FOOD CONCENTRATION AND TEMPERATURE EFFECTS ON LIFE CYCLE CHARACTERISTICS OF TROPICAL CLADOCERA (*Daphnia gessneri* Herbst, *Diaphanosoma sarsi* Richard, *Moina reticulata* (Daday)): I. DEVELOPMENT TIME.

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ABSTRACT — The effects of food concentration and temperature on embryonic and postembryonic duration of three tropical species, *Daphnia gessneri* (1.5mm), *Diaphanosoma sarsi* (1.2mm) and *Moina reticulata* (0.8mm), were investigated as part of life cycle studies which included growth, body size and reproduction. These are the very first experimental studies undertaken on these species. The long-term growth experiments were performed under controlled laboratory conditions at all combinations of temperature (22°C, 27°C and 32°C) and constant food concentration (0.03, 0.05, 0.10, 0.25, 0.50 and 1.00 mgC/L) of the unicellular green alga *Scenedesmus acutus*. Animals were examined twice daily throughout their life cycle from the neonate to third adult instar. In all three species, temperature exerted the most powerful influence on embryonic duration but there was also a smaller food effect. In *D. gessneri*, postembryonic durations remained more or less the same at food levels 0.25 mgC/L but were influenced by temperature. At food concentrations of 0.1 mgC/L or lower, postembryonic durations became increasingly prolonged, particularly at high temperatures. This threshold concentration is affected by temperature: in *D. gessneri*, it was 0.1 mgC/L at 22°C and 27°C but higher at 32°C (between 0.25 and 0.50 mgC/L). At the same temperature of 27°C, the food threshold level varied between species: it was higher (0.25 mgC/L) for *D. sarsi* and lower (0.05 mgC/L) for *M. reticulata* compared with *D. gessneri* (0.1 mgC/L). In both embryonic and postembryonic durations there is a body size effect as the absolute durations were longest in the largest species and shortest in the smallest species In all three species, prolongation of postembryonic duration at combinations of high temperature and lowered food levels was accompanied by increased **number of juvenile instars.**

Key- words: *Daphnia gessneri, Diaphanosoma sarsi, Moina reticulata*; Tropical; Food concentration-temperature experiments; Embryonic(D_e) ; Postembryonic development (D_j); Ratio (D_j/D_e); Food Threshold.

Efeitos da Temperatura e Concentração de Alimento na Historia de Vida de Tropical Cladocera (*Daphnia Gessneri* Herbst,*Diaphanosoma Sarsi* Richard, *Moina reticulata* Daday,)) I. Tempo de desenvolvimento.

RESUMO — Os efeitos de temperatura e concentração de alimento na duração do desenvolvimento embrionário e postembrionário de três espécies tropicais, *Daphnia gessneri* (1.5mm), *Diaphanosoma sarsi* (1.2mm) and *Moina reticulata* (0.8mm), foram investigados como parte dos estudos de ciclo de vida incluindo crescimento, tamanho do corpo e reprodução.Estes são os primeiros estudos experimentais realizados com estas espécies. Os experimentos de crescimento de longa duração foram feitos em condições controladas de laboratório em todas as combinações de temperature (22°C, 27°C e 32°C) e concentração constante de alimento (0.03, 0.05, 0.10, 0.25, 0.50 and 1.00 mgC/L) de alga verde unicelular *Scenedesmus acutus*. Os animais foram analisados duas vezes por dia durante o ciclo de vida desde neonata ao terceiro estádio

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adulto. Nas três espécies, a temperatura exerceu um maior efeito no tempo do desenvolvimento embrionário mas ocorreu também um pequeno efeito de concentração de alimento. Em *Daphnia gessneri*, a duração postembriônica permaneceu mais ou menos a mesma na concentração de alimento de 0.25 mgC/L mas foi influenciada pela temperature. Na concentração de 0.1 mgC/L ou mais baixa, a duração postembrionária tornou-se mais prolongada, particularmente em alta temperatura. O valor absoluto do nível crítico de concentração de alimento foi afetado pela temperatura: em *D. gessneri*, foi 0.1 mgC/L em 22°C e 27°C mas aumentou em 32°C (entre 0.25 e 0.50 mgC/L). Na mesma temperatura de 27°C, o nivel crítico de alimento variou entre as espécies: foi mais alta (0.25 mgC/L) para *D. sarsi* e mais baixa (0.05 mgC/L) para *M. reticulata* comparada com *D. gessneri* (0.1 mgC/L). Em ambos, no desenvolvimento embrionário e postembrionário ocorreu o efeito do tamanho do corpo visto que as durações absolutas foram mais longas nas espécies maiores e mais curtas na espécie menor. Nas três espécies, o prolongamento do desenvolvimento postembrionário nas combinações de alta temperatura e menores concentrações de alimento foi acompanhado por aumento no número de estádios juvenis.

