1}$ but not at the next lowest level of 0.5 $mgC.L^{-1}$. Rocha (1983) illustrated the effect of maternal body size on fecundity at 20°C and food concentrations of 0.01, 0.1, 1.0 and 5.0 $mgC.L^{-1}$ for Daphnia

magna, Daphnia pulicaria and Daphnia hyalina and found that the first two of these species did not have a significant relationship between the two characters at the lowest food level of 0.01 $mgC.L^{-1}$, while Daphnia hyalina had significant relationships at both 0.01 and 0.1 $mgC.L^{-1}$. Hence it is evident that the relationship between maternal body size and fecundity of cladocerans is affected by food limitation and, particularly in Moina micrura, the lower the temperature the greater the effect of food.

9.5 SIZE OF EGGS AND NEONATES

In the present study it was not possible to measure the egg size, so only neonate size was considered. No significant relationship between neonate size and food or temperature was found in the present study with Moina micrura except that small neonates were produced at the lowest food concentration (0.05 $mgC.L^{-1}$. Table 6.13). In Diaphanosoma excisum, however, a direct significant correlation was found between neonate size and food. Daphnia lumholtzi at 22°C, in contrast, had a significantly larger neonate at low food (0.1 $mgC.L^{-1}$) compared to the size at 1.0 and 0.5 $mgC.L^{-1}$. The similarity of these results for Daphnia lumholtzi to those of temperate daphnid species suggests that this may be a feature common to members of the genus Daphnia. Rocha (1983) also found the size of the egg and neonate to be inversely related to fecundity, temperature and food concentration in her three species of temperate Daphnia. In Daphnia magna the size of eggs and neonates were most strongly correlated with food and fecundity, but, in Daphnia pulicaria, these sizes were more strongly associated with temperature.

In the study by Green (1966) in which an attempt was made to relate fecundity to temperature, as discussed above, the author explained the occurrence of bigger eggs at low temperatures and vice versa as follows: the size of cladoceran eggs is determined by the balance of advantages and disadvantages at different temperatures. In cold water, larger eggs ensure that the neonate has a maximum chance of attaining maturity and reaches this state after fewer instar stages; in warmer waters, a greater number of smaller eggs ensures greater population growth. However, how these large, or small, eggs develop subsequently depends upon other environmental factors than temperature alone, one important one being the availability of food in limiting or non-limiting concentrations. Understanding the interactions of these two environmental variables upon cladoceran development and reproduction requires experimental investigation.

Rocha's (1983) studies on three temperate species of Daphnia which were reared throughout their life cycles at high and low temperatures, and in limiting and non-limiting food conditions, provides good experimental evidence for understanding some of these temperature-food interactions. In all three species, there was a decrease in egg size at 15°C and 20°C compared with 5°C and 10°C but within the size range for each temperature, the larger eggs were associated with food-limiting conditions and the smaller eggs with non-limiting concentrations. In most cases, there were significant, negative correlation coefficients to confirm these effects. Larger eggs produced larger neonates (as supported by significant positive correlation coefficients) but

there was no obvious trend for larger neonates to mature with fewer instar stages. On the other hand, there was a significant correlation between neonate size and the size of the primiparous female but it was a negative correlation - larger neonates producing smaller primipara. This is a complex effect associated with the very strong prolongation of the juvenile phase under the combined effects of low temperature and limiting food conditions which were the conditions under which large eggs and large neonates were produced. Any prolongation of the growth phase under food limiting conditions earns high cumulated respiratory costs and has to be paid for at the expense of growth and size.

In the present study in contrast to the temperate daphnids, Moina micrura and Diaphanosoma excisum did not indicate any direct effect of temperature on neonate size but larger neonates were associated with high levels of fecundity which is a result of high temperature and food levels above 0.1 $mgC.L^{-1}$. In addition, neonates of Diaphanosoma excisum showed a positive correlation with food concentration, and both species produced small neonates at the very low food level of 0.05 $mgC.L^{-1}$ (Table6.13).

In contrast to the above, the sparse results for Daphnia lumholtzi indicate that this species produced significantly smaller eggs at 0.1 $mgC.L^{-1}$ and 22°C than at higher food levels and this response resembles that of the temperate daphnids, but it is not possible to reach any firm conclusion without knowledge of the behaviour of this species at other temperatures.

In Diaphanosoma excisum the neonates produced by larger primipara tend to be bigger. Unlike temperate species, because the tropical species involved in the present study are small their sizes were difficult to measure critically and it was not possible to examine any other relationship.

In any case in Diaphanosoma excisum and Moina micrura the juvenile phase was prolonged at the lowest food level (0.05 $mgC.L^{-1}$) and this would involve a high cumulative respiratory cost, made still higher by tropical temperatures and this resulted in small neonates, in contrast to the temperate daphnids.

Changes in egg size of field populations of cladocerans were related to temperature by Burgis (1967) and Kerfoot (1974). In the former study a higher volume of eggs was recorded in Ceriodaphnia pulchella than in Ceriodaphnia reticulata. As it is now known that temperature-food interactions have an affect on egg size and also that food limitation on the mother affects the size of eggs produced in addition to the effect of temperature, there may have been a food effect which influenced the egg size in this study. Kerfoot (1974) investigated the effect of temperature on life-cycle parameters and found a negative relationship between egg size and temperature in a population of Bosmina longirostris, but not in the other species inhabiting the water body. Here too the food effect was obscured and, in addition, the food available in the reservoir at that time may not have been a good edible food for this species, which could explain why only this species was affected.

9.6 DURATION OF EMBRYONIC DEVELOPMENT

The manner in which the size of eggs/neonates produced by the mother is affected by food and temperature has already been discussed. However, the eggs which are produced into the brood pouch of a cladoceran have to develop under various environmental conditions.

In many earlier studies (Ingle et.al., 1937; Hall, 1964; Korinek, 1970; Weglenska, 1971; Kryutchkova, 1973; Bottrell, 1975a; and Bottrell et al, 1976; Magadza, 1977; Leveque & Saint-Jean, 1983) the duration of cladoceran embryonic development was found to be temperature-dependent. However, there is no evidence in these studies that embryonic duration is affected by food concentration available to the mother. This study presents evidence that not only decrease in temperature increases embryonic duration, but that eggs produced by the mother reared in low food levels have significantly increased embryonic duration: Moina micrura had significantly prolonged embryonic duration at low food concentration (Table 5.3b). Orcutt and Porter (1984) found that in Daphnia parvula both temperature and food level influenced embryonic duration. In their study, conducted at 15°C, 20°C and 25°C and with food concentrations of 0.02, 0.2 and 2.0 $mgC.L^{-1}$, the duration of embryonic development decreased with increase in food concentration at all temperatures but, at the lowest food level, temperature had no effect and the greatest prolongation of embryonic development was noted. Rocha (1983) working on three species of Daphnia, showed that Daphnia magna had a significantly prolonged embryonic duration at two low food levels (0.1 and 0.01 $mgC.L^{-1}$) but found no difference between the shorter duration at

two higher non-limiting food concentrations (0.5 and 1.0 $mgC.L^{-1}$). These results indicate that Daphnia magna significantly prolongs embryonic duration below 0.1 $mgC.L^{-1}$. No difference in duration with food concentration was found in Daphnia hyalina. No relationship was obtained for Daphnia pulicaria as data was available for only two temperatures.

Increase in egg size resulting in the prolongation of embryonic development associated with large eggs is known for both Cladocera (Munro, 1975; Bottrell, 1975a; Bottrell, 1975b; Vijverburg, 1976; Lynch, 1980b) and for copepoda (McLaren, 1965 & 1968; McLaren et.al. 1969). There is some evidence in daphnid species that larger eggs are produced by the mothers reared in lower food concentration (discussed above). Therefore, due to the larger size, prolongation of embryonic development of these eggs which were carried by the mothers fed with low food can be expected. The prolongation of duration of embryonic development in Moina micrura produced smaller neonates at this food level. Unlike other cladocerans, during embryonic development in the brood pouch of Moina micrura the embryo is not totally independent from the mother. The eggs are nourished by a unique placenta-like structure called "nahrboden" (Goulden, 1968; Goulden, cited in D'Abramo, 1980). This tissue, when fully developed, is composed of columnar cells which are believed to produce nutritive material for the developing embryos. Also unlike other daphnid eggs, the eggs of Moina micrura cannot develop if removed from the brood chamber of the mother (Fryer, personal communication). Hence prolongation of embryonic duration in

Moina micrura at 0.05 mgC.L^{-1} may be due to either a low level of nutritive material provided by the mother, or to the providing of this material at a slower rate. These interspecific differences imply that when calculating growth rates of field populations using mathematical models, two factors have to be taken into consideration:

(1) The reaction of the particular species to various food concentrations and the availability of edible food in the field must be known since low food levels affect embryonic duration in certain species.

(2) The temperature range within which the mathetical model operates has to be known to be applicable to the species concerned. There is evidence in the literature that both freshwater Cladocera and Copepoda are able to decrease the duration of their embryonic development to a minimum, beyond which the duration increases again. The temperature at which this minimum occurs is species-dependent. For example, the embryonic development of Daphnia ambigua was more prolonged at 30°C than at 25°C (Gelling, 1969) and the same occurs at temperatures beyond the minimum of Thermocyclops neglectus at 32°C (Burgis, 1970).

A comparison of the D_e for the temperate Daphnia magna (Rocha, 1983) and the tropical Diaphanosoma excisum and Moina micrura is made in Fig. 9.2. The two plots appear to fit together nicely but there is a suggestion of a lowered elevation in the two tropical species in relation to Daphnia magna and this needs to be tested statistically before we can say whether tropical species are not different from temperate ones.

9.7 SIZE AT FIRST REPRODUCTION

From the results of this study it is evident that both Diaphanosoma excisum and Moina micrura did not show any generally significant relationship between the size of the first reproductive female (primipara) and food concentration but the former did produce smaller primiparas at the lowest food level

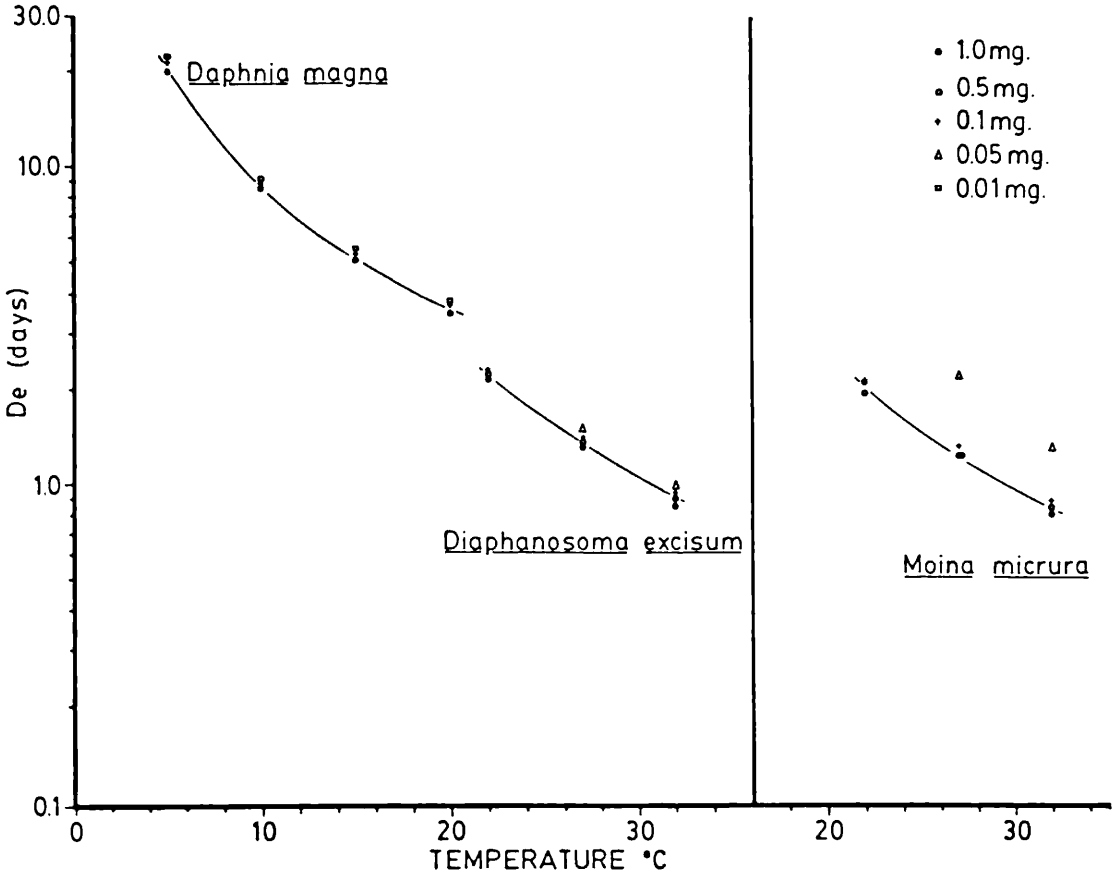


Figure 9.2 A comparison of the duration of embryonic development at different temperatures and food levels in the temperate Daphnia magna and the tropical Diaphanosoma excisum and Moina micrura.

(0.05 $mgC.L^{-1}$) at 32°C, and at both limiting food levels at 27°C. The latter species, on the other hand, showed a significant negative correlation between the size of primipara and temperature.

There is some evidence in the literature for increased primipara size at low food concentrations, at least in daphnids. Comparing the reproductive output of daphnids grown with natural and enriched (algae added to natural food) food, Green (1954), Hrbackova (1963, 1974) and Kerfoot (1974) all found larger primiparas when the food was enriched than in natural food. However, when Rocha (1983) carried out experiments with temperate daphnid species at constant defined temperature and food conditions which were similar to those used in the present study on tropical species and used the same unialgal food and experimental procedure she found that the size of the primiparous female increased with food concentration. But there was also a tendency for the primipara size to be smaller at lower temperatures, although only in limiting food levels (0.1 and 0.01 $mgC.L^{-1}$); not at all in non-limiting food conditions (1.0 and 5.0 $mgC.L^{-1}$).

Several other authors have also found an inverse relationship between temperature and primipara size; Ven kataraman et.al. (1980) recorded smaller primiparas in Daphnia carinata at 35°C (1.71mm) compared to larger primiparas (2.13mm) at 15°C.

Xiangfei (1984) found the primipara of the same species to be 1.712 $\pm$ 0.065mm, 1.859 $\pm$ 0.065mm, 1.859 $\pm$ 0.034mm, 1.640 $\pm$ 0.021mm, 1.500 $\pm$ 0.027mm and 1.473 $\pm$ 0.075mm at 15°C, 20°C, 25°C,

30°C and 35°C respectively. However, in both these cases the food level was not quantified so the decline in size might also be explained by a hidden food effect. This view is supported by the fact that in the latter study, the size of the primipara of Daphnia hyalina was found to decrease with increasing temperature and according to Rocha's results (see above) the animals were likely to be food-limited in the condition of their experiments.

