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The effect of temperature, and food quantity and quality on the growth and development rates in laboratory-cultured copepods and cladocerans from a Sri Lankan reservoir†

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Abstract

Length growth, instar durations, fecundity and mortality rates of five species of microcrustacean zooplankton from a tropical reservoir were measured in relation to food quantity and temperature in laboratory cultures. Three cladocerans (*Ceriodaphnia cornuta, Moina micrura, Diaphanosoma excisum*), one calanoid copepod (*Heliodiaptomus viduus*), and one cyclopoid copepod (*Mesocyclops thermocyclopoides*) were studied. Filtered seston (45 μm mesh) from a local pond was used for food. Two food concentrations were employed: (1) 10 μg chlorophyll l^{-1} (ca 0.25 mg Cl^{-1}), and (2) 50 μg chlorophyll l^{-1} (ca 1.25 mg C l^{-1}). Food levels and water temperature (22.5, 27.5, and 32.5 °C) used, roughly covered the ranges found in the reservoir. Although all the three growth parameters were often affected to some degree by temperature and food, the quantitative response of the species differed. Also, the species reacted differently to the three possible interactions (i.e. food × temperature, food × instar, and temperature × instar). This contributed to the overall differences in the species responses. For the cladocerans, instar durations were always affected by temperature. The food did not affect the duration time of the adults and that of the combined juvenile instars, the latter except in one case significantly. For the two copepods food level affected the duration times of naupliar and copepodite instars, but the effect of temperature was only significant for *H. viduus*. The development times observed for *H. viduus* were extraordinary long compared with values reported in the literature for other tropical calanoids. This suggests that food conditions in our culture were adversely affecting its growth rates.

Introduction

Zooplankton is a key element in the functioning of most lake and reservoir ecosystems in the temperate region. It controls the algal growth by its grazing, and is the resource base for the youngest ontogenetic stages of most fish species (Fernando, 1994). Furthermore, it sustains the growth and reproduction of older stages of obligatory and facultative zooplanktivorous species. In the temperate region, zooplanktivores usually dominate the fish community in terms of production (e.g. Mills et al., 1987; Vijverberg et al., 1990) and predation by fish can indirectly affect phytoplankton biomass and composition through predation on zooplankton (e.g. Lynch & Shapiro, 1981; Shapiro & Wright, 1984). Whether this is also true for tropical reservoir and lake ecosystems is still unresolved and two different views exist. Firstly, microcrustacean zooplankton of tropical waterbodies is generally of small size and less abundant than in the temperate region

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(Hebert, 1978; Dumont & Tundisi, 1984; Fernando, 1994). Besides, the zooplanktivorous fish guild seems to be less well represented, being largely replaced by the phytoplanktivores/detritivores (Fernando & Holcik, 1982; Bowen, 1988). This suggests an insignificant role of zooplanktivores in the lacustrine food web of the tropical region. Secondly, it can be argued, that this view is based upon sparse knowledge. Almost nothing is known about either the zooplankton production in tropical lakes or reservoirs and its utilization by zooplanktivorous fish. The notion that zooplanktivores are generally less important in the tropical region is largely based on the over representation of larger individuals of larger fish species in fishermen's catches. Moreover, the results of several studies which have included small species and/or the young of the larger species suggest that this view is not correct. It may be that zooplanktivores, and thus zooplankton, play a much more important role as was thought previously (Marshall, 1984; Newrkla & Duncan, 1984; Hecky, 1991; Witte et al., 1992; Hanna & Schiemer, 1993; Sirimongkonthaworn & Fernando, 1994; Piet et al., in press). In some large lakes and reservoirs in Africa, small pelagic zooplanktivorous fish play a key-stone role in the food web and contribute considerably to artisanal fisheries (Marshall, 1984).

To evaluate the functional role of microcrustacean zooplankton in tropical aquatic food webs, it is essential to quantify zooplankton production dynamics. Since microcrustaceans in tropical water bodies reproduce continuously, it is impossible to distinguish cohorts. Therefore, production has to be estimated by independent measures of growth rate and development time. Although there is a wealth of information about the effect of food and temperature on growth and development of zooplankton from the temperate region, this kind of information about tropical species is meagre. There are only a few quantitative studies on tropical species (e.g. Gras & Saint-Jean, 1976, 1978; Piyasiri, 1985; Duncan, 1989; Bonou et al., 1991; Hardy & Duncan, 1994).