Palavras chaves: *Daphnia gessneri, Diaphanosoma sarsi, Moina reticulata;* Tropical; Experimentos; Temperatura e concentração de alimento; Embrionico (D_e); Postembrionico (D_j); Ratío (D_e/D_j); Nível crítico de alimento.

INTRODUCTION

It has been reported many times that the duration of embryonic development in crustaceans is a function of temperature only (INGLE *et al.*, 1937; ESSLOVA, 1959; HALL, 1964; KORINEK, 1970; MUNRO & WHITE, 1975; review in BOTTRELL *et al.*, 1976; MAGADZA, 1977; LEVEQUE & SAINT-JEAN, 1983; HERZIG, 1984), whereas the duration of post-embryonic development is influenced by food level as well (HRBACKOVA-ESSLOVA, 1963; WEGLENSKA, 1971; KORINEK, 1971). Many authors have shown that cladocerans cultured at high food concentrations have a shorter duration of post-embryonic development than those cultured at low food levels (DE BERNARDI *et al.*, 1978; GRAS & SAINT-JEAN, 1978; LEI & ARMITAGE, 1980; VIJVERBERG, 1980; KANKAALA & WULFF, 1981; ROCHA, 1983; ORCUTT & PORTER, 1984; JAYATUNGA, 1986).

Most of the published information is for temperate species only, largely *Daphnia*, and there are few quantitative experimental studies on tropical species such as those of JAYATUNGA (1986) who investigated development times of Sri Lankan cladocerans. Authors working with tropical planktonic cladocerans such as GRAS & SAINT-JEAN (1978), MURUGAN (1975), JANA & PAL (1984,1985) and SAINT-JEAN & BONOU (1994) fed their experimental animals with undefined lake or pond water. Quantitative studies are important as developmental durations provide the time element in growth and production determinations. It is of interest to know whether the largely small-sized tropical cladocerans habituated to narrow temperature ranges respond like warmed-up temperate forms.

In this study, the influence of temperature and food concentration on the development times of three species of tropical cladocerans were studied experimentally, simultaneously and in

combination, using species commonly found in the Amazon "varzea" lakes: *Daphnia gessneri, Moina reticulata* and *Diaphanosoma sarsi,* with third instars adult sizes of 1.5mm, 0.8mm and 1.2mm. These are the first such experimental studies on these species and in Latin America. The experimental temperatures ranged from 22-32°C - high temperatures for temperate species but spanning the full range experienced by tropical forms. Food levels were selected to cover a likely range from limiting to non-limiting food concentrations in the light of JAYATUNGA's (1986) prior experience with Sri Lankan cladocerans.

MATERIALS AND METHODS

Stock cultures

The cladoceran species studied were collected from Lake Jacaretinga in the Amazon State, Brazil, and transported to Royal Holloway College where they were maintained in 1 litre-beaker cultures using filtered Alderhurst Pond water at 27°C, in an Astell Hearson incubator under a controlled 12 hour day/12 hour dark regime. These stock cultures of cladocerans were fed from stock cultures of the unicellular green alga, *Scenedesmus acutus* (Chlorophyceae, Chlorococcales) which were maintained in the exponential phase of growth by subculturing. In this condition, the food alga had cell dimensions of 10.34 x 4.68 μm, cell volume of 102 μm³· and cell carbon content of 11.78 pgC (ROCHA & DUNCAN, 1985). For about three weeks prior to

an experimental run, the stock cladoceran cultures were fed daily at a food level of 1.0 mgC/L.

Preparation of experimental food

Samples of *Scenedesmus acutus* taken from the 5-9 day old liquid medium culture in exponential phase of growth were centrifuged to sediment the cells and washed twice in distilled water by re- centrifugation and re-suspension to remove the nutrient medium. The carbon content of the final concentrate of algae collected quantitatively was determined by the wet-oxidation technique (COD) (MACKERETH *et al.*, 1978). Food medium with a concentration of 1.0 mgC/L was prepared by adding appropriate amounts of GFF-filtered pond water to the stock food suspension and other lower food levels were prepared by careful dilution of the 1.0 mgC/L suspension.