9.8 DURATION OF POST-EMBRYONIC DEVELOPMENT

Food and temperature affect not only the size at maturity but also the time taken to reach maturity. In the present study, it was found that the duration of juvenile development was influenced by both temperature and food concentration but, in the case of the latter, the effect was only observed below the limiting food level in both Diaphanosoma excisum and Moina micrura. In these circumstances the duration was prolonged as food concentration fell. Food limitation of juvenile duration has seldom been investigated since most authors have restricted their interest to the effect of temperature (Hall, 1964+ Hrbackova-Esslova, 1966; Bottrell, 1975; Landry, 1978; Kankaala & Wulff, 1981; Xiangfei, 1983, 1984; Herzig, 1985 and Magazda, 1977). In all these investigations an inverse relationship was demonstrated between juvenile duration and temperature.

Considerable prolongation of the duration of juvenile developmnent at and below limiting food concentrations has been observed in both freshwater (Weglenska, 1971; Kryutchkova, 1973; Neil, 1981; Elmore, 1982) and marine (Mullin and Brooks, 1970; Paffenhoffer, 1970; Landry, 1976 and Vidal 1980b) cladocerans and copepods, while Paffenhoffer and Harris (1976) and Harris and Paffenhoffer (1976) also found an effect (prolonged development) related to quality of food.

Weglenska (1971) demonstrated the effect of food limitation on the duration of juvenile development using dilutions of natural seston from the entrophic Mikolajskie lake. She found the limiting food concentration above which no decrease in the duration of juvenile development was observed. This limiting food concentration differed in the various species she studied: for Diaphanosoma brachyurum and Daphnia longispina it was 2.5 mg wet weight L^{-1} (algae) and for Daphnia cucullata and Chydorus sphaericus it was 3.7 mg wet weight L^{-1}. She also noted that Diaphanosoma brachyurum and Daphnia longispina at 1.0 mg wet weight L^{-1} had slow developmental rates and suggested that this may be caused by the slowing down of filtration rate, associated with the disturbance of normal feeding in Cladocera by clogging of the filtering appendages with large planktonic algae.

In all the above examples, the duration of the juvenile phase was determined either in several food concentrations at one temperature, or at various temperatures in excess food. Since both environmental variables influence the duration of the juvenile phase (which is the main phase of body growth), and both vary in field conditions, serious errors can result from the application of values of D_j, derived from laboratory studies and in excess food, to field production estimates. In general D_j will be too short, and so production will be over-estimated. Before reliable field estimates of zooplankton production can be obtained there needs to be critical experimental study of the simultaneous effect of these two variables on body size, developmental duration and growth such as has been carried out by Duncan (1985, on daphnids from Lake Washington) Rocha (1983, on London reservoir daphnids), Piyasiri (1984, on a tropical copepod) and in the present study.

One of the first attempts to examine the effects of both factors simultaneously was that of Neil (1981) who examined the effects of various combinations of temperature and food concentration on Daphnia rosea from Lake Placid, using naturally occurring sestonic material. The food was those particles which passed through a 73 μm filter and the duration of juvenile development was found to increase in low food concentrations at the highest temperature observed (21°C). Contrary to expectation, using the same food concentrations he found that duration was shorter at 12°C and 15°C. He suggested that the prolongation of juvenile development was due to the fact that the metabolic need for increased nutritive material at the higher temperature was greater than the ability of the juvenile organisms to obtain it, when food concentration was low. In this study, although an attempt was made to quantify the concentration of food, food quality was not considered. Natural seston was used and may have contained non-edible particles. Thus the food available for the animal may have been lower than the concentration offered, and also, because natural food quality varies, the amounts of edible food in the medium were not constant throughout the experiment. In addition, since experiments were carried out in standing jars, settling of the algae would result in changing food concentrations with time. Hence it is not possible to use the findings of this experimental study for comparision nor is it possible to draw conclusions on the real effects of temperature. Most of these draw-backs were overcome by Orcutt and Porter (1984) by using unialgal food,

Chlamydomonas reinhardi which is a flagellate. In their study no difference was noted in the duration of juvenile development at 0.2 and 2.0 mgC.L^{-1} at 15°C, 20°C and 25°C but there was a prolongation of G% at 0.02 mgC.L^{-1}. From these results it is evident that food is limiting at a concentration between 0.02 and 0.2 mgC.L^{-1} for Daphnia parvula within the temperature range of 10-25°C. In the present study on Diaphanosoma excisum and Moina micrura, the food concentration at which food limitation appeared was between 0.1 and 0.5 mgC.L^{-1} at the higher temperature range of 22-32°C. Rocha (1983) found interspecific differences in the limiting food concentration for juvenile duration in temperate daphnids. In her studies at 5, 10, 15 and 20°C and 5.0, 1.0, 0.1 and 0.01 mgC.L^{-1} Daphnia magna generally increased its juvenile duration with decrease in food levels; the juvenile duration of Daphnia pulicaria differed significantly only between the highest and the lowest food concentrations, while Daphnia hyalina significantly prolonged juvenile duration only at 0.01 mgC.L^{-1}. Thus in those three species of Daphnia, juvenile duration was most sensitive to food concentration in Daphnia magna and least so in Daphnia hyalina.

It is evident from the present results that Moina micrura can develop to maturity at a faster rate than Diaphanosoma excisum at all food concentrations and under similar conditions (Fig. 5.1). The maximum juvenile development rate of Diaphanosoma excisum (0.406 day^{-1}) at the highest experimental temperature (32°C) was only slightly higher than that for Moina micrura (0.379 day^{-1}) at the lowest experimental temperature (22°C). At 32°C the rate for Moina micrura was twice that of Diaphanosoma excisum (0.850 day^{-1}) at the same temperature.

In order to compare the effect of temperature on temperate and tropical species the Q_{10} values for temperate daphnids were computed from the results of Rocha (1983). For the temperature range of 10° - 20°C Q_{10} for Daphnia magna, Daphnia pulicaria and Daphnia hyalina was 3.66, 1.81 and 2.87 respectively and, for Diaphanosoma excisum and Moina micrura within the range of 22° and 32°C, it was 2.24 and 2.22. It is therefore evident that the effect of temperature on both temperate and tropical species was somewhat similar but the tropical species develop at a faster rate in their higher environmental temperatures.

Since it is now evident that both temperature and food concentration affect juvenile duration it is worth looking at how this prolongation occurs. Among the cladocerans both variable (Anderson, 1932; Anderson et.al., 1937; Anderson and Jenkin, 1942; Green, 1954 & 1956; Richman, 1958; Venkataraman & Job, 1980; Xiangfei, 1983; Rocha, 1983) and constant (Ingle et.al., 1937; Hrbackova-Esslova, 1962; Xiangfei, 1984) numbers of juvenile instars have been recorded in relation to increase in juvenile duration. However, increase in juvenile duration could result from either, having the same number of instar but prolonged instar duration, or constant duration with increased number of instars, or from both. In the present study prolongation of juvenile duration occurred not only by increasing the number of instars but also by prolonging the duration of juvenile instars (Table 5.5).

The results of the present study give contradictory evidence to the hypothesis put forward by Hall et.al. (1976) and Allan and Goulden (1980) who suggested a positive relationship between body size and the duration of juvenile development. At first, in terms of length, the larger size and longer duration of juvenile development in Diaphanosoma excisum compared with Moina micrura may appear to support this hypothesis. But, in terms of weight, it contradicts it. The heavier body weight per unit length of Moina micrura is associated with a juvenile development that is shorter than in Diaphanosoma excisum. Rocha (1983) also demonstrated the invalidity of this hypothesis for temperate daphnids by examination of body lengths. It seems more likely that intraspecific variations in rate of development are evolutionary adaptations of different species to their environments. Hrbáček and Hrbáčková (1978 & 1980) suggested that the increase in developmental rate is an adaptation to predation. They compared species from situations of low predation, high predation and waters of different trophic levels. They suggested that predation causes selection towards species of higher juvenile development rate, and with lower K_s values, which thus have a competitive advantage at low food concentrations. When this is taken into consideration Moina micrura is better adapted to tropical warm water bodies, in the presence of predators, than Diaphanosoma excisum.

There is evidence in the literature that some freshwater invertebrates have developed a strategy of maturing at a smaller size to cut down the cumulative metabolic cost which results from prolonged juvenile duration, when food is limited. For example, Schiemer et.al. (1980) and Schiemer (1982a & b) have shown that benthic nematodes grown at limiting food levels mature at a smaller size, as well as at an earlier age, thus reducing the high metabolic costs of a more prolonged juvenile phase to reach a larger body size. This idea was supported by energy budgets and respiratory measurements. Rocha (1983) also found smaller sized primipara in three species of Daphnia grown in limiting food concentrations which suggests a similar strategy for counteracting prolongation of the juvenile phase by maturation at a smaller body size. Since Diaphanosoma excisum also exhibits smaller sized primiparous females at 0.05 $mgC.L^{-1}$, which is known to be a limiting food concentration for all the tested temperature conditions, there is the possibility that this species has a similar strategy for survival under food limiting conditions, although an energy budget would be needed to confirm this. It should be noted that Moina micrura does not show a similar strategy when food is limited but tends to mature at a smaller size at high temperatures (there is an inverse relationship between primipara size and temperature) and this may be a strategy to reduce the higher respiratory cost at higher temperatures.

According to Klekowski et.al. (1975), a cumulative energy budget from time t_o to time t_n can be expressed as:

$$A_c = P_c + R_c$$

where A_c is the assimilated energy cumulated for the period $t_o - t_n$

P_c is the production energy cumulated for the period $t_o - t_n$ and

R_c is the respiratory energy costs cumulated for the period $t_o - t_n$

In such a cumulative energy budget under limiting food conditions, R_c must always be covered in order for the animal to survive. The value of A_c depends on the availability of food, which is assumed to be affected by food limitation, and P_c need not be provided for at all but an animal developing from the neonate to the primipara stage must channel a certain amount of energy into growth. Therefore in an animal developing towards maturity A_c must always be greater than R_c. When the duration of the juvenile phase is prolonged R_c increases and hence it is advantageous to mature at a smaller stage. Under non-limiting food conditions increase in filtering rate with increase in body size is evident in temperate daphnids (Burns, 1969) and this may compensate for the increased R_c resulting from the extra time necessary to reach the greater size. The balance of these factors in food-limited animals is not yet known. Energy budgets of tropical cladocerans under limiting and non-limiting food conditions, are required for a clarification of these life-history strategies.

As it is now evident that both temperature and food concentration affect duration of juvenile development a knowledge of the effect of factors applied simultaneously is essential before laboratory derived durations can be applied to field populations, in order to estimate population dynamics and production. Life-cycle studies, carefully carried out can provide accurate information on the duration of developmental phases and their body size both of which are essential in

calculating production. However, there is the problem of how to express the simultaneous effect of these two environmental factors on juvenile duration. In the present study a highly significant relationship was found between the natural log of juvenile duration and the reciprocal of food concentration and the natural log of temperature. However, the predicted values were not a good fit to the experimental data. The same problem was faced by Rocha (1983) who also found this relationship gave the best fit but did not predict the experimental results accurately. Further analysis of the present data found a significant relationship between the reciprocal of the juvenile duration and the reciprocals of both food concentration and temperature which did predict the experimental data, although the variance ratio (F value) was not as high as that of the former relationship, (details in Chapter 5). This suggests that the highest variance ratio itself is not always an adequate decider of the best relationship when considering biological processes. A similar situation was discussed by Lie and Armitage (1980). They found a highly significant relationship between embryonic development and temperature but realised that the model was not adequate to predict embryonic development properly at some temperatures. Therefore they too suggested that levels of significance alone are not adequate for judgement of a relationship, but that it is also necessary to determine by how much values predicted by that relationship deviate from the observed data.

9.9 RATIO OF EMBRYONIC TO POST-EMBRYONIC DURATION OF DEVELOPMENT

Comparing both D_e and D_j of the four species involved in the present study, Leveque & Saint-Jean (1983) calculated the ratio of D_j/D_e (results given in Table 9.4) and concluded that Moina micrura has a juvenile duration almost equal to its embryonic duration. Calculation of this ratio from the results of the present study (Table 9.4) indicates that this ratio is food dependent and at low food levels the juvenile duration of Moina micrura is double that of the embryonic duration. The tendency for the D_j/D_e ratio to increase is evident for all species at low food levels in the present study. Comparing the two sets of results (Leveque & Saint-Jean, 1983 and the present study) it is evident that the importance of the effects of food limitation on cladoceran life cycle characteristics was not taken into consideration by most of the investigators when looking into the effects of temperature. Since it is evident that an increase in the ratio D_j/D_e is due to food limitation, comparing the results for Ceriodaphnia cornuta from the present study with those of Leveque & Saint-Jean (1983) for the same species, suggests that this species was food limited in the present experiment. This further supports the view that Scenedesmus acutus is not a good food for Ceriodaphnia cornuta.

9.10 POSSIBLE EFFECTS OF SIZE-SELECTIVE PREDATION

In the present study it was shown that, in reservoir populations, three species out of the four studied tend to have smaller primipara than in the experiments and clearly demonstrated that it was not due to either food limitation or

Table 9.4 A comparison of Dj/De ratio from the results of the present study with those of Leveque & Saint-Jean, 1983.

Present study

Temp. °C	Food conc. mgC.L^{-1} 1.0	0.5	0.25	0.1	0.05
Diaphanosoma excisum					
22	3.30	2.70	-	6.75	-
27	2.73	3.15	-	3.78	4.97
32	2.69	3.09	3.14	6.67	6.18
Moina micrura					
22	1.20	1.49	-	1.99	-
27	1.28	1.43	-	1.48	2.50
32	1.05	1.24	1.45	2.01	2.05
Ceriodaphnia cornuta					
32	3.32	2.83	4.00	-	-
Daphnia lumholtzi					
22	3.53	2.78	-	4.15	-

Results from Leveque & Saint-Jean 1983

	Temp. °C 25°C	30°C
Diaphanosoma excisum	2.13	2.32
Moina micrura	1.20	1.26
Ceriodaphnia cornuta	1.80	1.98
Daphnia lumholtzi	2.63	3.01

temperature. It was suggested that this reduction in size of the primipara was probably due to size-selective predation. It is not possible to reach this conclusion without experimental evidence gained under defined conditions as it may also be due to other environmental factors such as parasitization, which was observed among the field populations on all sampling occasions.