For estimating zooplankton production in the Tissawewa reservoir in South-eastern Sri Lanka, we assessed the instar durations and growth in length of three dominant cladoceran species, *Ceriodaphnia cornuta, Moina micrura, Diaphanosoma excisum* and two dominant copepod species, *Mesocyclops thermocyclopoides*, and *Heliodiaptomus viduus* in the laboratory at three different temperatures and two different food levels. These temperatures and food levels covered the ranges for these factors prevailing in the Tissawewa reservoir.

Methods

Origin of animals

Zooplankton was collected from Tissawewa, an ancient low-land reservoir, impounded in 35 AD and located in the dry-zone of South-eastern Sri Lanka (6 °18′ N, 81 °17′ E). It is small (250 ha), shallow (Z_{mean} = 1.7 m; Z_{max} = 3.5 m), exposed to winds and is characterised by strong fluctuations in water level. It is also eutrophic, with a mean gross primary production 9.5 g O_2 m^{-2} d^{-1} (Piet et al., in press). The reservoir was sampled in 1993 with a 120 μm townet in January for cladocerans and in February for copepods. The animals were immediately resuspended in lake water, and transported directly to the laboratory. Individual animals were picked out and placed individually in 100 ml test tubes, These were placed in thermostatically controlled water baths, which were kept at three temperatures of 22.5, 27.5 and 32.5 ($\pm$ 0.2) °C.

Culture conditions

The growth and reproductive rates of the five zooplankton species were measured at two food levels and at each of the three temperatures (22.5, 27.5, 32.5 °C) under a light: dark regime of 12:12 h. The chlorophyll-*a* content of this water was 100 $\pm$ 5 μg l^{-1}. The algal species present consisted of 90% *Oocystis elliptica* (Chlorophyceae) (90%), and 10% of *Oscillatoria* spp. (Cyanophyceae), *Trachydiscus* spp. (Xanthophyceae) and pennate diatoms (Bacillariophyceae). *Oocystis elliptica* was present as 1-, 2-, 4- and 8-cell clusters in proportions of 57%, 34%, 8% and 1%, respectively. *O. elliptica* cells have an ellipsoid shape, with a length-width ratio of ca 17:12 μm (biovolume 1300 μm^{-3}). In size and shape, it is similar to *Scenedesmus* spp. (Chlorophyceae) which are commonly used as food for freshwater cladocerans in laboratory studies and which are known to support good growth and reproduction (Vijverberg, 1989). Two food concentrations were used: (1) Low (L)-medium, 10 μg chlorophyll-*a* l^{-1} (ca 0.25 mg C l^{-1}; Bailley-Watts, 1974) and (2) High (H)-medium 50 μg chlorophyll-*a* l^{-1} (ca. 1.25 mg C l^{-1}). To attain these chlorophyll levels, the 45 μm filtered pond water was diluted quantitatively with the pond water filtered through glass fibre filters

(Schleicher & Schull no. 6; pore size 0.5–1.5 μm). The chlorophyll content of the pond water was measured at regular intervals during the study period and was found to maintain a rather constant level.

The experiments with the cladocerans were started with F_2 newborn not older than 24 h. The length of the neonates was measured and animals were transferred individually into 100 ml tubes. Approximately twenty such individuals were cultured per temperature-food treatment. Every day, the media were replaced, while at the same time the length of the animals was measured using a micrometer eyepiece to the nearest 0.01 mm, from the anterior end of the head to the posterior margin of the valve. The number of juvenile instars and the time and length at maturity were noted. Once the animals reached maturity, both the number of eggs and newborns were counted, and the length of the individual newborns was measured. These animals were then discarded. Growth and reproduction of the cladocerans were followed for about 21 days and nine adult instars. The intrinsic rate of population increase (r) was estimated using the Euler equation:

$$1 = \sum_{x=0}^{N} \mathrm{e}^{-rx} l_x m_x ,$$

where r = per capita daily rate of increase for the population (d^{-1}), x = age class (0,1....N), l_x = probability of surviving to age x, and m_x = fecundity at age x. Since r is a population parameter it was not possible to compute directly standard errors. Hence the standard error of r was computed using a Jack knife method (Meyer et al., 1986). As only one value of r was available per treatment, it was not possible to compute the significance of the interaction between temperature and food concentration. We circumvented this problem by splitting the series to obtain two independent values for r for each temperature-food treatment. These values were analysed using analysis of variance.