Experimental chambers

The experimental animals were reared for their whole life cycle, from neonates to third adult instar using two culture techniques both designed to provide a constant food concentration. One technique was the continuous flow system described by LAMPERT (1975) which provided a constant food concentration by slow replacement from a stirred known food supply at a rate controlled by a Watson-Marlow peristaltic pump (Figure 1). Six chambers of 100 ml capacity and containing up to 20 individuals in each were
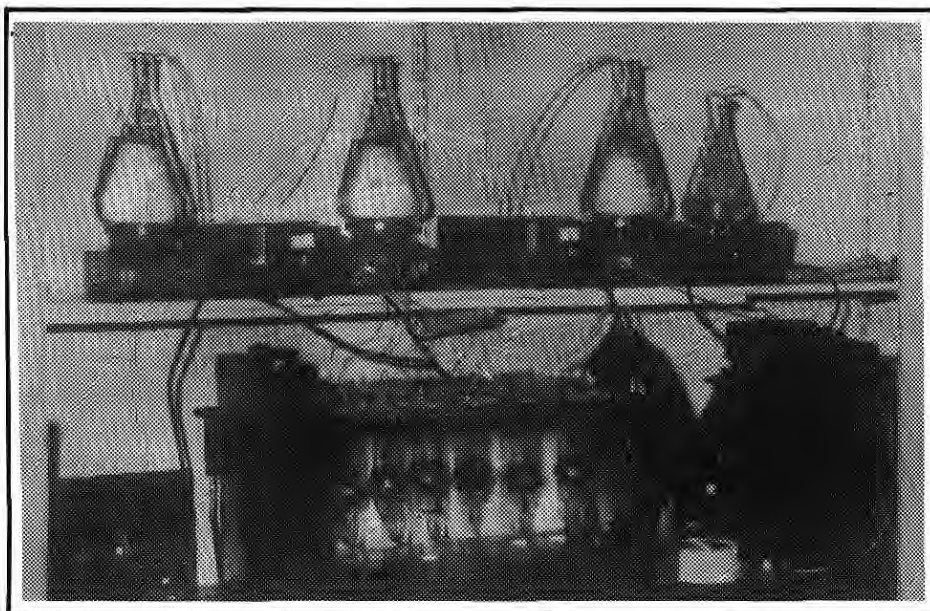
Figura 1. Continuous flow system food concentration and temperature effects on the life cycle characteristics of tropical cladocera *Daplinia gessneri* Herbst, *Diaphanosoma sarsi* Richard, *Moina reticulata* (Daday): I Development time. E.R. HARDY & A. DUNCAN.

immersed in a temperature-controlled water-bath which maintained both the animals and the pumped food supply at the experimental temperature under natural daylight conditions. The other kind of experimental system adopted was batch culture using 250 ml bottles whose food medium was changed daily. These bottles were turned on their short axes at 1 rpm inside an Astell Hearson incubator with light and temperature control. Each bottle contained one individual and there were 4 replicates.

Experimental design and procedure

Daphnia gessneri was reared at three temperatures (22", 27" and 32"C) and five food concentrations of *Scenedesmus acutus* (1.0, 0.5, 0.25,

0.1 and 0.05 mgC/L), giving 15 experimental conditions; *Diaphanosoma sarsi* and *Moina reticulata* were cultured at the same food levels but only one temperature (27"C). An additional food level (0.03 mgC/L) was tested in *M. reticulata*.

The life cycle studies at one temperature and five food concentrations were started with first instar neonates not older than 12 hours. A number of these (up to 20) were sorted, measured and placed in a chamber (Figure 1). Twice daily (at 8.00 and 17.00), the contents of each chamber was gently poured through a mesh which retained the animals and from which they could be removed by means of a large-bore pipette for quick examination. This involved date and time of observation, observation of state of health, mea-

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surement of length, determination of instar stage (carapaces) and counting the number of eggs or embryos in the brood pouches of adults. The duration of the adult instars gave embryonic durations. Animals were replaced into a clean flow-through chamber filled with freshly prepared food medium. With experienced handling, mortalities were almost nil amongst well-fed animals at the higher food concentrations but attained levels of 30% (*Daphnia*) to 40% (*Moina*) at the lowest food levels (0.05 mgC/L) mostly during ecdysis when animals were especially vulnerable. Any accidental deaths or losses of animals were not replaced in flow cultures but neonates were replaced in batch cultures.

RESULTS

Embryonic development

Table 1 gives the mean durations of embryonic development in the three cladoceran species at each of the experiment food-temperature combina-tions in flow culture. These means, which were calculated from between 6-12 observations of all the individuals (up to 20) in one flow chamber, represent the time from release of oocytes from the ovary into the brood pouch to the release of first instars from the brood pouch, as detected by twice daily observation.

In *D. gessneri* with the most complete data set, it is clear that temperature has a powerful influence, doubling the embryonic duration with a 10°C decrease in temperature. There is also a food effect which results in a less pronounced prolongation of embryonic duration as food levels become more limiting. Moreover, the absence of D_e values for 0.05 mgC/L at 27°C and for 0.10 and 0.05 mgC/L at 32°C is because all the juveniles died before maturing to an adult with adult instars. The shortest duration occurred at 32°C and 1.0 mgC/L and the most prolonged embryonic duration at 22°C and 0.25 mgC/L. There is a similar food effect on embryonic

Table 1. The duration of the embryonic development (D_e) of *Daphnia gesseneri, Moina reticulata* and *Diaphanosoma sarsi* in various combinations of temperature and food concentration in flow culture Duration in hours; mean±Standart deviation; number of observations in brackets.