In the literature the decrease in body size of the primipara in field populations has often been explained in terms of size-selective predation without considering other environmental effects that could act upon the size of primipara. A good example can be obtained by examining the predictions of Brambilla (1982). In that study he found Daphnia pulex producing smaller primipara (0.8-1.0mm) and fewer small eggs (5-6 eggs; $0.0011mm^3$ each) at 18°C late in the season than earlier in the season at 10°C when the size of the primipara was 1.5-1.6mm and there were 30-40 eggs per clutch ($0.0026mm^3$ each). He showed that in the early season mortality was due to invertebrate predation on animals <0.75mm which were roughly the first and second instars. Hence larger eggs enabled these two instars to be completed more quickly than when the eggs were smaller. In the late season Ambystoma larvae preyed on larger sizes, and at the same time Diacyclops predated the animals <0.5mm, which is about the first instar. As the animals left that instar the mortality risk due to Diacyclops was no longer there, so reduced primipara size may have evolved to reduce mortality by Ambystoma. If this suggestion is correct, it seems odd, since a smaller neonate is not a prerequisite for a smaller primipara, that it did not

overcome the Diacyclops predation by having larger neonates (eggs) and the Ambystoma predation by having a smaller primipara, instead producing smaller eggs. Consideration of possible food effects is quite impossible because the cell counts given at weekly intervals do not give a clear indication of whether the animals were food limited or not at this period. It is evident from the literature that fecundity is food dependent as discussed above, and in addition, Rocha (1983) showed that temperate daphnids tend to produce larger but fewer eggs under conditions of food limitation and also have a tendency to produce larger eggs at lower temperatures. Therefore, the production of fewer eggs in the late season could have been a result of food limitation and the smaller size might have been due to the effect of high temperature. Hence, there is no knowledge of this species under predator-free conditions at that time. This could have been tested by carrying out experiments in field enclosures or in the laboratory using natural food. In the absence of such experiments it cannot be said that this effect is definitely due to size-selective predation only.

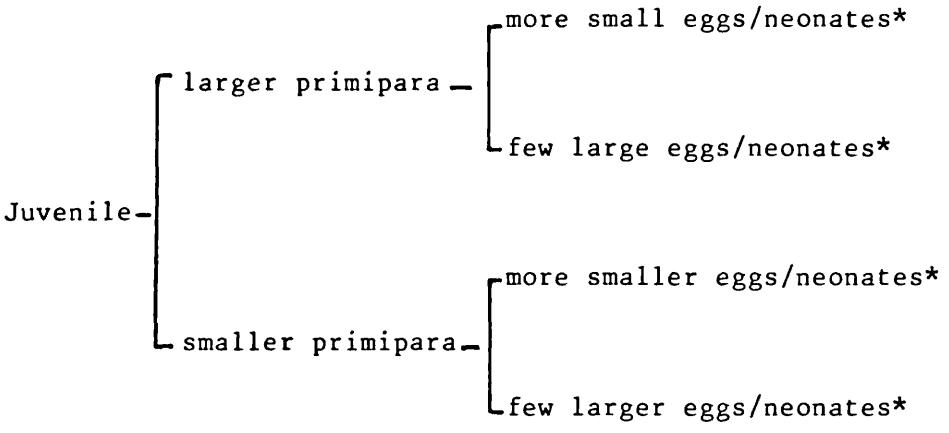
Green (1967) also concentrated only on size-selective predation in explaining changes in body and egg size of cladocerans and did not consider possible effects of food. He found that the Daphnia lumholtzi in Lake Albert were of different size in different regions of the lake. The small helmeted forms, with smaller eggs, were found in the marginal areas of the lake where there was a dense fish population, while the larger monacha form, carrying fewer but larger eggs, occurred in mid-lake. He

proposed that the small eggs developed into small helmeted forms which were less susceptible to fish predation whereas the larger eggs resulted in the large monacha form which was more vulnerable to fish predation and was therefore only found where fish densities were low. From the results of the present study it is evident that Daphnia lumholtzi produces larger eggs at low food concentrations but it is not possible to extend this information due to the paucity of results. Nevertheless it can be said that the larger eggs carried by the monacha forms in open water of Lake Albert may also have been due to low food availability, and it is not possible from the data given to eliminate the possible effect of food and to conclude that the results were solely due to the effect of predation.

These two examples, and many more in the literature, concentrate on size-selective predation in explaining differences in the life cycle characteristics of field populations without considering other environmental factors such as food and temperature which act upon the animal simultaneously. It is now evident that, in order to understand properly the effect of predation on life cycle characteristics, controlled experimental evidence is further required, and that shifts in the size of the primipara to a smaller range, or production of larger eggs and neonates, cannot necessarily be explained as due to size-selective predation alone.

9.11 FACTORS INFLUENCING SIZE AT MATURITY, SIZE OF EGGS AND SIZE OF NEONATES

From experimental results and field observations recorded in the literature it is clear that a number of envrionmental effects influence the size of the primipara, eggs and neonates. Stern (1976) states that under conditions of limited energy supply, allocation of energy to various biological processes is linked by various trade-off models. Following this idea, and using evidence from the literature as well as from the present study, it is suggested that a juvenile cladoceran could follow any of the paths given in the following scheme:



*not only at primipara stage but throughout reproduction.

Using the amount of energy available for reproduction a primipara can produce either more small eggs and neonates or few large eggs and neonates. According to Brambilla (1982), the reproductive success of a parent is measured by the total number of offspring surviving to reproduce: such success is influenced

both by offspring size and offspring number. Because a small neonate takes a longer time to attain a certain size of primipara (ie. D_j is prolonged) than a larger neonate, smaller cladoceran species having a relatively large neonate may be exposed more to invertebrate predation, both in the presence and absence of fish. (Lynch, 1980a). Where this is so a larger neonate may nevertheless be advantageous, because it soon becomes mature. On the other hand, in a small cladoceran species, the size range of the neonates will be small so, in spite of the necessary slight prolongation of D_j, a larger number of smaller neonates may enhance the number which will suvive predation by invertebrates. According to Brambilla (1980) Daphnia pulex becomes free from Diyacyclops predation after about the second juvenile instar. Therefore for larger species in such a situation it may be an advantage to produce larger neonates which quickly become large enough to avoid invertebrate predation. However, from the same work, it is evident that maturing at a smaller size can avoid the danger of vertebrate predation (by Amblystoma) on animals before they attain maturity. Moreover, early maturity is also advantageous in that the cumulated energy required for a neonate to attain a smaller primipara size is less than that for a larger one (Schiemer et.al., 1980; Rocha, 1983; Pilarska, 1977). Further, that a larger neonate can result in a smaller primipara in conditions of low food, compared to that reared in an enriched medium, is evident from the work of Hrbackova (1974). Although it is possible to mature at a smaller size there is a draw-back; a smaller primipara is less fecund than larger ones due to the direct influence of maternal size on fecundity (discussed above).

According to Neil (1975) and Lynch (1979), when food supply is limited, its most critical effect is on juvenile mortality, and this may lead to the decline of a species population resulting from a failure of its juveniles to reach maturity. This might be overcome by producing a larger egg. Rocha (1983) found a tendency for her daphnids to produce larger eggs in low, limiting food concentrations and in low temperature conditions. Green (1966) and the present study give further evidence for the production by daphnids of bigger eggs in low food conditions. Further field observations of winter breeding forms having fewer and larger eggs than summer breeding forms, in different taxonomic groups have been recorded by McLaren (1965), Mc Laren et.al. (1969), Steel and Steel (1975), Bagenal (1971) and Southward and Demir (1974). This is explained as an evolutionary adaptation to the larger juvenile mortality caused by low growth rates at low temperatures. Therefore production of larger eggs under conditions of severe food limitations, as well as low temperature conditions, may be a strategy to counter-balance what would otherwise be very prolonged juvenile duration. Both Green (1954, 1956) and Hrbáčková (1963) found the number of pre-adult instars to be dependant on neonate size, larger neonates having fewer pre-adult instars.

Whether or not to mature at a larger or smaller adult size must also depend on the ratio of the increase in cumulative assimilated energy with time to the increase in cumulative respiratory cost plus cumulative production energy, for the particular species at a particular temperature as discussed

above. If cumulative increase in assimilated energy is high it is advantageous to reproduce at a bigger size and vice versa. When temperature is also taken into consideration the energy loss due to metabolism increases with temperature, but the time required to attain maturity decreases, at a particular food level. Thus, the increased energy loss at a higher temperature is normally compensated for by an increase in food consumption (discussed above).

Taking all these factors into consideration it seems that it is advantageous for small Cladocera living in a tropical water body to have a smaller primipara and produce more small eggs throughout the reproductive period. Some results of the present study support this consideration. Moina micrura matured at a smaller size at higher temperatures, irrespective of the amount of food available. Also at very low food levels, with the comparatively lower excess energy available for reproduction compared to high food levels, they produce smaller offspring, which may be an adaptation to get maximum output with the small amount of energy available. Diaphanosoma excisum attempted to produce the maximum number of neonates possible, when energy available for reproduction decreased with decreasing food, by producing smaller neonates as food level decreased. Also at very low food levels this species attained maturity at a smaller size. In contrast, the rather larger Daphnia lumholtzi responded quite differently, producing larger neonates in low food.

Lynch (1980a) has put forward a theoretical model to predict optimal size at maturity and the optimal compromise between offspring size and number of organisms living in different environments. This model predicts that, in the absence of size selective predators and in an enriched environment, the species which mature at a relatively large (but not their largest) size will be selected by the predator and the size of their offspring will be intermediate. Small species in such an environment will be best advantaged by producing the smallest offspring possible thus allowing a maximum clutch size. This is because, in small species which grow only a little before maturity, a reduction in offspring size produces only a minor increase in the age at maturity. This pattern will, however, vary in different environmental conditions. The present results do not agree with this view because both Diaphanosoma excisum and Moina micrura at high food levels (comparable to an "enriched" medium) do not produce the smallest possible neonates: the smallest are produced in the lowest food concentrations. Daphnia lumholtzi does the opposite. Therefore, as Stearn(1976) says, there are several reacting complexities of gene interaction and linkage, plus physiological and morphological limitations, why the theoretical optimal life history may never evolve in real organisms.

However, from examination of the past literature, it is evident that food, temperature and predation pressure all have effects on the reproduction parameters of cladocera and it is also clear that these cannot be considered in isolation because

there is interaction between them. There is no possibility of isolating each of these effects directly from field data, as a zooplankton sample obtained at a particular time will contain overlapping generations in most environments (Allan & Goulden, 1980). In a flow-through water body continuous input and output of water changes food regimes, in addition to the effects of normal grazing. The animals face diurnal vertical migration exposing them to variable temperature and food conditions. In addition they are susceptible to predator pressure. Therefore to understand these complicated interactions in nature experimental knowledge of the animal's growth and reproduction under defined conditions is required. In the present study the effects of food and temperature are not always the same in the genera studied. There are both inter and intra specific variations in the effect of food and temperature conditions and experimental knowledge is required at species level in order to understand the events occurring in nature.

9.12 THE STRUCTURE OF THE FILTERING LIMBS

a) Do they filter or not?

Discussing the filtering mechanism of daphnids, Porter et.al. (1983) and Ganf and Shiel (1985 a & b) have put forward a hypothesis that the third and fourth limbs play no role in the filtering processes but act only as paddles in creating a water current. If this is true, there is no point in examining the possibilities of filtration and the ultrastructure of the filtering comb. Hence, it is necessary to look into this matter first. Their hypothesis was put forward on the basis of the Reynolds number and boundary layer thickness around the setule.

According to their calculations the boundary layer surrounding the setules extend across the intersetular distance such that no water would pass through. These proposed properties are based on mathematical models but have not been experimentally proved with daphnid filtering.

Reynolds number is a measure of the ratio of inertial to viscous forces and, to break the laminar layering, Reynolds number has to be greater than one. These parameters are related as follows:

Inertia force $Fi = \rho SU^2$, where ρ = density

S = area

U^2 = velocity $^{-2}$

Viscous force $Fv = SU\mu/L$, where μ = kinematic viscocity

U = velocity

L = distance between two plates

Reynolds Number $(R_e) = \frac{Fi}{Fv} = \frac{\rho SU^2}{SU\mu} .L$

Therefore $R_e = \frac{\rho UL}{\mu}$

μ varies with temperature according to the following equation (Smith, 1975):

$\mu = 0.0178/(1 + 0.0337Q + 0.00022Q^2)$ where Q = temperature.

According to Vogel (1981) L is a measure called "characteristic length" and for a solid immersed in fluid L is taken as the greatest length of the solid in the direction of the flow.

The authors who formulated this hypothesis for Daphnia limbs gave the formula of R_e Number as here, but the values they used have some drawbacks.

(a) Porter et al. (1983) calculated the speed of the setular movement by using several assumptions. They used the beat rate measured previously (Porter et.al., 1982) in terms of cycles per second. The distance moved by the limb was calculated by assuming that the leading distal edge of the appendage completed a cycle through an arc of 60°, as 1/3 of the circumference of a circle with a diameter equal to the setal length (length of the filter comb). There is no evidence to show that daphnid limbs move in this manner.

The velocity, which is a vector property, involves the direction of movement. The speed of the limb need not be equal to the velocity of the setules as the setules are attached to the setae at an angle (Fig.3.3b, Plate 4, Watts, 1981) and setae in turn are attached to the gnathobase somewhat perpendicularly. As the limb is moving in a medium of water, using the values of speed of limb movement, which was calculated using several assumptions, and assuming that value to be in turn equal to setular velocity, can generate an error in the calculated Reynolds Number.

Ganf and Shiel (1985a & b) used the same appendage beat rate values from Porter (1982) for an entirely different species despite the fact that Porter et.al. (1982) showed that beat rate is species dependent. Also the filtering rate, which is dependent on appendage beat, varies with both temperature and food concentration. (McMahon, 1965; Burns, 1969; Rigler, 1971; Harward and Gallup, 1976; McMahon and Rigler, 1965). Thus, it is necessary to obtain real figures for setular velocity when

calculating the Reynolds number and the boundary layer associated with the setule. An example indicating that the rate of movement is an important value in these calculations is quoted by Zaret (1980) who said that the Reynolds Number associated with a zooplankton may range from 0.1 for a sinking Bosmina to 3 for a swimming Bosmina.

(b) The calculations were made taking into consideration only the appendage beat which implies that water in the filter chamber is not subjected to any other forces. According to Fryer (1968) in some chydorids a water current is set up by the exopod pump of trunk limbs 4 and 5, which enters the carapace chamber, and ultimately the filter chamber anteriorly and ventrally. If such water currents are created in other groups there are resultant forces that act on the setules. Without taking these forces into consideration it is not possible to calculate a precise Reynolds Number associated with the setules. Further, according to Geller (personal communication) a "one way valve system" is operating within the filter chamber that could force the water through the setules by breaking the boundary layer.

(c) The value of L used in the calculation was the diameter of the setule. According to Vogel (1981) L should be the greatest length of the solid in the direction of the flow. As the setule is attached at an angle to the seta (Fig 3.3b) the direction of the water flow is not from the forefront to the setule but at an angle. Therefore in such a case L is always greater than the diameter of the setule.

It is essential to take all these factors into consideration when calculating the Reynolds Number. At this stage it is not possible to conclude that the limb is acting at a Reynolds Number less than one. It is necessary to examine the functional morphology of the whole limb system, the action of the carapace (the carapace gape) and the geometry of the filtering chamber to determine the forces acting on setules in addition to the beat of the "filtering limbs" to reach a proper calculation of the Reynolds Number. It is clear that it is still not possible to accept or reject the hypothesis that the third and fourth limbs of daphnids act only as paddles.