For the two copepod species a slightly different experimental procedure was used. Newborn nauplii were collected within 12 hours after birth, two of which were measured for length, and the rest of the batch were transferred into a tube. The medium in these tubes was replaced daily with fresh seston. To reduce handling mortality, the nauplii were measured systematically only after they reached their fourth naupliar instar. Their naupliar stage was then assessed and their length was measured. For the copepodite stages, cephalothorax length was recorded. The sex of the animals was established in the fifth copepodite stage. Adult copepods were measured and then discarded.

Results

Mortality

The mortality of copepods and cladocerans in the culture varied predominantly according to species and instar stage, but was to a lesser degree also affected by the temperature treatment; food effects were less clear (Table 1). The lowest mortalities were observed for *C. cornuta* (0–4% d^{-1}), somewhat higher mortalities for *M. micrura* (2–13% d^{-1}), and high mortalities for *D. excisum* (6–19% d^{-1}). The mortalities of *H. viduus* and *M. thermocyclopoides* (1–15% d^{-1}) were similar to those observed for *M. micrura*. For cladocerans, mortality in the juvenile phase was generally higher than in the adult phase, whereas in copepods, naupliar mortality was generally higher than mortality of juvenile copepodites. The highest mortalities were often observed at the highest temperature.

Cladocerans

Both temperature and food concentration were found to affect the growth in length of the cladocerans, although the extent of these effects differed between species (Figure 1). Growth patterns were analysed including the length measurements of the different instars as repeated measurements, with food and temperature as independent factors (Table 2). The overall growth pattern of *C. cornuta* was significantly affected by temperature and food, but not in *M. micrura*. In *D. excisum* only temperature significantly affected growth. For all three species none of the interaction effects between food and temperature differed significantly from zero. But, for *M. micrura* the interaction of instar with food and temperature, respectively, were both significant, whereas for *C. cornuta* only the interaction of instar with temperature was significant.

The effect of food level and temperature on the clutch size of the different adult instars is shown in Figure 2. For *C. cornuta* fecundity at low food levels was independent of temperature, whilst for the high food levels there was a marked effect of temperature, the lowest fecundity being observed at the highest temperature. Fecundity pattern of *M. micrura* showed a more clear food effect, than temperature effect. Clutch size of *D. excisum* decreased after the first adult instar and

Table 1. Mortality (% .d^{-1}) and number of individuals with which the culture was started between brackets, for three cladoceran and two copepod species at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C) and two food levels (L: 10, H: 50 μg chlorophyll.l^{-1}). Cladoceran mortality is given separately for combined juvenile instars (JUV) and for adults instars 1–8 (ADULT), copepod mortality is given for combined naupliar instars (NAUP) and for combined juvenile copepodite instars (COP).

Species	Stage	LA	HA	LB	HB	LC	HC
C. cornuta	JUV	1.5 (18)	1.2 (18)	0.0 (18)	0.0 (18)	0.0 (18)	0.0 (18)
	ADULT	1.6 (17)	4.2 (17)	1.2 (18)	0.0 (18)	0.0 (18)	1.9 (18)
M. micrura	JUV	5.8 (19)	5.3 (21)	3.5 (19)	6.2 (18)	11.1 (19)	13.1 (19)
	ADULT	3.5 (16)	3.5 (18)	2.1 (18)	1.9 (17)	5.4 (17)	4.4 (17)
D. excisum	JUV	10.2 (19)	11.5 (22)	12.3 (19)	10.5 (19)	19.4 (20)	10.8 (20)
	ADULT	6.3 (2)	6.3 (5)	6.4 (8)	7.8 (12)	9.7 (9)	6.9 (13)
H. viduus	NAUP	2.7 (17)	5.1 (12)	1.3 (10)	1.9 (10)	13.3 (15)	2.4 (14)
	COP	2.6 (12)	1.3 (7)	2.4 (9)	0.6 (9)	3.3 (2)	1.5 (12)
M. thermocyclopoides	NAUP	3.1 (25)	3.9 (43)	7.7 (26)	10.0 (45)	3.6 (45)	15.9 (50)
	COP	7.2 (19)	1.0 (33)	1.2 (14)	2.9 (26)	3.8 (34)	1.3 (19)