TEMPERATURE	FOOD CONCENTRATION (mgC.L⁻¹)					
°C	1,0	0,5	0,25	0,1	0,05	0,03
Daphnia Gessneri						
22	50±4,6 (12)	56±10.6 (12)	63±11,5 (12)	58±16,3 (9)	54±10,0 (7)	-
27	40±14,7 (12)	38±16,0 (12)	54± 10,8 (12)	54±16,9 (12)	*	-
32	24±0,0 (12)	28±9,7 (12)	31±11,5 (12)	*	*	-
Moina Reticulata						
27	24±0,0 (12)	24±0,4 (12)	24±0,4 (12)	25±3,4 (12)	31±7,0 (3)	51±2,5 (7)
Diaphanosoma Sarsi						
27	24±0,0 (12)	29±10,4 (9)	34±12,4 (9)	40±11,4 (6)	44±20,4 (11)	-

* The females did not complete three broods.

- Not tested.

duration in *M. reticulata* and *D. sarsi* (Table 1). The embryonic duration of *Moina* at food levels 1.0 to 0.25 mgC/L was constant at 24 hours and was both much shorter and less variable than those of the other two species (Table 1).

A comparison of the three species at one temperature shows considerable variation in embryonic durations at optimal food levels, that of the largest species (*Daphnia*) being much longer than the two smaller species. At the most severe food level of 0.05 mgC/L, the durations vary with species size (54 hr, 1.5mm; 44 hr, 1.2mm; 31 hr, 0.8mm) (Table 1).

Table 2 gives the results of fitting a curvilinear regression of the form D=a.T^{-b} to describe, for *Daphnia gessneri* only, the relationship between embryonic development (D in hours) and temperature (T in degrees Celsius) for each tested food level. Statistically significant regressions were obtained for food levels from 1.00 to 0.25 mgC/L but not for 0.1 mgC/L (P=0.25) or

for 0.05 mgC/L in which the life cycle was completed under flow conditions at 22°C only (Table 1). Table 3 gives the results of covariance analysis of the three significant regressions in Table 2. This shows that the regression coefficients (slopes) were not significantly different but that the elevation of the regression for 0.25 mgC/L was significantly lower than the other two higher food levels.

An attempt was made to fit similar curvilinear regressions to quantify the relationship between embryonic development and food concentration for each tested temperature and species. Only one significant relationship was found, that for *Diaphanosoma sarsi*: lnY = 3.19 - 0.179lnX (df=1,42; F=130; P=0.001).

Post-embryonic development

The duration of post-embryonic development (=D$_j$ juvenile duration) was taken to extend from the release of neonates from the female's brood pouch (time 0) to the first appearance

Table 2. Curvilinear regressions relating the duration of embryonic development to temperature at various food concentrations for *Daphnia gessneri* in flow culture.

Regression equation lny = lna - 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

FOOD LEVEL (MgC. L⁻¹)	ln a	b	df	F	P
Daphnia gessneri					
1,0	9,76	-1,88	1,29	27,9	0.001
0,5	9,86	-1,90	1,32	87,6	0.001
0,25	10,03	-1,88	1,31	5,7	0.025
0,1	8,87	-1,54	1,18	2,6	0.25
0,05	*				

* regression coud not be calculated

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Table 3. Covariance analysis of the significant regressions of duration of embryonic development on temperature at various food levels for *Daphnia gessneri*. 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.

FOOD LEVEL mgC.L⁻¹	GROUP	REGRESSION COEF.±SE	Comparisons of Slopes			
			df	F	P	SS-STP
1,0	1	-1.88±0.30	2,95	0.001	0,99	<u>1 2 3</u>
0,5	2	-1.90±0.33				
0,25	3	-1.88±0.33				
mgC.L⁻¹	GROUP	ADJUSTED MEAN+SE	Copararison of Elevations			
			df	F	P	S-N-K
1,0	1	3.59±0.15	2,98	8.61	0.000	<u>1 2 3</u>
0,5	2	3.64±0.15				
0,25	3	3.85±0.15				

of the primiparous female carrying her first brood. This was easiest to observe on individuals in batch culture. Thus the duration of the juvenile stage also estimates the age of the primipara female. In practice, the actual moment of release of neonates or oocytes was only rarely observed but were recorded at the next observation time. The presence of an ecdysed carapace

Table 4. Duration of post-embryonic development (hours) to primipara and of instars (in parenthesis) attained in various combinations of food and temperature in, *Daphnia gessneri*, *Moina reticulata* and *Diaphanosoma sarsi*. N = 4. Batch culture.