Porter et.al. (1983) suggested that the second limb may be involved in food capturing while Ganf and Shiel (1985a) experimentally demonstrated this. While it is possible to accept that the second limb does play such a role, it is not yet clear whether or not this is the only limb with such a role. If only the second limb actually captures food in daphnids, the question arises in Diaphanosoma excisum, where the first five pairs of limbs have filter structures, if they only act as paddles creating water current, how food collection is achieved in Ctenopods.

From examination of intersetular distance recorded in the literature (Table 9. 5) it is clear that this cannot be considered as a species-specific character. Korinek and Machack (1980) and Korinek et.al. (1981) claim that the density of setules per 100μm setal length in populations from lakes and reservoirs, with low amounts of seston, was smaller than those

Table 9.5 The range of intersetular distance (ISD) in cladoceran species found by different authors: (1) Geller & Muller (1981), (2) Porter et.al. (1983), (3) Hessen (1985), (4) Ganf & Shiel (1985), (5) Brendelberger & Geller (1985), (6) Present study.

Species		ISD (μm)	Body length (mm)
Daphnia magna	(1)	0.24 - 0.64	2.00 - 3.00
	(5)	0.26 - 0.54	0.88 - 2.70
	(2)	1.00 ± 0.02	2.36 ± 0.02
Daphnia cucullata	(1)	0.23 - 0.45	0.70 - 0.80
	(5)	0.76 - 0.45	0.90 - 1.50
Daphnia galeata	(1)	0.32 - 1.00	1.70 - 2.10
	(5)	0.30 - 0.74	0.60 - 2.10
Daphnia pulicaria	(1)	0.45 - 1.40	1.80 - 2.40
	(5)	0.40 - 1.11	0.68 - 3.20
Daphnia hyalina	(1)	0.56 - 1.80	1.70 - 2.20
	(5)	0.28 - 0.54	0.70 - 1.90
Daphnia longispina	(3)	0.50 - 0.90	1.40
	(5)	0.28 - 0.78	0.68 - 2.25
Daphnia pulex	(5)	0.20 - 0.90	0.53 - 2.80
Daphnia carinata	(4)	0.23 ± 0.02	2.34 ± 0.07
Daphnia lumholtzi	(6)	0.15 ± 0.46	0.57 - 1.90
Ceriodaphnia quadrangula	(1)	0.24 - 0.40	0.40 - 0.50
	(3)	0.40 - 0.80	adult
	(4)	0.14 - 0.16	0.67 ± 0.01
Ceriodaphnia cornuta	(6)	0.16 - 0.20	0.25 - 0.53
Moina micrura	(6)	0.13 - 0.40	0.55 - 0.70
Daphnaosoma brachyurum	(1)	0.16 - 0.24	0.80
	(3)	0.20 - 0.30	adult
Diaphanosoma excisum	(6)	0.14 - 0.26	0.50 - 0.96
Holopedium gibberum	(1)	1.43 - 4.30	1.00 - 1.50
	(3)	1.80 - 3.90	1.50
Sida crystalina	(1)	0.90 - 4.20	1.50 - 1.70

living in ponds and laboratory cultures with a high seston content. Geller and Muller (1981) supported this observation but there is no comparative data given in any of these papers. Brendleberger and Geller (1985) compare the relationship between body length and mesh size of Daphnia hyalina and Daphnia galeata from a mesotrophic habital and eutrophic pond. In both species the mesh size of the mesotrophic population was greater than in the eutrophic population. The slope of the regression line was also greater for the population from the mesotrophic habitat than that from the eutrophic pond. This difference was more marked in Daphnia hyalina than Daphnia galeata. Another remarkable feature of Daphnia hyalina was that the intersetular distance of the animals from the eutrophic pond was restricted to between 0.3 - 0.34 μm while in the mesotrophic population it increased from 0.4 - 1.4 μm, within the same body length range of approximately 0.6 - 1.9 mm. This indicates that the increase in mesh size with body length is also not a constant species specific character. If the intersetular distance also does not play any role in food capturing one would expect it to be stabilized within a species. But the difference in intersetular distance at different localities suggest that this morphological difference has some significance. If it s sole responsibility is to create a water current when the limbs act as paddles, one would expect stabilization at an optimum size which is most beneficial to the animal at least within a species. In the present study the similar mesh size found throughout the size range of Ceriodaphnia cornuta may not therefore be a character which is confined to

this species. It is obviously necessary to examine more specimens from different habitats. However, it is the only exception in the present study, in that the other three species, which originated from the same lake did show increasing mesh size with body length.

The results of Gophen and Geller (1984) provide evidence that inter-setular distance does play a role in food capturing. They accept the explanation of Rubenstein and Koehl (1977) for the capture of particles smaller than the inter-setular distance. The piggybacking mechanism (small particles attached to larger particles) and the stickiness of particle surfaces suggested by Porter et.al. (1983) explain the uptake of smaller particles only in the presence of larger ones. Uptake of 0.5μm beads, which were smaller than the inter-setular distance of Holopedium giberum as observed by Hessen (1985) can be explained either by the mechanism suggested by Rubenstein and Koehl's (1977) or by Porter et al's (1983) view, or by looking into the nature of the mesh. The structures revealed by the electron microscopes in the species studied here, and those which are shown in the literature, reveal that it is not a rigid structure but there is a certain amount of flexibility. According to Horn (1985) the filter apparatus can be regarded as a sieve with different hole sizes. The electron micrographs support this view showing that the setules are not rigid, forming a rigid mesh but are indeed flexible. Assuming that these structures do indeed act as filters, all the limbs which bear setae and setules together form the total filter area.

(b) The filter area and inter-setular distance

Previous workers, who have recorded significant regressions between filtering area and body length, worked with temperate species of cladocerans and only on Daphnidae. The present study has provided some information on tropical species of cladocerans, both in Daphnia and other genera. Table 9.6 collects together the information available on this relationship and it is graphically presented in Fig. 9.3. This shows that the relationship for these tropical species fall within the spread of those for temperate species. The rather steep regressions for Ceriodaphnia cornuta and Moina micrura may be related to the small range of body sizes available for these species (0.25 - 0.5mm and 0.45 - 0.75 mm respectively).

It is evident from the literature that a higher filtering rate is not always a result of larger comb area. Egloff and Palmer (1971) found relationships between comb area and body length for Daphnia rosea and Daphnia magna and by relating these to the relationship between filtering rate and body length which was recorded by Burns (1967) and McMahon and Rigler (1965) demonstrated a positive correlation between filtering rate and comb area for these two species. Since Daphnia rosea of the same body length as Daphnia magna had larger filtering rates and comb areas, they suggested that their higher filtering rate was dependent on their larger comb area. In contrast Arunda (1983) found that Daphnia similis, with a comb area similar to that of Daphnia parvula of the same body length, filtered at a much lower rate. However, since it is now evident that maximum filtering

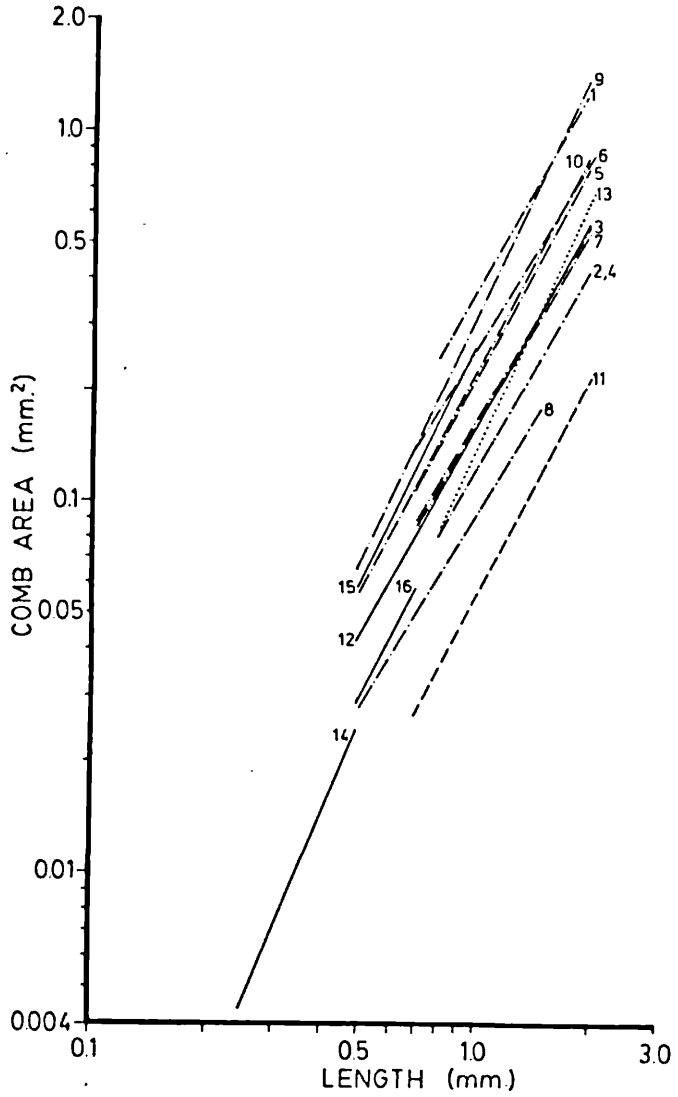


Figure 9.3 A comparison of the relationship between total comb area (mm^2) and body length in planktonic Cladocera. Total refers to the sum of limbs 3 + 4, except in Diaphanosoma excisum in which 5 limb areas are summed.

KEY:- Egloff & Palmer (1971): Daphnia rosea (13), Daphnia magna (4). Korinek & Machacek (1980): Daphnia pulicaria (2). Ganf & Shiel (1985): Daphnia carinata (11). Brendelberger & Geller (1985): Daphnia magna (1), Daphnia curvirostris (5), Daphnia pulex (6), Daphnia pulicaria (3), Daphnia longispina (7), Daphnia cucullata (8), Daphnia hyalina (9), Daphnia galeata (10). Jayatunga (present study): Daphnia lumholzi (12), Ceriodaphnia cornuta (14), Diaphanosoma excisum (15), Moina micrura (16).

Table 9.6 The relationship between total filtering area and body length found by different authors. Regression equation: $Y = aX^b$ (Y = total filtering area (mm^2), X = body length (mm), r = correlation coefficient). (1) Brendelberger & Geller, 1985; (2) Egloff & Palmer, 1971; (3) Gnaf & Shield, 1985; (4) Korinek & Machacek, 1980; *(5) Present study.

Species		a	b	r
Daphnia magna	(1)	0.362	1.74	0.97
	(2)	0.121	1.77	
Daphnia pulicaria	(1)	0.164	1.73	0.99
	(4)	0.121	1.77	0.99
Daphnia rosea	(2)	0.144	2.22	
Daphnia curvirostris	(1)	0.212	1.87	0.99
Daphnia pulex	(1)	0.250	1.63	0.97
Daphnia longispina	(1)	0.159	1.71	0.98
Daphnia cuculata	(1)	0.089	1.68	0.86
Daphnia hyalina	(1)	0.030	2.23	0.96
Daphnia galeata	(1)	0.215	1.92	0.97
Daphnia carinata	(3)	0.054	2.00	0.97
Daphnia lumholtzi	*(5)	0.154	1.86	0.98
Ceriodaphnia quadrangula	(3)	0.072	1.59	0.92
Ceriodaphnia cornuta	*(5)	0.142	2.55	0.99
Diaphanosoma excisum	*(5)	0.257	2.16	0.88
Moina micrura	*(5)	0.123	2.13	0.97

rate is not a result of maximum filtering area and vice versa, although Daphniaosoma excisum bears the highest filtering area it may not be the most efficient filtrator out of the four species investigated.

In the present study a significant increase in mesh size with body length was found only for Diaphanosoma excisum, Moina micrura and Daphnia lumholtzi; no significant increase was evident for Ceriodaphnia cornuta, although all four species exhibit a significant relationship between filtering area and body length. Brendleberger and Geller (1985) related the mesh size of four daphnids to filtering area and found three types of relationships such that when body size increased (a) filtering area also increased but there was no increase in mesh size, (b) both filtering area and mesh size increased, (c) there was an increase in mesh size but only a very small increase in filtering area. In the present study Daiphanosoma excisum, Moina micrura and Daphnia lumholtzi belong to category (b) while Ceriodaphnia cornuta belongs to category (a).

(c) Summary

(1) Tropical species are similar to temperate species in their filter structure and filter area in spite of their smaller body size.

(2) Being a ctenopod Diaphanosoma excisum has a greater filter area compared to anomopods but the relationship between filter area and body size is similar to that in anomopods.

(3) Moina micrura, a member of the Moinidae, has a resemblance to members of the Daphnidae in its filter structure but the increase in mesh size relative to body size in this species is much more pronounced than in Daphnids.

(4) There is still no definite evidence to reject the hypothesis that in Daphnidae the third and fourth limbs play a role in food capture.

(5) Inter-setular distance seems more of a habitat specific character than a species specific character.

(6) The four species studied here can be classed as microfiltrators according to the classification of Geller (1981) ie. they can feed on particles less than 1μm in diameter.

(7) The filter structure (inter-setular distance) does not explain why Ceriodaphnia cornuta is not able to grow in Scenedesmus acutus, but the smaller range of mesh sizes throughout the body length range reveals that they can feed on very small particles such as bacteria.

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Appendix 1

Algal culture medium CHU12 (Stein,1973)

Stock solutions.

K_2HPO_4	$50gl^{-1}$
$MgSO_4\ 7H_2O$	$50gl^{-1}$
Na_2CO_3	$20gl^{-1}$
$FeCl_3$	$4gl^{-1}$
$Ca(NO_3)_2$	$200gl^{-1}$
Na_2SiO_4	$125gl^{-1}$

As ordinary chemicals which contain tracer elements as impurities were used in the preparation of the medium it was not necessary to add trace elements to the medium.

To make the culture medium 1 ml of each of these solutions was added to 1l water and autoclaved for 15 minutes.

Appendix 2

Determination of organic carbon

(a) Wet-oxidation method

The carbon content of the stock algal food (page) and field sestonic carbon (page) was determined by wet dichromate oxidation method. (Mackereth et.al., 1978).

Principle

The organic matter reacts at 100°C with a strong oxidizing mixture (Potassium dichromate and sulphuric acid) and the decrease in oxidant (Potassium dichromate) can be determined by titration with a ferrous salt. The accuracy of the end point can be increased by detecting it amperometrically.

When organic matter is oxidized it yields its inorganic constituents according to the following equation and the amount of carbon can be calculated from the amount of oxygen used.

$$C_x H_{2y} O_z + \left(x + \frac{x - z}{2}\right) = O_2 \text{ -- } xCO_2 + yH_2O.$$

The values of x, y and z can be determined only if the composition of the organic matter is known.