Table 2. Summary table of the analyses of variance with the lengths of the different instars included as repeated measurements, with food and temperature as independent factors.

Effect	Error df	*Moina micrura*		Error df	*Ceriodaphnia cornuta*		Error df	*Diaphanosoma excisum*	
		F	P		F	P		F	P
Food	48	1.62	0.209	75	6.95	0.010	33	0.73	0.397
Temperature	48	2.06	0.139	75	18.93	<0.001	33	12.41	<0.001
Instar	192	671.37	<0.001	300	225.0	<0.001	66	30.64	<0.001
Food × Temp	48	1.09	0.345	75	0.04	0.960	33	0.24	0.786
Food × Instar	192	4.81	0.001	300	1.30	0.270	66	5.73	0.005
Temp × Instar	192	9.94	<0.001	300	3.33	0.001	66	1.59	0.187
3-way interaction	192	1.21	0.293	300	0.71	0.687	66	1.06	0.382

remained low in the subsequent instars. Effects of food level and temperature on clutch size of the different instars was analysed using ANOVA, after square root transformation of the clutch size of the different adult instars, included as repeated measurements, and with food and temperature as independent factors (Table 3). In both *M. micrura* and *C. cornuta* fecundity was significantly affected by both temperature and food level, but not in *D. excisum*. In *M. micrura* there was a significant temperature-instar interaction, indicating that the fecundity of the instars were differently affected by temperature. *C. cornuta* showed two significant interactions: one between food and temperature, indicating that fecundity was differently affected by temperature at the two different food levels (see also Figure 2a), and an other between food level and instar number. The absence of significant interactions observed for *D. excisum* is probably partly due to the low number of juveniles reaching maturity.

In all three cases development time was highly influenced by the temperature (Table 4; Figure 3), but no significant interaction effects were observed between food and temperature. The food level affected juvenile and adult instars differently. For all three species, duration time of adult instars was not influenced by the food level, whereas the effect on the duration of the combined juvenile instars was species dependent. *C. cornuta* showed a significant food effect but, unexpectedly, higher food levels resulted in longer and not shorter duration times. The food effect for *D. excisum* was almost significant on the 0.05 level (Table 4), whereas no significant food effect was observed for *M. micrura*. In two of the three species the observed food effect on the duration time of the combined juvenile instars can at least be partially explained by variation in the number of juvenile instars. *C. cornuta* and *D. excisum* showed a substantial variation in number of juvenile instars among treatments, while this variation was small in *M. micrura* (Table 5). For

Table 3. Summary table of the analyses of variance with the square-root transformed values of the clutch size of the different instars included as repeated measurements, with food and temperature as independent factors.

Effect	Error	*Moina micrura*		Error	*Ceriodaphnia cornuta*		Error	*Diaphanosoma excisum*	
	df	F	P	df	F	P	df	F	P
Food	48	54.97	<0.001	75	4.36	0.040	31	0.34	0.564
Temperature	48	3.25	0.047	75	8.51	<0.001	31	2.25	0.122
Instar	192	13.39	<0.001	300	6.50	<0.001	62	13.14	<0.001
Food × Temp	48	0.02	0.977	75	8.22	<0.001	31	0.69	0.508
Food × Instar	192	2.36	0.055	300	3.32	0.011	62	0.10	0.904
Temp × Instar	192	2.97	0.004	300	1.48	0.165	62	1.00	0.415
3-way interaction	192	0.13	0.998	300	1.90	0.059	62	0.38	0.819

Table 4. Summary table of analyses of variance of development times of the three cladoceran species, with food level and temperature as independent factors, and the time to reach maturity (JUV), and the average time of the first three adult instars (for *D. excisum* the first two adult instars) (ADULT) as dependent ones.