TEMPERATURE °C	FOOD CONCENTRATION (mgC.L⁻¹)					
	1,0	0,5	0,25	0,1	0,05	0,03
Daphnia gessneri						
22	177±11.4 (V-VII)	186±12.0 (VI-VII)	186±20.7 (VIII)	207±35.8 (VIII)	240±56.7 (VII-X)	-
27	174±12.0 (V-VII)	156±16.9 (V-VII)	162±12.0 (VI-VII)	192±0.0 (VIII-IX)	198±22.8 (IX)	-
32	132±0.0 (IV-VI)	156±0.0 (V.VI)	162±12.0 (V-VI)	207±18.0 (VI-VII)	246±53.2 (VII-VIII)	-
Moina reticulata						
27	54.0±0.0 (III)	48.0±0.0 (III)	48.0±0.0 (III)	48.0±0.0 (III)	60.0.±0.0 (III-IV)	90.0±12.0 (IV-V)
Diaphanosoma sarsi						
27	72.0±13.8 (III-IV)	72.0±13.8 (IV)	90.0±12.0 (IV-V)	116.0±13. (V)	126.0±12. (V-VI)	-

- not tested

Food concentration and ...

accompanies the release of the first instar neonates and permits the identification of the instar stage of the female.

Table 4 gives the mean post-embryonic durations (± SD) for the three species at the same temperature and food level combinations as before but taken from the batch culture experiments. In *D. gessneri*, there is a general pattern of prolongation of post-embryonic development with decrease in temperature, with some variability at the higher food levels. in Table 5, three significant curvilinear regressions of duration on temperature could be fitted to the data for the top food levels but not for 0.1 and 0.05 mgC/L. Covariance analysis in Table 6 of the three significant regressions
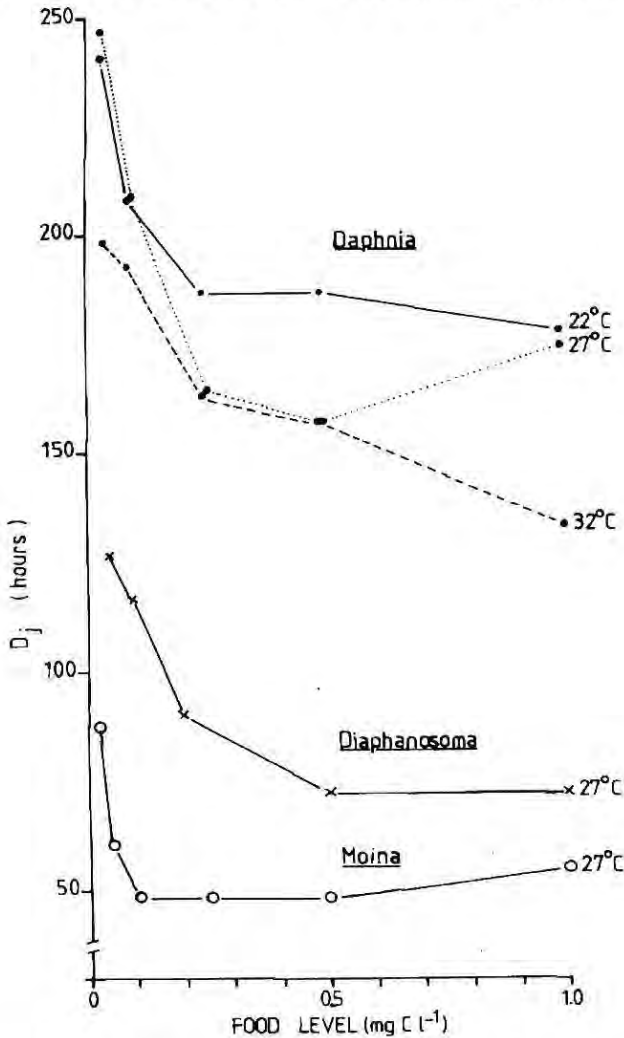

Figura 2. The effect of food concentration on the mean duration of postembryonic development of *D. gessiri, D. sarsi* and *M. reticulata* at various temperatures.

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showed that neither the slopes (P=0.24) nor the elevations (P=0.26) of these differed. This means that temperature influences juvenile duration in *Daphnia gessneri* in the higher food levels but not in the lower ones.