Considering

$$C_6H_{12}O_6 \text{ --} \blacktriangleright 6CO_2 + 6H_2O$$

1 mg of $O_2 \equiv 0.375$ mgC

When dichromate is used as the oxidant

1 ml of 0.125 $K_2Cr_2O_7$ 1 mg O_2

1 ml of 0.125 $K_2Cr_2O_7$ 0.375 mg C

1 ml of 0.01 $K_2Cr_2O_7$ 0.8 ml of 0.125 N $K_2Cr_2O_7$

1 ml of 0.01 $K_2Cr_2O_7$ 0.030 mgC

as 1 ml of 0.01 $K_2Cr_2O_7$ 1 ml of 0.01 N FAS

(ferrous ammonium sulphate)

1 ml of 0.01 FAS 0.030 mgC (30μgC)

Using glucose as a substrate at 5 concentrations, it was determined that 1 ml of 0.01 N FAS was equivalent to 30.7 μgC. The results are given at the end of this Appendix. The 0.7 μgC difference compared to the theoretical value may be due to experimental error but is <2.5%.

Reagents used

(a) O.2N Potassium dichromate ($K_2Cr_2O_7$)
Analytical grade was oven dried at 60°C to exclude water. This was done by weighing it to a constant weight. O.9808g was then dissolved in 100ml water.

(b) Silver sulphate - sulphuric acid (Ag_2SO_4-H_2SO_4)
O.24g of analytical grade was dissolved in 20ml of analytical grade sulphuric acid

(c) O.01N Ferrous ammonium sulphate ($FeSO_4.(NH_4)_2SO_4.6H_2O$)
O.1N Ferrous Ammonium Suphate stock solution was prepared by dissolving 9.8g of analytical grade reagent in 100ml of double distilled water and 5ml of acid, which was then diluted up to 250ml. The stock solution was brought to O.01N by further diltion.

Apparatus:

(a) 50 ml beakers with solid glass covers. These were cleaned in hot chromic acid and double distilled water and kept inside a desiccator to prevent contamination by dust.

(b) Thermostatically controlled water bath.

(c) 2 ml and 1 ml pipettes washed in hot chromic acid and double distilled water and kept under cover.

(d) 10 ml Metrohm piston burette.

(e) Narrow stemmed platinum-calomel electrode (type EA 234 of Metro hm).

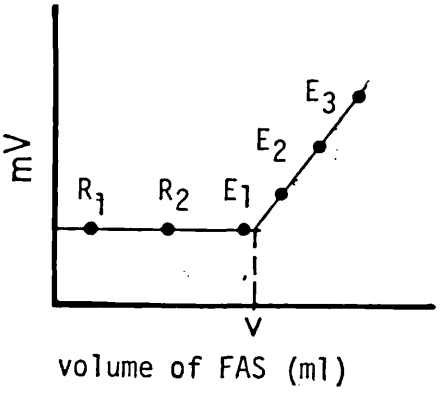
(f) Magnetic stirrer and follower.

(g) Corning research pH meter with an expanded scale.

Experimental procedure:

A known amount of algae or reservoir water was filtered on to a GF/F pad of 2.5 cm diameter which had been muffled at 500°C for 2 hours to remove background carbon, and stored in muffled aluminium foil. Each filter was put into a 50 ml beaker containing 1 ml of 0.2N $K_2Cr_2O_7$ and 2 ml of Ag_2SO_4/H_2SO_4 mixture. At least three replicates were carried out and in addition, two blanks (muffled GF/F pads without algae) at the same time. The beakers were well covered with glass tops and the samples were digested for two hours in a 100°C water bath. If, during digestion, a blue green colour appeared, indicating the absence of $K_2Cr_2O_7$, another 1 ml of $K_2Cr_2O_7$ and 2 ml of Ag_2SO_4/H_2SO_4 was added. After digestion, samples were cooled and 15 ml of double distilled water was added to each sample. Subsequently a magnetic follower was added to the sample and it was kept on a magnetic stirrer with a constant speed. This was important, as it was found that the slight variation in stirring resulted in variable readings. Then the electrode was immersed in the sample during the titration against ferrous ammonium sulphate. As the end-point was approached, the needle of the meter started to flicker and came back to the original reading. After this point ferrous ammonium sulphate was added in very small volumes. Just

after the end-point, each addition of ferrous ammonium sulphate showed a fixed, but increased, reading. Three consecutive readings were taken and plotted against the volume of ferrous ammonium sulphate used. The readings prior to the end-point and after the end-point were joined separately and the volume of ferrous ammonium sulphate used at the end-point was obtained as in the following illustration.



R_1, R_2, R_3 = readings prior to the end-point

E_1, E_2, E_3 = consecutive readings after the end-point

V = Volume of FAS use at end-point

It should be noted that the multimeter TM9B of Level Electronics which is recommended by Mackerth et.al. (1978) did not work in Sri-Lanka due to the effect of high humidity. This meter was then substituted by a corning research pH meter with an expanded scale.

The ferrous ammonium sulphate used in the titrations was standardized frequently using 1 ml 0.2 $K_2Cr_2O_7$ and 2 ml H_2SO_4/Ag_2SO_4 without heating to 100°C.

Calculation of results

Volume of FAS used in standardization = V ml

(against 1 ml 0.2 $K_2Cr_2O_7$)

Volume of 0.01 N FAS required to oxidize 1 ml 0.2 $K_2Cr_2O_7$ = 20 ml

Volume of FAS used in blank = B ml

Volume of FAS used in algal sample = A ml

Volume of algal sample = S ml

$\mu gC.ml^{-1}$ sample $= (B - A) \times \frac{20}{V} \times \frac{1}{S} \times 30$

The sensitivity of the method was tested by using different concentrations of analar grade glucose in addition to using the relationship of 1 ml 0.01 FAS being equivalent to 30 µgC.

Procedure

Analar grade glucose solution containing 600 µgC was prepared by dissolving 1.285 mg glucose in a litre of double-distilled water. By serial dilution with double-distilled water solutions which contained 300, 150, 75, and 37.5 $\mu gC.ml^{-1}$ were prepared. Using 1 ml of each solution, titration was carried out according to the procedure described above. Three replicates were done for each concentration and the means of the three replicates were calculated. At each concentration µgCarbon equivalent to 1 ml of 0.01 FAS was calculated.

The results are presented in Table A.1.

Concentration of ugC in solution	ugC equivalent to 1 ml of 0.01N FAS
600.0	30.673
300.0	29.720
150.0	30.808
75.0	30.787
37.5	31.566

Mean value of ugC equivalent to 1 ml 0.01N FAS = $\underline{30.71 \pm 0.66 \text{ ugC}}$

(b) Modified micro-titration method for determinati of the carbon content of field animals.

The principle is similar to that described above but that procedure, as followed for algal carbon, required a minimum of 30µgC per sample. To use the same procedure for the determination of animal carbon it would have required about 150-200 neonates per sample. The aim of the animal carbon determination was to establish a length/carbon weight relationship and it was difficult to sort enough animals of a specific length to give >10µg. Talling (1983, personal communication).

The following modifications were therefore used:

(1) Animals of known length were placed by a needle on to small GF/F pads which were cut into 0.8 cm circles. These had been previously muffled at 500°C for two hours.

(2) Samples were digested in covered 6 cm test tubes with a diameter of 1.5 cm, and covered with specimen tubes of 4 cm with a diameter of 2 cm. The digestion was carried out using a temperature controlled dry block heater.

(3) The concentration of $K_2Cr_2O_7$ used was 0.1 N to enhance sensitivity.

(4) The volume of digestants used was 0.1 ml of $K_2Cr_2O_7$ and 0.2 ml of H_2SO_4/Ag_2SO_4 per sample. Eppendof micro pipettes were used to pipette those volumes accurately.

(5) An Agla syringe attached to a micrometer was used instead of a piston burette so that the volume of FAS introduced could be controlled to the nearest 20µl.

(6) The number of blank samples used on each occasion was increased to 5.

The calculations were carried out in a similar manner to those for the algal carbon but the results were expressed as $\mu gC.animal^{-1}$.

APPENDIX 3

Measurement of Chlorophyl a content of Kalawewa Reservoir at the time of field sampling

The algal chlorophyll a was determined according to the hot methanol extraction procedure (described by Talling in Vollenweider, 1969).

Phytoplankton samples of known volume were filtered onto GF/F pads and these were placed in test-tubes with 5 ml of 90% methanol. The test-tubes were heated by immersion in a water bath for about two minutes until the pigment was extracted from the algae. The samples were then cooled under a black cover and the pad and cell residuals were sedimented by centrifugation. The volume of extracted pigment was made up to 10 ml in volumetric flasks. The samples were poured into 4 cm cuvetts (4 cm path length was used as readings fell below 0.1) and absorbance was recorded at optimal densities of 665 and 750 mm using a Pye-unicam Sp 6 - 450 uv/visible spectrophotometer.

Chlorophyll a concentration was calculated according to the equation (Talling & Driver, 1963).

$$\text{Chlorophyll a mg.l}^{-1} = K\left(\frac{D_{665} - D_{750}}{L} \times \frac{v}{V} \times \frac{1}{1000}\right)$$

K = 13.9 for methanol

L = path length (4 cm)

v = volume of extracted chlorophyll a

V = volume of alga in litre

Appendix 4

Carbon weights of individual animals: original data by a dry combustion method (Salonen, 1979)

emb = embryos

Diaphanosoma excisum at 1.0 mgC.L^{-1} food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.50		42	16.636	0.396
0.60		37	37.935	1.025
0.60		22	18.362	0.835
0.70		14	14.270	1.019
0.55		33	17.760	0.538
0.55		29	15.119	0.521
0.80	emb	12	19.179	1.598
0.80	emb	14	25.498	1.821
0.70		6	12.007	2.001
0.70		8	15.811	1.986
0.85	emb	7	15.492	2.213
0.85	emb	5	15.671	3.134
0.85	emb	3	11.226	3.742
1.00	emb	3	15.603	5.201
1.31	emb	2	20.939	10.469
1.22	emb	2	23.033	11.547

Diaphanosoma excisum at 0.25 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.792		4	6.84	1.71
0.883		3	5.31	1.77
0.850		2	3.22	1.61
0.873		2	3.34	1.67
0.806		3	5.49	1.83
0.835		1	1.44	1.44
1.087	emb	1	4.44	4.44
1.123	emb + ovary	1	7.38	7.38
1.037	emb	2	3.98	1.99
1.152	emb	1	3.31	3.31
1.037	emb + ovary	2	8.66	4.33
1.238	emb	2	11.30	5.65
1.080	emb	1	5.81	5.81
1.022	emb	2	5.42	2.71
1.080	emb	2	5.82	2.91
1.138	emb	2	6.32	3.18
0.504		4	2.04	0.51
0.560		3	1.62	0.54
0.620		4	3.20	0.80
0.900	ovary	4	9.92	2.48

Diaphanosoma excisum at 0.1 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg C ind^{-1}
0.756		4	3.20	0.80
0.835	ovary	2	2.84	1.42
0.922	ovary	3	9.75	3.25
0.933	emb	2	8.82	4.41
1.000	emb	2	8.82	4.41
0.950	emb	1	1.65	1.65
1.018	emb	2	6.90	3.45
0.936	emb	3	8.70	2.90
1.080	emb + ovary	1	4.78	4.78
0.763	emb	2	2.14	1.07
0.907	emb	4	13.00	3.25
0.550		3	1.77	0.59
0.550		5	1.75	0.35
0.760		3	3.00	1.00
0.812		2	2.60	1.30
0.530		3	10.05	3.35
0.560		4	2.24	0.56
0.760		2	2.60	1.30
0.840		2	2.20	1.10
0.830		2	2.80	1.40
0.880		3	4.56	1.52
0.920	emb	1	1.80	1.80
0.980	emb	2	4.20	2.40
0.940	emb	4	4.80	2.40
1.030	emb	2	5.00	2.50

Moina micrura at 1.0 mgC.L^{-1} food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.40		42	22.88	0.544
0.40		68	33.57	0.494
0.60	e emb	8	25.63	3.204
0.63	eggs	7	11.94	1.706
0.82	emb	4	28.40	7.101
0.60	no emb	4	20.19	5.047
0.56		18	16.03	0.890
0.50		22	17.08	0.776
0.50		25	25.59	1.024
0.63	develop em + o	5	33.25	6.651
0.44		57	30.64	0.538
0.55		12	14.83	1.236
0.79	emb	2	21.94	10.974
0.65	eggs	6	10.60	1.767

Moina micrura at 0.25 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.667	emb	3	4.740	1.580
0.792	emb	2	4.600	2.300
0.727	emb	4	5.160	1.290
0.632	emb	3	4.891	1.630
0.773	emb	2	4.841	2.429
0.720	emb	1	6.020	6.020
0.792	emb	1	4.441	4.440
0.763	emb	4	18.121	4.530
0.792	egg	3	9.750	3.250
0.748	egg	2	3.160	1.580
0.792	emb	1	3.180	3.180
0.816	emb	2	5.180	2.590
0.430		4	1.604	0.401
0.430		5	2.225	0.445
0.430		4	1.880	0.470
0.500		3	1.800	0.600
0.470		4	2.768	0.692
0.560	egg	2	2.400	1.200
0.580	emb	4	5.600	1.400
0.640	emb	2	3.300	1.650
0.670	emb	1	1.550	1.550
0.810	emb	1	2.300	2.300
0.820	emg	1	2.600	2.600
0.820	egg	1	3.400	3.400
0.820	emb	1	3.710	3.710

Moina micrura at 0.1 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg C ind^{-1}
0.662	emb	3	9.751	3.250
0.634	emb	4	3.040	0.760
0.720	emb	2	3.141	1.570
0.698	emb	3	5.311	1.770
0.677	emb	2	2.920	1.460
0.648	emb	3	5.160	1.720
0.655	egg	4	4.600	1.150
0.749	emb	2	3.620	1.810
0.763	emb	2	3.440	1.720
0.749	egg	3	4.680	1.560
0.778	emb	4	7.720	1.930
0.677	emb	2	9.920	4.960
0.533		5	3.730	0.746
0.590		4	4.520	1.130
0.821	emb	2	6.160	3.080
0.749	emb	2	6.560	3.280
0.432		4	1.160	0.290
0.460		6	2.340	0.390
0.500		4	2.008	0.502
0.640	egg	2	1.780	0.890
0.830	emb	3	8.101	2.701

Ceriodaphnia cornuta at 1.0 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.25		52	10.683	0.205
0.30		37	15.060	0.407
0.35		32	12.463	0.389
0.35	ovary	25	14.747	0.589
0.40	ovary	38	23.682	0.623
0.40	eggs	22	15.257	0.693
0.45	eggs	17	14.655	0.862
0.45	eggs	19	14.641	0.770
0.50	eggs	10	22.487	2.248
0.50	emb	9	18.429	2.047
0.50	develop emb	7	21.016	3.002
0.50	develop emb	8	15.665	1.958
0.55	eggs	9	22.347	2.483
0.55	emb	7	24.193	3.456
0.55	develop emb	5	22.666	4.533