Instar		Error	Food		Temperature		Food × Temp	
		df	F	P	F	P	F	P
M. micrura	JUV	98	0.01	0.936	94.70	<0.001	1.05	0.353
	ADULT	86	0.22	0.638	536.20	<0.001	0.09	0.914
C. cornuta	JUV	99	4.57	0.035	19.85	<0.001	1.71	0.187
	ADULT	91	0.02	0.897	142.98	<0.001	0.87	0.423
D. excisum	JUV	44	3.87	0.055	25.26	<0.001	2.60	0.085
	ADULT	35	0.54	0.467	33.50	<0.001	0.19	0.827

both these species food effects were strongest at the lowest temperature, but the two species reacted differently. *D. excisum* showed a marked increase in the number of instars at the lower food level at the lowest temperature, in contrast, *C. cornuta* showed its lowest number of juvenile instars at this treatment. The two species also reacted differently to temperature: whereas in *C. cornuta* the number of juvenile instars tended to increase with temperature *D. excisum*, to the contrary, showed a decrease.

The rate of population increase (r) of the three cladocerans was estimated for the laboratory conditions. Temperature effect on 'r' is highly significant, whereas food affected *M. micrura* and *D. excisum*, but not *C. cornuta* (Table 6, Figure 4). Significant interactions between food and temperature were not observed.

Copepods

The effects of food and temperature on length growth of the two copepod species, *H. viduus* and *M. thermocyclopoides* were highly variable and not clear (Figure 5). These effects as a whole on instar length were non significant ($P>0.05$). A three-way ANOVA showed only for *M. thermocyclopoides* a significant interaction, between food and instar ($F=2.42$; $P<0.05$), indicating that instar length was affected differently by food depending on instar stage.

There was a clear effect of food on the duration times of the combined naupliar and the combined copepodite instar of the two copepod species, but the effect of temperature on instar duration was less pronounced (Figure 6). *H. viduus* showed a clear temperature effect only at low food level and at the high food level between 22.5 and 27.5 °C, whereas *M. thermocyclopoides* only showed a temperature effect at the high food level between 22.5 and 27.5 °C. When relating the factor temperature as a whole to instar duration the effect was not significant for *M. thermocyclopoides*, but it was significant for *H. viduus* (Table 7). Food affected the instar durations of both copepods significantly, but also instar stage did so (Table 7). In both cases, only the interaction between food and instar was significant. Thus food affects on the instar durations of the different instar stages differed.