The striking effect in Table 4 is the prolongation of the post-embryonic duration in the lower food levels from 0.1 mgC/L downwards. in *D. gessneri*, from 0.25 mgC/L downwards in *D. sarsi* and from 0.05 mgC/L downwards in *M. reticulata*. Table 7 gives the results for fitting curvilinear regression of juvenile duration on food concentration at each tested temperature. It proved possible to fit significant regressions for *D. gessneri* at all three temperatures and for *D. sarsi* at 27°C but not for *M. reticulata* (P=0.14). This last species shows a rather different pattern of response with a constant juvenile duration of 48 hours for food levels from 0.5 to 0.1 mgC/L (Table 4). However, a significant regression could be fitted to the data for food levels 0.1, 0.05 and

0.03 mgC/L; this was $\ln Y = 1.26 - 1.08\ln X$ (d.f. 1,7; F=249; P=<0.001).

Figure 2 illustrates these relationships between D_j and food concentration in the three cladocerans and shows several interesting features. One is a body size effect, namely that post-embryonic duration was longest in the largest species *D. gessneri* (3rd instar adult size, 1.5mm), shortest in the smallest species *M. reticulata* (0.8mm) and was intermediate in *D. sarsi* (1.2mm). This figure also shows that the food threshold level at which food limitation starts (= marked prolongation of D_j) is much lower in *Moina* (<0.1 mgC/L), higher in *Diaphanosoma* (0.25 mgC/L) than in *Daphnia* (at 0.1 mgC/L) in a manner which parallels embryonic duration. The aberrant point at 27°C/1.0 mgC/L in Figure 2 comes from a high degree of variability in individual D_j values and general lower "fitness" ("difficult" ecdysis) in what seems to be "excess" food for this species.

Table 4 also gives the instar stage of the primiparous females for each

Table 5. Curvilinear regressions relating the duration of post-embryonic development to temperature at various foog concentration for *Daphnia gessneri* in batch culture.

Regression equation $\ln Y = \ln a - \ln X$

Y = post-embryonic development in hours; X = temperature in °C

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

FOOD CONCENTRATION (mgC.L⁻¹)	ln a	b	df	F	P
Daphnia gessneri					
1	7,55	-0,75	1,10	19,75	0.001
0,5	6,67	-0,47	1,10	9,96	0.010
0,25	6,34	-0,37	1,10	4,72	0.05
0,1	5,25	0,01	1,8	0,006	0.94
0,05	5,01	0,09	1,10	0,059	0.81

temperature-food condition. The general pattern of response in *D. gessneri* is that the females became primiparous at instar IV-V at the higher temperatures and higher food levels but were not able to produce a brood of young until instars VIII-IX-X at lower temperatures and low food levels. In both *Diaphanosoma* and *Moina* at 27°C, primiparous females appeared at instar III in the highest food levels but at later and later instars with declining food levels. *Diaphanosoma* matured at instars V-VI in 0.05 mgC/L and *Moina* at instar IV in 0.03 mgC/L. Only in *Moina*, the pattern of response differed in that there was no change in the duration of postembryonic development until they were reared in food levels of 0.05-0.03 mgC/L. In general, the response to severe conditions of both high temperature and low food level appears to involve delaying the primipara's stage of development and, inevitably, increasing her age.

Table 8 gives values of the ratio for D_j/D_c for all three species reared in batch culture at the comparable temperature of 27°C. The pattern shown by *Moina reticulata* is the simplest - the ratio increases with greater prolongation of D_j as food levels became limiting and, as shown in Table 4, this is associated with an increased number of juvenile instars from III to V. The pattern of D_j/D_c with food level is more complex in the other two species: the lowest ratio occurs at 0.25 mgC/L and it increases on either side of this food level. The increased ratios at food levels lower than 0.25 mgC/L are due to food limitation,

together with associated increased number of juvenile instars, as in *Moina:* from V to IX in *Daphnia* and from IV to VI in *Diaphanosoma*. We do not know the causes of the larger ratios in food levels larger than 0.25 mgC/L in these two species, except that these are not associated with increased number of juvenile instars (Table 4). In general, the replicated individuals reared in the food levels of 0.5 and 1.0 mgC/L were more variable in D_j and D_c and less "fit".

The relationship of post-embryonic duration to both temperature and food concentration was examined for *D. gessneri* by means of multiple regression analysis (Table 9). The best fit to the data was obtained using the equation $1/D_j = a - b. 1/T + c.\ln F$. An analysis of the variance associated with food concentration was three times greater than that due to temperature.

DISCUSSION

The three species studied are all truly tropical (Lake Jacaretinga, 3°S). *Daphnia gessneri* was first described in 1965, has been recorded only from northern South America and is the only *Daphnia* species recorded in the well-worked Lake Jacaretinga and its other localities. Both *Moina* and *Diaphanosoma* are commoner genera in the South America, particularly in the turbid Amazonian lakes, but little is known about their distribution elsewhere.