Ceriodaphnia cornuta at 0.25 $mgC.L^{-1}$ food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.20		12	0.756	0.061
0.25		7	0.644	0.092
0.30		13	3.055	0.235
0.30		4	0.652	0.163
0.35	ovary	7	1.393	0.199
0.35	ovary	3	0.540	0.180
0.35	egg	8	1.800	0.225
0.40	emb	2	1.044	0.522
0.40	egg	4	1.844	0.461
0.45	emb	3	1.866	0.622
0.45	emb	5	3.565	0.713
0.50	emb	4	5.252	1.313
0.50	emb	2	2.056	1.028
0.50	emb	1	1.973	1.973
0.50	emb	3	2.658	0.886
0.45	emb	3	1.701	0.567

Daphnia lumholtzi at 1.0 mgC.L^{-1} food concentration

Length in mm	Reproductive status	No. of animals $sample^{-1}$	µg C $sample^{-1}$	µg ind^{-1}
0.55		31	18.612	0.600
0.55		22	13.226	0.601
0.55		13	14.367	1.105
0.55		12	12.739	1.061
0.70	young ovary	5	33.390	6.679
0.80	young ovary	7	40.124	5.732
0.90	young ovary	3	17.959	5.986
0.90	mature ovary	4	27.442	6.860
0.90	mature ovary	2	24.531	12.265
1.00	eggs	3	15.478	5.159
1.00	young ovary	4	29.781	7.445
1.00	eggs	2	18.175	9.087
1.20	emb	3	29.980	9.993
1.20	eggs	2	22.214	11.107
1.20	emb	2	33.321	16.660
1.30	emb	1	10.974	10.974

May 1984. Diaphanosoma excisum

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.45		30	6.042	0.201
0.50		26	7.221	0.278
0.55		19	6.813	0.358
0.60		22	8.810	0.400
0.65	ovary	13	7.660	0.589
0.70	ovary	15	12.840	0.856
0.70	ovary	13	13.800	1.062
0.70	emb	1	1.281	1.281
0.70	egg	4	4.656	1.164
0.80	ovary	6	8.710	1.452
0.80	emb	9	13.940	1.549
0.80	emb	7	12.790	1.826
0.85	emb	1	2.070	2.070
0.90	emb	4	8.300	2.075
0.95	emb	8	10.692	1.337
0.90	emb	8	18.691	2.336
0.90	emb	1	2.471	2.471
0.95	emb	6	11.380	1.897
0.95	emb	3	6.210	2.070
1.00	emb	2	5.928	2.964
1.00	emb	1	2.167	2.167

November 1984. Diaphanosoma excisum

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.45		34	12.025	0.353
0.55		9	11.955	1.329
0.60		10	12.360	1.236
0.65		10	10.452	1.045
0.70	eggs or emb	9	10.126	1.125
0.75	eggs or emb	11	15.124	1.374
0.75	eggs or emb	6	11.258	1.876
0.80	eggs or emb	14	25.170	1.797
0.80	ovary & ebp	4	9.553	2.388
0.85	eggs or emb	7	15.180	2.168
0.90	eggs or emb	5	13.758	2.751
1.00	eggs or emb	3	12.844	4.281

February 1985. Diaphanosoma excisum

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.45		50	13.076	0.261
0.45		48	11.094	0.231
0.50		13	11.845	0.911
0.60		16	15.606	0.975
0.60		20	16.258	0.812
0.75	ovary	7	13.445	1.920
0.80	emb 1-2 or ovary	14	24.118	1.722
0.85	emb, eggs or ovary	17	24.020	1.412
0.90	eggs or emb 2	12	19.253	1.604
0.95	eggs or emb 2	13	21.994	1.691
1.00	eggs or emb 2	6	12.065	2.010
1.10	eggs or emb 2	7	24.510	3.501

August 1984. Moina micura

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.4		60	18.816	0.313
0.4		50	17.506	0.350
0.4		50	17.471	0.349
0.4		40	13.977	0.349
0.55	emb	16	19.174	1.198
0.60	emb	20	17.869	0.893
0.60	emb	20	18.586	0.929
0.60	emb	18	19.804	1.100
0.65	emb	21	18.579	0.884
0.65	emb	7	12.249	1.749
0.70	emb	17	21.381	1.257
0.70	emb	8	11.624	1.453
0.75	emb	10	16.930	1.693

November 1984. Ceriodaphnia cornuta

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.20		50	3.000	0.060
0.20		100	5.293	0.052
0.25		22	9.856	0.740
0.30		14	10.368	0.740
0.30		24	13.351	0.556
0.35	eggs or emb	30	15.906	0.530
0.35	eggs or emb	20	16.977	0.848
0.35	eggs or emb	18	11.443	0.635
0.40	eggs or emb	25	15.481	0.619
0.40	eggs or emb	25	15.598	0.623
0.40	eggs or emb	20	14.053	0.702
0.40	eggs or emb	20	15.235	0.761
0.40	eggs	11	11.609	1.055
0.40	ebp	10	9.995	0.999

February 1985. Ceriodaphnia cornuta

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.20		47	9.156	0.194
0.20		50	11.245	0.224
0.35		30	18.701	0.623
0.35		16	13.507	0.844
0.35		9	10.130	1.125
0.35		10	9.712	0.971
0.40	eggs or emb	30	29.999	0.999
0.40	eggs or emb	20	16.317	0.815
0.40	eggs or emb	25	21.640	0.865
0.40	eggs or emb	25	18.237	0.729
0.40	eggs or emb	30	25.286	0.842
0.45	eggs or emb	9	9.981	1.109
0.45	eggs or emb	10	10.657	1.065
0.45		8	9.290	1.161

August 1984. Daphnia lumholtzi

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.40		25	7.855	0.314
0.45		10	5.276	0.527
0.50	ovary	23	19.076	0.829
0.55	ovary	13	20.101	1.546
0.60	ovary	23	52.156	2.267
0.65	ovary	28	58.231	2.079
0.70	ovary	25	58.534	2.341
0.75	eggs	10	23.340	2.340
0.75	emb	11	32.955	2.995
0.80	eggs	12	39.138	3.261
0.80	emb	12	45.412	3.784
0.80	late emb + ovary	9	41.431	4.603
0.85	o or eggs	7	41.811	5.973
0.85	emb	12	50.254	4.187
0.85	late emb	11	47.734	4.33948907
0.90	o or egg	12	50.914	4.242843952
0.90	emb	9	28.707	3.189699426
0.95	ovary	3	11.228	3.742690059
0.95	ovary	3	12.728	4.242843952
0.95	emb	5	25.826	5.165281676

February 1985. Daphnia lumholtzi

Length in mm	Reproductive stage	No. of animals sample^{-1}	Total µg C sample^{-1}	µg C ind^{-1}
0.40		30	9.006	0.300
0.40		32	10.276	0.321
0.50		13	11.130	0.856
0.60		18	23.938	1.329
0.65	ovary	12	15.709	1.309
0.65	ovary	15	20.958	1.397
0.70	ovary	16	23.254	1.453
0.70	ovary	15	26.472	1.764
0.75	ovary & egg	9	20.445	2.271
0.80	eggs or emb 1-2	16	34.889	2.180
0.80	eggs or emb 1	5	13.823	2.764
0.85	eggs or emb 1-2	14	34.560	2.468
0.85	eggs or emb 1-2	8	17.992	2.249
0.85	eggs or emb 1-2	7	16.854	2.407
0.90	eggs or emb 1-2	7	22.477	3.211
0.90	eggs or emb 1-2	9	27.604	3.067
0.90	eggs or emb 1-2	8	22.505	2.813
0.95	eggs or emb 1-2	7	24.691	3.527

Appendix 5

Body length and egg production of individual animals at each temperature and food concentration

o = ovary e = eggs em = early embryos ebp = empty brood pouch in adult female

(a) Diaphanosoma excisum at 32°C and 1.0 $mgC.L^{-1}$ food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.533	0.490	0.533	0.533
0.833	0.590	0.590	0.648	0.605
1.167	0.590	0.677	0.720	0.605
1.833	0.648	0.749 (o)	0.792	0.706
2.167	0.734 (o)	0.936 (3em)	0.806 (o)	0.792 (o)
2.833	0.893 (o)	1.080 (9em)	0.907 (6em)	0.893 (3em)
3.167	0.979 (5em)	1.080	0.994 (6em)	0.893
3.834	1.109 (6em)	1.123 (8em)	1.094 (11em)	1.080 (12em)
4.167	1.109	1.123	1.094	1.080
4.833	1.238 (8em)	1.224 (10em)	1.152 (9em)	1.109 (8em)
5.167	1.238	1.253	1.152	1.109
5.833	1.267 (9em)		1.224	1.253 (9em)
6.167	1.267			1.253
6.833	1.310			1.325

(a) Diaphanosoma excisum at 32°C and 0.5 $mgC.L^{-1}$ food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.533	0.504	0.533	0.533
0.833	0.648	0.590	0.590	0.605
1.167	0.778	0.648	0.720	0.605
1.833	0.778	0.792 (o)	0.792	0.792 (o)
2.167	0.864 (o)	0.792	0.864	0.893 (6e)
2.833	0.864	0.965 (7em)	0.864 (o)	0.893
3.167	1.037 (6e)	0.965	1.008 (6e)	1.066 (5e)
3.833	1.109 (8e)	1.080 (7em)	1.123 (6e)	1.066
4.167	1.109	1.080	1.123	1.181 (8e)
4.833	1.224 (8e)	1.152 (7em)	1.195 (10em)	1.181
5.167	1.224	1.152	1.195	1.224 (10em)
5.833	1.296 (11e)	1.224 (8em)	1.296 (11em)	1.224
6.167	1.296	1.267	1.296	1.267
6.833	1.310	1.267	1.310	

(a) Diaphanosoma excisum at 32°C and 0.25 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.533	0.533	0.504	0.533
0.833	0.590	0.590	0.576	0.605
1.167	0.634	0.619	0.677	0.648
1.833	0.749	0.778	0.778 (o)	0.677
2.167	0.749 (o)	0.821 (o)	0.778	0.749 (o)
2.833	0.950 (4e)	0.936 (4em)	0.950 (6e)	0.749
3.167	0.950	0.936	0.950	0.922 (5e)
3.833	1.022 (6e)	1.037 (6em)	1.008 (6e)	0.922
4.167	1.022	1.037	1.008	1.037 (6em)
4.833	1.138 (7e)	1.138 (6em)	1.080 (6e)	1.037
5.167	1.138	1.138	1.108	1.123 (7em)
5.833	1.181 (8e)	1.181 (7em)	1.138 (6e)	1.123
6.167	1.181	1.181	1.138	1.181 (9em)
6.833	1.210	1.224	1.210	1.181
7.167				1.224

(a) Diaphanosoma excisum at 32°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.490	0.490	0.490
0.833	0.504	0.490	0.490	0.490
1.167	0.504	0.533	0.533	0.533
1.833	0.547	0.533	0.533	0.533
2.167	0.547	0.533	0.533	0.634
2.833	0.634	0.533	0.533	0.634
3.167	0.634	0.590	0.634	0.648
3.833	0.634	0.590	0.634	0.749
4.167	0.734 (o)	0.706	0.706 (o)	0.749
4.833	0.734	0.706	0.706	0.864 (o)
5.167	0.734	0.864 (o)	0.878 (o)	0.936 (2em)
5.833	0.922 (3em)	0.936 (3em)	0.936 (2e)	0.936
6.167	0.922	0.936	0.936	1.008 (2em)
6.833	0.936 (3em)	0.994 (2em)	1.008 (4e)	1.008
7.167	0.936	0.994	1.008	1.051 (3em)
7.833	0.994 (3em)	1.008 (4em)	1.066 (2e)	1.051
8.167	0.994 (3e)	1.051 (3em)	1.066	1.080 (4em)
8.833	0.994	1.051	1.520 (4e)	1.080
9.167	0.994	1.152	1.152	1.123
9.833			1.152	

(a) Diaphanosoma excisum at 32°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.533	0.531	0.533	0.533
0.833	0.533	0.562	0.533	0.561
1.167	0.533	0.562	0.590	0.561
1.833	0.605	0.634	0.590	0.619
2.167	0.605	0.634	0.662	0.619
2.833	0.648	0.634	0.662	0.648
3.167	0.648	0.648	0.662	0.648
3.833	0.677	0.648	0.720	0.706
4.167	0.677	0.677	0.720	0.706
4.833	0.720	0.677	0.763	0.806
5.167	0.720	0.749 (o)	0.763	0.806
5.833	0.792 (o)	0.749	0.821 (o)	0.864 (o)
6.167	0.792	0.850 (1e)	0.821	0.864
6.833	0.792	0.850	0.864 (3em)	0.878 (1e)
7.167	0.907 (3e)	0.864 (1e)	0.864	0.878
7.833	0.907	0.864	0.893 (1em)	0.979 (1e)
8.167	0.994 (3e)	0.893 (1e)	0.893	0.979
8.833	0.994	0.893	0.893 (1em)	1.008 (3e)
9.167	1.008 (3e)	0.936(4e)	0.893	1.008
9.883	1.008	0.936	1.008 (3em)	1.051 (4e)
10.167	1.008 (2e)	0.936	1.051	1.051
10.833	1.008	0.979		1.08
11.167	1.051			

(a) Diaphanosoma excisum at 32°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.504	0.490	0.504
0.833	0.504	0.504	0.490	0.504
1.167	0.504	0.504	0.504	0.504
1.833	0.504	0.504	0.504	0.504
2.167	0.504	0.504	0.504	0.504
2.833	0.504	0.562		0.504
3.167		0.562		
3.833		0.562		

(a) Diaphanosoma excisum at 27°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.490	0.490	0.504
0.833	0.533	0.533	0.504	0.533
1.167	0.533	0.533	0.504	0.533
1.833	0.662	0.648	0.662	0.662
2.167	0.662	0.648	0.778	0.662
2.833	0.835 (o)	0.727 (o)	0.778 (o)	0.763
3.167	0.936 (4e)	0.864 (3em)	0.893 (3e)	0.864
3.833	0.936	0.864	0.893	0.864 (o)
4.167	0.936	1.008 (4em)	0.893	0.936 (4e)
4.833	1.022 (6e)	1.008	0.893 (o)	0.936
5.167	1.022	1.080 (6em)	1.008 (4em)	1.022 (6e)
5.833	1.080 (8e)	1.080	1.008	1.022
6.167	1.080	1.080	1.066 (6e)	1.066 (6e)
6.833	1.094	1.152 (6e)	1.066	1.066
7.167	1.138 (6em)	1.152	1.080 (6e)	1.080
7.833	1.138	1.152	1.080	1.109 (5em)
8.167	1.167	1.195	1.152	1.109
8.833				1.109