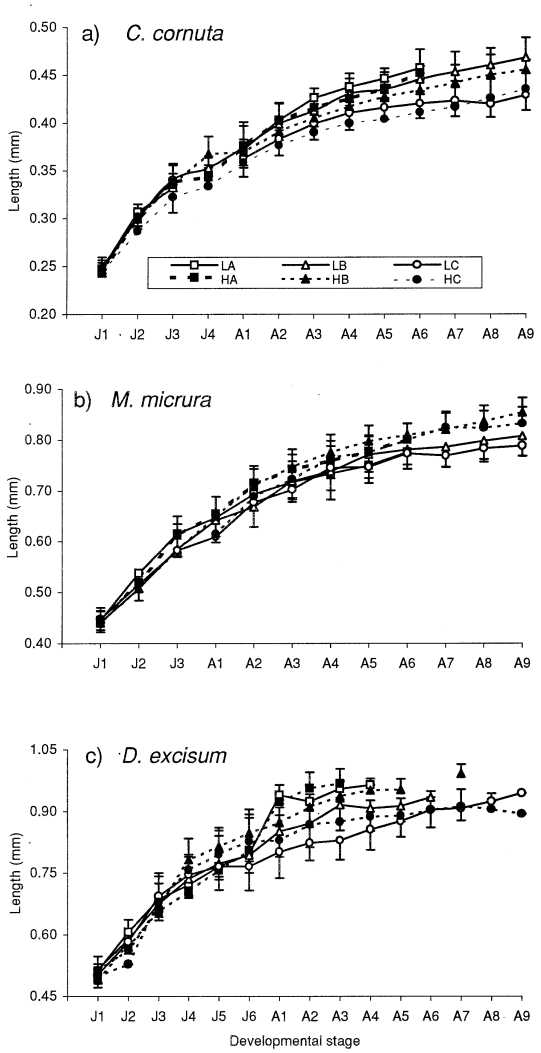

Figure 1. Length increment per instar of different cladoceran species at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C) and two food levels (L: 10, H: 50 μg chlorophyll l^{-1}) for (a) *Ceriodaphnia cornuta*, (b) *Moina micrura*, and (c) *Diaphanosoma excisum*. The error bars in the graphs indicate the standard deviations of the measurements.

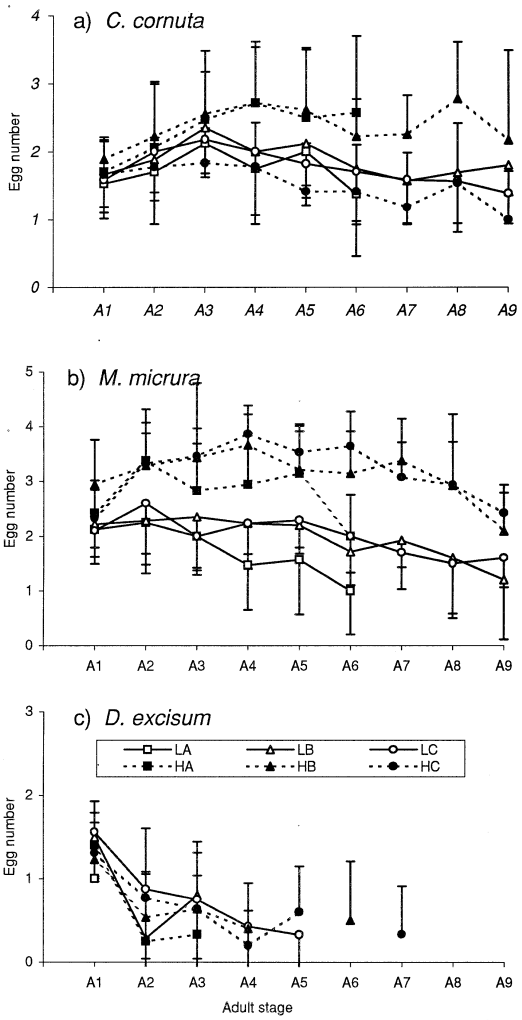

Figure 2. Clutch size per adult instar of different cladoceran species at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C), and two food levels (L: 10, H: 50 μg chlorophyll l^{-1}) for (a) *Ceriodaphnia cornuta*, (b) *Moina micrura*, and (c) *Diaphanosoma excisum*. The error bars in the graphs indicate the standard deviations of the measurements.

Discussion

Although morphological differences between the sexes were large enough to distinguish sexes only from the fourth copepodite instar onwards, significant size differences between juvenile males and juvenile females were found for *M. thermocyclopoides* already in the first copepodite instar (Table 8, Figure 5). In contrast, in *H. viduus* only a small, but significant, size difference was observed in the adult phase.

Ideally, growth and development times used in production studies should be measured under conditions simulating the natural conditions which the populations inhabit. In the Tissawewa reservoir, unfortunately, logistics prevented such an approach, i.e. made it impossible to use fresh natural seston from the reservoir as food and forced us to use seston from a nearby pond at the University campus. This probably biased