The main findings of this study are that the quantity of food is as im-

portant factor as temperature for the duration of development of tropical planktonic cladocerans as of temperate species. There is a significant prolongation of development as either temperature or food level decreases as well as temperature-food synergistic effects reported by ORCUTT & PORTER, (1984). These effects are shown for *D. gessneri, Diaphanosoma sarsi* and *Moina reticulata* for the first time.

Embryonic duration

Temperature was the main factor controlling the embryonic duration of *Daphnia gessneri* reared on the higher food levels, with a halving of the duration with a 10°C increase in temperature (Table 1). However, at lower food levels there is some evidence of a food-level effect on D_e shown by longer D_e values which caused non-significant regressions of D_e on temperature or inability to measure D_e in tested food levels under flow conditions (Tables 1, 2). Both of these effects may be due to the poor nutritional condition of the mother under food limiting condition, particularly at the two higher temperatures. ORCUTT & PORTER (1984), who quantified their experimental food levels (0.02 to 2.0 mgC/L), have also found a significant food-level effect as well as synergistic temperature-food effects on embryonic development of the Georgian (USA) *Daphnia parvula.* Compared with *Daphnia,* the food-level effect on D_e of *Moina reticulata* and *Diaphanosoma sarsi* was both

Table 6. Covariance analysis of the regressions comparing the duration of post-embryonic development on temperature at different food concentrations for *Daphnia gessneri,* The regression coefficient were compared by the SS-Stp test and difference between elevations by the S-N-K test. Regression coeficients and mens underlined are not significantly different at P = 0.05 level.

Regression equation $\ln Y = \ln a - b \ln X$

Y = duration of post-embryonic development in hous; X = food concentration in mgC.L^{-1};

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

FOOD LEVEL mgC.L^{-1}	GROUP	REGRESSION COEFF.±SE	COMPARISON OF SLOPES			
			df	F	P	SS-STP
1.0	1	-0.75±0.17	2,30	1,45	0,24	3 2 1
0.5	2	-0.47±0.15				
0.25	3	-0.37±0.17				
mgC.L^{-1}	GROUP	ADJUSTED MEAN±SE	COMPARISON OF ELEVATIONS			
			df	F	P	S-N-K
1.0	1	5.07±0.05	2,33	1.39	0.26	1 2 3
0.5	2	5.10±0.05				
0.25	3	5.13±0.05				

Table 7. Curvilinear regressions relating the duration of post-embryonic development to food concentration at various temperature for *Daphnia gessneri, Moina reticulata* and *Diaphanosoma sarsi* in batch culture

Regression equation $\ln Y = \ln a - b \ln X$

Y = post-embryonic development in hours; X = food concetration in $mgC.L^{-1}$

TEMPERATURE °C	ln a	b	df	F	P
Daphnia gessneri					
22	5.14	-0.087	1,18	8.63	0.008
27	5.07	-0.060	1,16	6.89	0.018
32	4.89	-0.160	1,18	50.70	0.000
Moina reticulata					
27	43.38	-8.05	1,18	3.22	0.14
Diaphanosoma sarsi					
27	4.20	-0.21	1,17	45.1	0.000

larger and started at lower food levels (Table 1). JAYATUNGA (1986), who is the only other worker on tropical cladocerans using defined food levels, found a significant prolongation of embryonic duration in the Sri Lankan *Moina micrura* at 0.05 mgC/L) in both 27°C and 32°C).

Postembryonic duration

The dependence of juvenile duration on food concentration has been shown for all three cladoceran species but how temperature affects this response could be demonstrated only for *Daphnia*.

The pattern of response in *Daphnia gessneri* is that the juvenile phase prolongation was continuous as food level declined, was greatest at the two lower food levels and most severe at the highest temperature. This pattern of D_j on food level was statistically significant at each temperature (Table 7), there is no statistical difference between regressions for D_j on temperature (Table 6) and the multiple regression in Table 9 shows that the variance associated with food concentration was three times greater than that due to temperature. The most severe food limitation occurred in the combination of low food and high temperature which represents extreme conditions for a tropical species whose normal temperature range in the field is a few degrees either side of 27°C.

A similar pattern of response to that of *Daphnia* described above was shown by *Diaphanosoma sarsi* at 27°C. Here too a significant regression of D^j on food was obtained and the greatest prolongation of the juvenile phase occurred at the lowest food of 0.05 mgC/L. Only in *Moina reticulata* at 27°C was there no significant relationship between D_j and food concentration including all six tested levels. The D_j remained constant down to 0.1 mgC/L and a significant relationship could only be obtained from the three lowest food levels. *Moina reticulata* is noteworthy in being the only tested species whose juveniles survived to maturity at 0.03

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Table 8. The mean values of the ratio D_j/D_c and standard deviation for *Daphnia gessneri, Moina reticulata* and *Diaphanosoma sarsi* in batch culture. Values in brackets gives the number of observations.