(a) Diaphanosoma excisum at 27°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.490	0.564	0.490	0.490
0.833	0.561	0.533	0.533	0.562
1.167	0.561	0.584	0.590	0.562
1.833	0.677	0.590	0.590	0.648
2.167	0.677	0.691	0.734	0.648
2.833	0.763	0.778 (o)	0.893	0.734
3.167	0.763	0.778	0.893	0.734
3.833	0.850 (o)	0.864 (2e)	0.893	0.806 (o)
4.167	0.850	0.864	0.893	0.806
4.833	0.950 (4e)	0.907 (3e)	0.950 (4e)	0.864 (2e)
5.167	0.950	0.907	0.950	0.864
5.833	1.022 (4e)	0.907	1.008 (5e)	0.893 (2e)
6.167	1.022	0.907	1.008	0.893
6.833	1.066 (6e)	0.907 (2e)	1.037 (6e)	0.893
7.167	1.066	0.907	1.037	0.922 (4e)
7.833	1.109 (6e)	0.923 (2e)	1.080	0.922
8.167	1.109	0.923		0.922
8.833	1.109	0.923		0.922 (3e)
9.167	1.138	0.923		0.922
9.833		0.950		0.922
10.167				0.950

(a) Diaphanosoma excisum at 27°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.490	0.504	0.504	0.482
0.833	0.547	0.547	0.504	0.547
1.167	0.547	0.605	0.562	0.547
1.833	0.605	0.662	0.562	0.634
2.167	0.605	0.662	0.662	0.634
2.833	0.691	0.720	0.662	0.720
3.167	0.691	0.720	0.821 (o)	0.720
3.833	0.763	0.763	0.821	0.806 (o)
4.167	0.763	0.763	0.821	0.806
4.833	0.792 (o)	0.835 (o)	0.821	0.878 (2e)
5.167	0.792	0.835	0.893 (2e)	0.878
5.833	0.864 (2e)	0.864 (1e)	0.893	0.878
6.167	0.864	0.864	0.893	0.907 (2e)
6.833	0.936 (4em)	0.864	0.922 (e)	0.907
7.167	0.936	0.936 (2e)	0.922	0.907
7.833	0.965 (2e)	0.936	0.922	0.929 (2e)
8.167	0.965	0.936	0.950 (2e)	0.929
8.833	0.965	0.965 (3e)	0.950	0.929
9.167	0.994 (4e)	0.965	0.950	0.936 (2em)
9.833	0.994	0.965	0.979 (2em)	0.936
10.167	0.994	0.979 (3e)	0.979	0.950
10.833	0.979	0.979	0.994	
11.167		0.979		

(a) Diaphanosoma excisum at 27°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.490	0.490	0.475	0.49
0.833	0.490	0.540	0.562	0.49
1.167	0.562	0.540	0.562	0.533
1.833	0.562	0.605	0.662	0.533
2.167	0.648	0.605	0.662	0.533
2.833	0.648	0.648	0.706	0.562
3.167	0.706	0.648	0.706	0.562
3.833	0.706	0.691	0.72	0.562
4.167	0.763 (o)	0.691	0.72	0.590
4.833	0.763	0.706	0.771	0.590
5.167	0.763	0.706	0.771	0.634
5.833	0.763	0.778 (o)	0.835 (o)	0.634
6.167	0.893 (2e)	0.778	0.835	0.662
6.833	0.893	0.835 (2em)	0.907 (2em)	0.662
7.167	0.893	0.835	0.907	0.749 (o)
7.833	0.923 (ebp)	0.835	0.907 (1e)	0.749
8.167	0.923	0.850 (ebp)	0.907	0.749
8.833	0.923 (2e)	0.850	0.907	0.749
9.167	0.930	0.850		0.878 (1e)
9.833	0.930	0.871 (1em)		0.878
10.167	0.950 (o)	0.871		0.878
10.833	0.950	0.871		0.878 (ebp)
11.167	0.950			0.878
11.833	0.965 (o)			0.878
12.167	0.965			0.878
12.833	0.965			
13.167	0.965			
13.833	0.965			

(a) Diaphanosoma excisum at 27°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.490	0.490	0.490	0.504
0.833	0.490	0.490	0.490	0.504
1.167	0.490	0.519	0.490	0.562
1.833	0.490	0.519	0.490	0.562
2.167	0.547	0.533	0.490	
2.833	0.547	0.533	0.490	
3.167		0.533	0.504	
3.833			0.504	

(a) Diaphanosoma excisum at 27°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	5	6	7	8
0.167	0.490	0.490	0.490	0.490
0.833	0.490	0.490	0.490	0.490
1.167	0.490	0.519	0.504	0.533
1.833	0.490	0.519	0.504	0.533
2.167		0.547	0.547	0.533
2.833		0.547	0.547	0.533
3.167		0.547	0.547	0.562
3.833		0.547		0.562
4.167		0.562		0.590

(a) Diaphanosoma excisum at 27°C and 0.01 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.489	0.504	0.532
0.833	0.489	0.504	0.532
1.167	0.509	0.562	0.532
1.833	0.509	0.562	0.532
2.167	0.509	0.562	0.532
2.833	0.509	0.562	0.532
3.167	0.509	0.562	0.532
3.833	0.509	0.562	0.532
4.167		0.562	
4.833		0.562	

(a) Diaphanosoma excisum at 27°C and 0.01 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.490	0.489	0.504
0.833	0.490	0.489	0.504
1.167	0.504	0.562	0.562
1.833	0.504	0.562	0.562
2.167	0.504	0.562	0.562
2.833	0.504	0.562	0.562
3.167	0.504	0.562	
3.833	0.504	0.562	

(a) Diaphanosoma excisum at 22°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.475	0.518	0.533	0.504
1.167	0.533	0.533	0.562	0.562
2.167	0.605	0.533	0.648	0.619
3.167	0.605	0.576	0.648	0.706
4.167	0.662	0.576	0.720	0.734
5.167	0.749	0.720	0.835	0.850 (o)
6.167	0.850	0.763	0.878 (o)	0.936 (4em)
7.167	0.878 (o)	0.864 (o)	0.994	0.936
8.167	0.979 (3em)	0.936 (4em)	0.994	0.979 (4e)
9.167	0.979	0.936	1.051 (4e)	0.994
10.167	1.008 (6em)	0.994 (4em)	1.051	0.994
11.167	1.008	1.008	1.094 (5e)	1.037 (4e)
12.167	1.080 (6em)	1.008	1.094	1.037
13.167	1.080	1.080 (6em)	1.138 (4em)	1.094 (6e)
14.167	1.080	1.080	1.152	1.094
15.167	1.123 (4em)	1.138 (6e)	1.166	1.138
16.167	1.123	1.138		
17.167	1.152	1.152		

(a) Diaphanosoma excisum at 22°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.533	0.504	0.504
1.167	0.533	0.562	0.533	0.533
2.167	0.605	0.605	0.576	0.605
3.167	0.766	0.677	0.662	0.706
4.167	0.792	0.734	0.706	0.806
5.167	0.850 (o)	0.835 (o)	0.806 (o)	0.850 (o)
6.167	0.922 (3e)	0.893 (2em)	0.907 (2em)	0.922 (2e)
7.167	0.922	0.893	0.907	
8.167	0.994 (4e)	0.965 (3e)	0.922	
9.167	0.994	0.965	1.008 (4em)	
10.167	1.022 (5e)	0.965	1.008	
11.167	1.022	1.008 (4e)	1.066 (5em)	
12.167	1.022	1.008	1.066	
13.167	1.094 (6e)	1.051 (6e)	1.094 (6em)	
14.167	1.094	1.051	1.094	
15.167	1.152	1.080	1.123	

(a) Diaphanosoma excisum at 22°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.504	0.533	0.457
1.167	0.504	0.533	0.562	0.504
2.167	0.533	0.605	0.562	0.533
3.167	0.605	0.605	0.590	0.605
4.167	0.605	0.605	0.634	0.605
5.167	0.622	0.648	0.662	0.648
6.167	0.622	0.648	0.720	0.648
7.167	0.622	0.677	0.778	0.706
8.167	0.720	0.677	0.806	0.734
9.167	0.778	0.720	0.850	0.792
10.167	0.835	0.763	0.878 (o)	0.850
11.167	0.850	0.763	0.878	0.878
12.167	0.893	0.850 (o)	0.907	0.936 (1e)
13.167	0.922 (o)	0.850	0.907	0.936
14.167	0.922 (1e)	0.878 (o)	0.994 (2e)	0.936
15.167	0.922	0.936 (o)	0.994	0.950 (1e)
16.167	0.965 (1e)	0.936	0.994 (ebp)	0.950
17.167	0.965	0.979 (o)	0.994	0.994 (o)
18.167	0.965	1.008 (o)	0.994	0.994
19.167	0.994 (1e)	1.037 (3e)	0.994 (o)	1.008 (1e)
20.167	0.994	1.037	0.994	1.008
21.167	1.051 (2e)	1.037	1.008 (o)	1.008
22.167	1.051	1.066 (3e)	1.008	1.008 (o)
23.167	1.051	1.066	1.008 (3e)	
24.167	1.051	1.066 (2e)	1.008	
25.167		1.066	1.051	
26.167		1.066 (o)		
27.167		1.066		

(a) Diaphanosoma excisum at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.504	0.504	0.475	0.533
1.167	0.533	0.533	0.504	0.605
2.167	0.533	0.533	0.562	0.605
3.167		0.533	0.562	0.605
4.167			0.562	

(a) Diaphanosoma excisum at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.533	0.475	0.446
1.167	0.533	0.564	0.578
2.167	0.533	0.504	0.578
3.167	0.533	0.5504	0.619
4.167	0.533	0.605	0.619
5.167	0.533	0.605	0.619
6.167		0.605	

(a) Diaphanosoma excisum at 22°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.475	0.504	0.504
1.167	0.520	0.533	0.547
2.167	0.547	0.533	0.547
3.167		0.533	0.547
4.167		0.533	0.547
5.167			0.605

(a) Diaphanosoma excisum at 22°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.432	0.504	0.475
1.167	0.504	0.533	0.475
2.167	0.533	0.533	0.504
3.167	0.533	0.547	0.533
4.167	0.533	0.547	0.576
5.167	0.533		0.634

(b) Moina micrura at 32°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.432	0.432
0.833	0.518 (o)	0.518 (o)	0.504 (o)	0.504 (o)
1.167	0.634 (6em)	0.619 (5em)	0.605 (5e)	0.605 (4em)
1.833	0.634	0.619	0.605	0.691 (7e)
2.167	0.691 (8e)	0.691 (8e)	0.691 (8e)	0.691
2.833	0.734 (12e)	0.720 (13e)	0.720 (10e)	0.720 (11em)
3.167	0.792 (15e)	0.792 (14e)	0.763 (12e)	0.763 (12e)
3.833	0.792	0.792	0.763	0.763
4.167	0.821	0.821	0.821	0.806
4.833	0.821	0.821		

(b) Moina micrura at 32°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.446	0.446
0.833	0.518 (o)	0.533 (o)	0.533 (o)	0.533 (o)
1.167	0.634 (4e)	0.634 (4e)	0.634 (6e)	0.648 (5e)
1.833	0.634	0.691 (8em)	0.634	0.648
2.167	0.691 (8em)	0.691	0.691 (8em)	0.706 (7em)
2.833	0.734 (11em)	0.720 (8em)	0.734 (10em)	0.706
3.167	0.734	0.720	0.734	0.778 (11em)
3.833	0.749 (10em)	0.763 (12e)	0.749 (11em)	0.792 (12em)
4.167	0.749	0.763	0.749	0.792
4.833	0.792	0.792	0.792 (em)	0.806

(b) Moina micrura at 32°C and 0.25 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.432	0.432
0.833	0.504	0.504 (o)	0.518 (o)	0.518
1.167	0.576 (3e)	0.605 (5e)	0.605 (4e)	0.605 (o)
1.833	0.576	0.605	0.677 (4e)	0.677 (3e)
2.167	0.576	0.677 (7em)	0.677	0.677
2.833	0.605 (4em)	0.691 (8e)	0.691 (5em)	0.720 (3em)
3.167	0.605 (8e)	0.691	0.734 (5e)	0.749 (10e)
3.833	0.648	0.720 (8em)	0.734	0.749
4.167	0.691 (8e)	0.720	0.763	0.778 (8em)
4.833	0.691	0.792		0.778
5.167	0.720			0.821

(b) Moina micrura at 32°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.461	0.475	0.461	0.446
0.833	0.533	0.533	0.533	0.446
1.167	0.590 (o)	0.533 (o)	0.594 (o)	0.475
1.833	0.619 (3em)	0.576 (2e)	0.648 (4e)	0.562 (o)
2.167	0.619	0.576	0.648	0.605 (3e)
2.833	0.691 (3em)	0.634 (2em)	0.691(4em)	0.605
3.167	0.691	0.634	0.691	0.648 (2e)
3.833	0.706 (4em)	0.634 (6em)	0.706 (4em)	0.648
4.167	0.706	0.634	0.706	0.691 (6e)
4.833	0.720 (6em)	0.677 (5em)	0.749 (7em)	0.691
5.167	0.720	0.677	0.749	0.706 (5e)
5.833	0.749	0.706	0.749	0.706
6.167				0.720

(b) Moina micrura at 32°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.461	0.461	0.475	0.461
0.833	0.518	0.518 (o)	0.518	0.518
1.167	0.590 (o)	0.576 (2e)	0.518	0.605 (o)
1.833	0.634 (2e)	0.590	0.576 (o)	0.634 (2em)
2.167	0.634	0.590	0.605 (1e)	0.648
2.833	0.662 (1e)	0.605 (2em)	0.605	0.677 (2em)
3.167	0.662	0.605	0.648 (1em)	0.677
3.833	0.691 (2e)	0.619 (1em)	0.662 (2em)	0.691 (2e)
4.167	0.691	0.619	0.662	0.691
4.833	0.691 (2e)	9.634 (1em)	0.677 (1em)	0.691 (2e)
5.167	0.691	0.634	0.677	0.706
5.833	0.706	0.677	0.691	0.720

(b) Moina micrura at 27°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.432	0.432
0.833	0.547 (o)	0.523 (o)	0.547 (o)	0.533 (do)
1.167	0.605 (5e)	0.523	0.547	0.605 (4e)
1.833	0.605	0.619 (4e)	0.648 (2e)	0.605
2.167	0.619	0.619	0.648	0.619
2.833	0.648 (5e)	0.677 (4e)	0.648	0.648 (3e)
3.167	0.691	0.677	0.677 (7e)	0.648
3.833	0.691 (6e)	0.706 (6e)	0.677	0.648
4.167	0.691	0.706	0.734 (6e)	0.619 (6e)
4.833	0.706 (6e)	0.706	0.734	0.619
5.167	0.706	0.734 (5e)	0.734 (6e)	0.734 (8e)
5.833	0.749 (e)	0.734	0.734	0.734
6.167		0.763 (e)	0.792 (em)	0.749 (e)