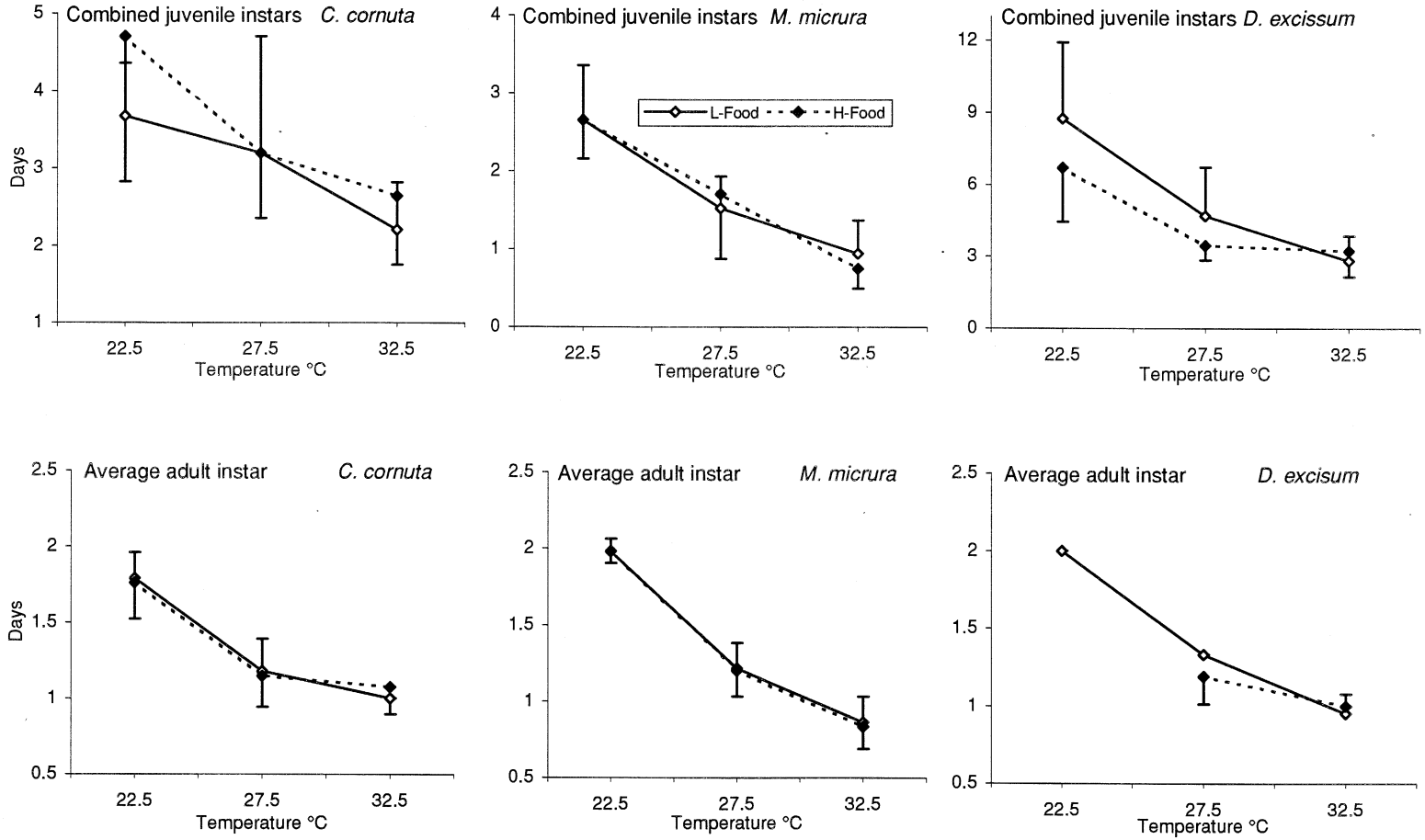

Figure 3. Duration of the combined juvenile instars, and of the average duration of the first three adult instars at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C), and two food levels (L: 10, H: 50 μg chlorophyll l^{-1}), of *Ceriodaphnia cornuta* (left panels), *Moina micrura* (middle panels), and *Diaphanosoma excisum* (right panels). The error bars in the graphs indicate the standard deviations of the measurements.

Table 5. Number of juvenile instars (mean and range) of the three cladoceran species at different experimental conditions. Three temperatures (°C), and two food levels (μg chlorophyll l^{-1}).