T°C	FOOD CONCENTRATION ou mgC,L⁻¹					
Species	1.00	0.50	0.25	0.10	0.05	0.03
Daphnia gessneri						
27°C	3.01 ±1.55(5)	2.16 ±0.52(5)	1.71 ±0.20(6)	3.13 ±0.87(3)	2.70 ±0.86(4)	-
Diaphanosoma sarsi						
27°C	2.75 ±0.58(4)	2.71 ±0.47(3)	2.07 ±0.53(3)	3.19 ±0.0(2)	2.83 ±0.89(4)	-
Moina reticulata						
27°C	1.17 ±0.0(3)	1.17 ±0.0(3)	1.42 ±0.0(3)	1.36 ±0.44(3)	1.99 ±0.44(3)	1.97 ±0.0(3)

mgC/L. *Daphnia parvula* can complete her life cycle in a food concentration as low as 0.02 mgC/L, according to ORCUTT & PORTER (1984).

Food thresholds

In the process of food limitation, there are two food threshold levels : that at which onset of food limitation starts (= beginning of developmental prolongation) and that below which maturation of juveniles becomes impossible. This defines the concentration range of food limitation. At the comparable temperature of 27°C, these three species differed both these food

threshold levels. They were lowest in *Moina reticulata* and higher in the other two species. This is a finding that is opposite to ROMANOVSKY's (1985) prediction that ruderal species like *Moina* have "higher food thresholds" than competitor species like *Daphnia* and *Diaphanosoma*. Temperature also affected food threshold levels at which juveniles could not complete their maturation: for *D. gessneri*, these were <0.25 mgC//L at 32°C, <0.1° mgC/L at 27°C and <0.05 mgC/L at 22°C. The absolute concentration of these tropical food thresholds for development are similar to those reported for temperate

Table 9. Parametrs of the multiple regressions relating the effect of food concentration and temperature on the duration of post-embryonic development of *Daphnia gessneri*.

Regression equation: $1/D_j = a - b, 1/T + \ln F$

D_j = juvenile duration in hours; T = temperature in °C; F = food concentration in mgC.L⁻¹

df = degress of fredom; F = variance ratio; P = level of significance.

Daphnia gessneri						
	a	b	c	df	F	P
	0.0087	-0.611	0.00054	2,55	24.37	0.001
Due to food concentration					36.39	0.001
Due to temperature						

and other tropical species (ROCHA, 1983; ORCUTT & PORTER, 1984, JAYATUNGA, 1986).

Numbers of juvenile instars

LEI & ARMITAGE (1980) suggest that an increase in postembryonic development time could arise either from an increase in the duration of the individual juvenile instars without an increase in their number or from an increase in the number of juvenile instars without an increase in their individual duration or from an increase in both duration and numbers of juvenile instars. From our evidence in Table 4, all three species lengthen their juvenile phase mainly by adding more juvenile instars and that this occurs only at limiting food levels. However, at 32^0C *Daphnia gessneri* is capable of increasing both duration and numbers of juvenile instars as the food level is changed from 1.0 mgC/L to 0.05 mgC/L.

The ratio D_j/D_e

BONOU *et al.* (1991) and SAINT-JEAN & BONOU (1994) show very clearly the relationship between the number of juvenile instars and the size of the D_j/D_e ratio in *Moina micrura* from fish ponds in the Ivory Coast . Both papers show that in the 30^0C experiments all individuals attained the primipara stage with 2 instars and a D_j/D_e ratio of 0.98 in 1991 paper and 0.89 in the 1994 paper. Whereas there were 2-instar, 3-instar and 4-instar primiparas present in the 26^0C experiments, with D_j/D_e ratios of 1.12 and 1.03, respectively. From our results in Table 6, these were well-fed *Moina* but there was probably some difference between the food conditions in these two periods as well as a temperature difference. In general, the response to food limitation appears to involve delaying the primipara's development stage thus, inevitably, increasing her age.

Body size effect

Within the three tropical cladocerans studied here, the smallest *Moina reticulata* attained maturity in the fastest time at all food levels and under similar conditions compared with *Diaphanosoma sarsi* and *Daphnia gessneri*, both larger animals.

This confirms its classification by ROMANOVSKY (1985) and SAINT - JEAN & BONOU (1994) as an opportunistic ruderal species.

It provides some evidence to support the hypothesis that body size and duration of postembryonic development of cladocerans are positively related (HALL ET A., 1976; ALLAN & GOULDEN, 1980).

ACKNOWLEDGMENTS

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