(b) Moina micrura at 27°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.446	0.432
0.833	0.518	0.526	0.547 (o)	0.576
1.167	0.518	0.526	0.619 (6e)	0.576
1.833	0.612 (4e)	0.619 (e)	0.619	0.662 (8e)
2.167	0.612	0.619	0.619	0.662
2.833	0.648 (3e)	0.662 (4e)	0.648 (4em)	0.662
3.167	0.648	0.662	0.648	0.706 (9e)
3.833	0.677 (5e)	0.706 (5e)	0.706 (7e)	0.706
4.167	0.677	0.706	0.706	0.778 (10e)
4.833	0.677	0.706	0.706	0.778
5.167	0.720 (8e)	0.749 (6e)	0.763 (8em)	0.802 (9e)
5.833	0.720	0.749	0.763	0.802
6.167	0.749	0.792 (8e)	0.763 (em)	0.802(ebp)

(b) Moina micrura at 27°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.461	0.461	0.461	0.440
0.833	0.562 (o)	0.576 (o)	0.533 (o)	0.562
1.167	0.562	0.576	0.533	0.562
1.833	0.662 (3e)	0.679 (4e)	0.634 (2n)	0.648 (4e)
2.167	0.663	0.679	0.634	0.648
2.833	0.663	0.679	0.634	0.648
3.167	0.706 (2e)	0.706 (2e)	0.663 (3n)	0.679 (3em)
3.833	0.706	0.706	0.663	0.679
4.167	0.749 (6em)	0.763 (5e)	0.713 (4n)	0.734 (5em)
4.833	0.749	0.763	0.713	0.734
5.167	0.749	0.763	0.734 (4n)	0.734 (3e)
5.833	0.763 (3e)	0.778 (2e)	0.734	0.734
6.167	0.763	0.778	0.734	0.778 (epb)
6.833	0.763	0.778	0.777	
7.167	0.763	0.821		

(b) Moina micrura at 27°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.461	0.432	0.432	0.461
0.833	0.540	0.432	0.504	0.533
1.167	0.540	0.475	0.504	0.533
1.833	0.590 (o)	0.475	0.576 (o)	0.590 (o)
2.167	0.590	0.475	0.576	0.590
2.833	0.634 (1e)	0.475	0.634 (1e)	0.633 (2e)
3.167	0.634	0.518	0.634	0.633
3.833	0.634	0.518	0.634	0.633
4.167	0.676 (e)	0.576	0.662 (2e)	0.633
4.833	0.676 (emb)	0.576	0.662	0.648 (1e)
5.167	0.691	0.634 (o)	0.662	0.662
5.833	0.706 (2e)	0.634	0.677 (1e)	0.662
6.167	0.706	0.634	0.677	0.706 (1e)
6.833	0.706	0.648 (1em)	0.677	0.706
7.167	0.720 (ebp)	0.648	0.677	0.706
7.833	0.720	0.706 (3e)	0.706 (1e)	0.720 (2e)
8.167	0.720	0.706	0.706	0.720
8.833	0.720 (e)	0.734 (3em)	0.706	0.720
9.167		0.734	0.706	0.720
9.833		0.734	0.720 (ebp)	0.720 (em)
10.167		0.734 (3em)		
10.833		0.734		
11.167		0.734		
11.833		0.778		

(b) Moina micrura at 27°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.446	0.446	0.461
0.833	0.533	0.446	0.518
1.167	0.533	0.533	0.518
1.833	0.533	0.533	0.518
2.167	0.533	0.533	0.518
2.833	0.533	0.533	0.533
3.167	0.533	0.547	0.533
3.833	0.533	0.547	0.533
4.167	0.547	0.547	0.533
4.833	0.547	0.547	0.533

(b) Moina micrura at 27°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.461	0.418	0.475
0.833	0.490	0.418	0.475
1.167	0.490	0.418	0.547
1.833	0.490	0.432	0.547
2.167	0.533	0.432	0.590
2.833	0.533	0.432	0.590
3.167	0.533		0.590
3.833	0.533		0.590
4.167	0.533		0.590
4.833			0.590

(b) Moina micrura at 27°C and 0.01 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.432	0.432	0.432	0.446
0.833	0.482	0.468	0.432	0.504
1.167	0.482	0.468	0.432	0.504
1.833	0.504	0.468	0.432	0.504
2.167	0.504	0.468	0.468	0.504
2.833	0.504	0.468	0.468	0.504
3.167		0.468		0.504
3.833		0.518		0.504
4.167		0.518		
4.833		0.518		

(b) Moina micrura at 22°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.401	0.461	0.461	0.432
1.167	0.523 (o)	0.533	0.533 (o)	0.489
2.167	0.691 (6e)	0.619 (o)	0.634 (4e)	0.562 (o)
3.167	0.691	0.677 (2em)	0.634	0.634 (3e)
4.167	0.691	0.677	0.634	0.634
5.167	0.720 (5em)	0.691 (6em)	0.634 (3e)	0.662 (4e)
6.167	0.720	0.691	0.648	0.677
7.167	0.749 (8em)	0.691 (4em)	0.706 (4em)	0.677 (1e)
8.167	0.749	0.691	0.706	0.691
9.167	0.749 (4e)	0.720 (5e)	0.713 (3e)	0.691
10.167	0.749	0.720	0.713	0.691 (2em)
11.167	0.778	0.720	0.713	0.691
12.167			0.713	

(b) Moina micrura at 22°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.446	0.461	0.461	0.446
1.167	0.533 (o)	0.564	0.526	0.504
2.167	0.648 (4e)	0.562 (o)	0.619 (o)	0.562
3.167	0.648	0.619 (3e)	0.706 (6em)	0.648 (o)
4.167	0.677 (2e)	0.619	0.706	0.706 (4e)
5.167	0.677	0.648 (4e)	0.734 (4em)	0.706
6.167	0.706 (5e)	0.648	0.749	0.749 (4e)
7.167	0.706	0.677 (4e)	0.792 (6e)	0.749
8.167	0.720 (4e)	0.677	0.792	0.778 (6e)
9.167	0.720	0.691 (2e)	0.792 (6e)	0.778
10.167	0.720	0.691	0.792	0.778
11.167	0.763	0.691	0.804	0.778 (2e)
12.167		0.691		0.778
13.167				0.821

(b) Moina micrura at 22°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.446	0.446	0.432	0.446
1.167	0.489	0.504	0.504	0.518
2.167	0.489	0.590 (o)	0.576 (o)	0.518
3.167	0.533	0.662 (3e)	0.576	0.590 (o)
4.167	0.547	0.662	0.634 (2em)	0.648 (3e)
5.167	0.619	0.662	0.634	0.648
6.167	0.677 (3e)	0.677 (2e)	0.662 (2em)	0.648
7.167	0.691	0.677	0.662	0.677 (2em)
8.167	0.691	0.691 (2e)	0.662 (2em)	0.677
9.167	0.720 (2e)	0.691	0.691	0.677 (2em)
10.167	0.720	0.706 (2e)	0.691 (2em)	0.691
11.167	0.720 (2e)	0.720	0.706	0.720 (2em)
12.167	0.720	0.720	0.706	0.720
13.167	0.734 (2e)	0.720	0.706	0.734
14.167	0.734			
15.167	0.792			

(b) Moina micrura at 22°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.461	0.432	0.461
1.167	0.489	0.489	0.504
2.167	0.489	0.489	0.504
3.167	0.489	0.526	0.504
4.167	0.489	0.526	0.504
5.167		0.526	0.504
6.167			0.504

(b) Moina micrura at 22°C and 0.03 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.461	0.446	0.446
1.167	0.489	0.489	0.489
2.167	0.489	0.489	0.489
3.167	0.489	0.489	0.489
4.167	0.489	0.489	0.489
5.167			0.489

(b) Moina micrura at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.446	0.446	0.446
1.167	0.518	0.504	0.504
2.167	0.562	0.504	0.504
3.167	0.562	0.562	0.533
4.167	0.590	0.562	0.533
5.167	0.619	0.576	0.562
6.167	0.619	0.576	0.562
7.167	0.619	0.619	
8.167		0.619	
9.167		0.619	

(b) Moina micrura at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.446	0.446	0.446
1.167	0.489	0.504	0.489
2.167	0.489	0.518	0.576
3.167	0.518	0.562	0.619
4.167	0.518	0.562	0.619
5.167	0.518	0.619	
6.167	0.526	0.619	
7.167		0.619	
8.167		0.619	
9.167		0.619	

(c) Ceriodaphnia cornuta at 32°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.288	0.274	0.274	0.274
0.833	0.331	0.274	0.317	0.274
1.167	0.331	0.331	0.317	0.224
1.833	0.374	0.403	0.403	0.403
2.167	0.374	0.403 (o)	0.403 (o)	0.403
2.833	0.418	0.432 (2e)	0.432 (2e)	0.432 (o)
3.167	0.468 (o)	0.432	0.432	0.432 (2em)
3.833	0.468 (4e)	0.461 (2e)	0.446 (1e)	0.461 (2e)
4.167	0.475	0.461	0.446	0.461
4.833	0.497 (4e)	0.482 (2e)	0.475 (2e)	0.497 (2e)
5.167	0.497 (2e)	0.482	0.475	0.497
5.833	0.497	0.504 (4e)	0.505 (4e)	0.584 (4e)
6.167	0.504 (2e)	0.504	0.505	0.584
6.833	0.504	0.518	0.518	0.584
7.167	0.518			

(c) Ceriodaphnia cornuta at 32°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.274	0.274	0.274	0.288
0.833	0.346	0.274	0.274	0.302
1.167	0.346	0.346	0.356	0.302
1.833	0.403	0.403	0.418	0.331
2.167	0.403 (o)	0.418 (o)	0.418 (o)	0.331
2.833	0.432 (2e)	0.461 (2e)	0.461 (2e)	0.331
3.167	0.432	0.461	0.461	0.331
3.833	0.432 (ebp)	0.461 (2e)	0.475 (2e)	0.331
4.167	0.432	0.461	0.475	0.331
4.833	0.489 (1e)	0.482 (2e)	0.499 (4e)	0.331
5.167	0.489	0.482	0.489	0.331
5.833	0.489 (2e)	0.482 (3e)	0.511 (2e)	0.331
6.167	0.489	0.482	0.511	0.331
6.833	0.489	0.482	0.518	

(c) Ceriodaphnia cornuta at 32°C and 0.25 $mgC.L^{-1}$ food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.274	0.274	0.274	0.276
0.833	0.331	0.331	0.274	0.302
1.167	0.331	0.331	0.302	0.302
1.833	0.374	0.360	0.302	0.331
2.167	0.374	0.360	0.302	0.331
2.833	0.374	0.360	0.302	0.331
3.167	0.418	0.403	0.346	0.389
3.833	0.418 (o)	0.403 (o)	0.346	0.389
4.167	0.461 (le)	0.439 (le)	0.346	0.446
4.833	0.461	0.439	0.346	0.446
5.167	0.475 (le)	0.461 (le)		
5.833	0.475	0.461		
6.167	0.482 (le)			
6.833	0.482			
7.167	0.497			

(d) Daphnia lumholtzi at 22°C and 1.0 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.576	0.576	0.576	0.576
1.167	0.576	0.576	0.576	0.691
2.167	0.691	0.720	0.691	0.878 (o)
3.167	0.691	0.720	0.691	1.024 (o)
4.167	0.835 (o)	0.864 (o)	0.849 (o)	1.024
5.167	0.979 (o)	1.008 (o)	0.996 (o)	1.253 (6e)
6.167	0.979	1.008	0.996	1.253
7.167	1.138 (3e)	1.152 (3e)	1.152 (4e)	1.354
8.167	1.138	1.152	1.152	1.354
9.167	1.224 (4e)	1.224 (6e)	1.267 (8e)	1.354
10.167	1.224	1.224	1.267	
11.167	1.224	1.224	1.267	
12.167	1.339 (5e)	1.324 (6e)	1.360 (8e)	
13.167	1.339	1.324	1.368	
14.167	1.397	1.324	1.451 (7e)	
15.167			1.451	
16.167			1.451	
17.167			1.451	

(d) Daphnia lumholtzi at 22°C and 0.5 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals			
	1	2	3	4
0.167	0.576	0.576	0.576	0.576
1.167	0.576	0.576	0.576	0.720
2.167	0.749	0.691	0.691	0.922
3.167	0.878	0.691	0.821 (o)	1.037
4.167	0.878	0.792 (o)	0.821	1.037
5.167	1.065	0.792	0.821	1.209 (4e)
6.167	1.065	0.878 (o)	0.849 (o)	1.209
7.167		0.878	0.849	1.325 (6e)
8.167		0.964 (o)	0.948 (o)	1.325
9.167		0.964	1.094 (2e)	1.325 (8e)
10.167		1.080 (2e)	1.094	1.451
11.167		1.080	1.094	1.451
12.167		1.109	1.094	1.472
13.167			1.195 (2e)	
14.167			1.195	
15.167			1.195	
16.167			1.195	

(d) Daphnia lumholtzi at 22°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.576	0.547	0.562
1.167	0.576	0.677	0.634
2.167	0.648	0.749	0.634
3.167	0.648	0.749	0.634
4.167	0.706	0.965	0.720
5.167	0.706	0.965	0.720
6.167	0.720	1.080 (2e)	0.792
7.167	0.763	1.080	0.792
8.167		1.080	0.864
9.167			0.878
10.167			0.950
11.167			0.950
12.167			1.066
13.167			1.066
14.167			1.152
15.167			1.152
16.167			1.238 (1e)
17.167			1.238
18.167			1.238
19.167			1.267 (2e)
20.167			1.267
21.167			1.267
22.167			1.267
23.167			1.267 (1e)

(d) Daphnia lumholtzi at 22°C and 0.1 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.571	0.542	0.542
1.167	0.571	0.634	0.633
2.167	0.662	0.664	0.662
3.167	0.662	0.720	0.662
4.167	0.734	0.734	0.691
5.167	0.734	0.806	0.691
6.167	0.749	0.806	0.778
7.167	0.749	0.864	0.778
8.167	0.763		0.849
9.167			0.849
10.167			0.936
11.167			0.936
12.167			0.936

(d) Daphnia lumholtzi at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	1	2	3
0.167	0.576	0.576	0.576
1.167	0.706	0.648	0.576
2.167	0.720	0.648	0.691
3.167	0.720	0.648	0.691
4.167	0.720	0.706	0.691
5.167	0.734	0.706	0.720
6.167	0.734	0.706	0.720
7.167		0.763	0.720
8.167			0.720
9.167		0.763	
10.167		0.763	

(d) Daphnia lumholtzi at 22°C and 0.05 mgC.L^{-1} food concentration

Age in days	Length in mm of individual animals		
	4	5	6
0.167	0.576	0.576	0.576
1.167	0.576	0.576	0.634
2.167	0.691	0.634	0.634
3.167	0.691	0.634	0.634
4.167	0.691	0.634	0.634
5.167	0.734	0.691	0.634
6.167	0.734	0.691	0.706
7.167	0.734		0.777
8.167	0.734		0.777
9.167	0.734		0.777
10.167	0.734		0.777
11.167			0.776