Species	Cult. regime	Food	Temp.	Mean	Range	*N*
M. micrura	LA	10	22.5	2.3	2–3	16
	HA	50	22.5	2.3	2–3	18
	LB	10	27.5	2.1	2–3	18
	HB	50	27.5	2.2	2–4	17
	LC	10	32.5	2.1	2–3	17
	HC	50	32.5	2.0	2	17
C. cornuta	LA	10	22.5	2.4	2–3	17
	HA	50	22.5	3.2	2–6	17
	LB	10	27.5	3.0	2–7	18
	HB	50	27.5	2.8	2–4	18
	LC	10	32.5	3.1	3–4	18
	HC	50	32.5	3.1	3–4	18
D. excisum	LA	10	22.5	8.0	7–9	2
	HA	50	22.5	5.8	4–7	5
	LB	10	27.5	5.0	3–9	8
	HB	50	27.5	4.4	4–6	12
	LC	10	32.5	3.8	3–6	9
	HC	50	32.5	4.3	3–7	13

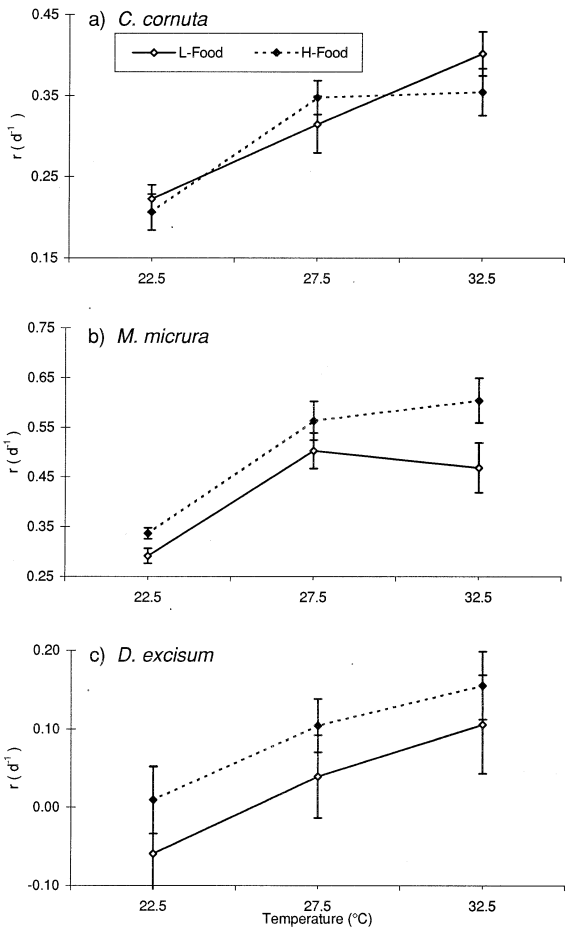

Figure 4. Intrinsic rates of population increase (*r*), at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C), and two food levels (L: 10, H: 50 μg chlorophyll l^{-1}), for (a) *Ceriodaphnia cornuta*, (b) *Moina micrura*, and (c) *Diaphanosoma excisum*. The error bars in the graphs indicate the standard deviations of the measurements.

the results of our laboratory observations, although we simulated realistic levels of temperature and algal biomass.

Mortality rates in the culture varied widely. They were low for *C. cornuta*, i.e. similar to what is usually observed for hardy species like *Daphnia* spp. in well kept cultures (Vijverberg, 1989), generally moderately low for *M. micrura*, *H. viduus*, and *M. thermocyclopoides*, but high for *D. excisum*. We cannot explain why the survival rate of this species was so low in comparison with the other species in the culture.

Although all three growth parameters of the five study species (i.e. length, instar duration, fecundity) were often affected in some degree by temperature and food, the quantitative response of the species to these factors was quite different. Besides, the species reacted often differently to the three possible interactions, food × temperature, food × instar, and temperature × instar. This contributed further to the overall differences in response of the species. Instar duration time of cladocerans was always affected by temperature, food significantly affected the duration time only of the combined juvenile instars in one species only. For the copepods food level affected the duration times of the naupliar and copepodite instars of both species. But the temperature effect was only significant for *H. viduus*. Apparently both species were severely food limited at the lowest food level.

Threshold Food Concentration

The Threshold Food Concentration for growth, a concentration just sufficient to cover maintenance requirements but insufficient for growth and development (Lampert, 1977), is an ecological important food concentration. Duncan (1989) compared the threshold values for tropical cladocerans with those of temperate cladocerans and observed that for tropical species threshold food level was an order of magnitude high-

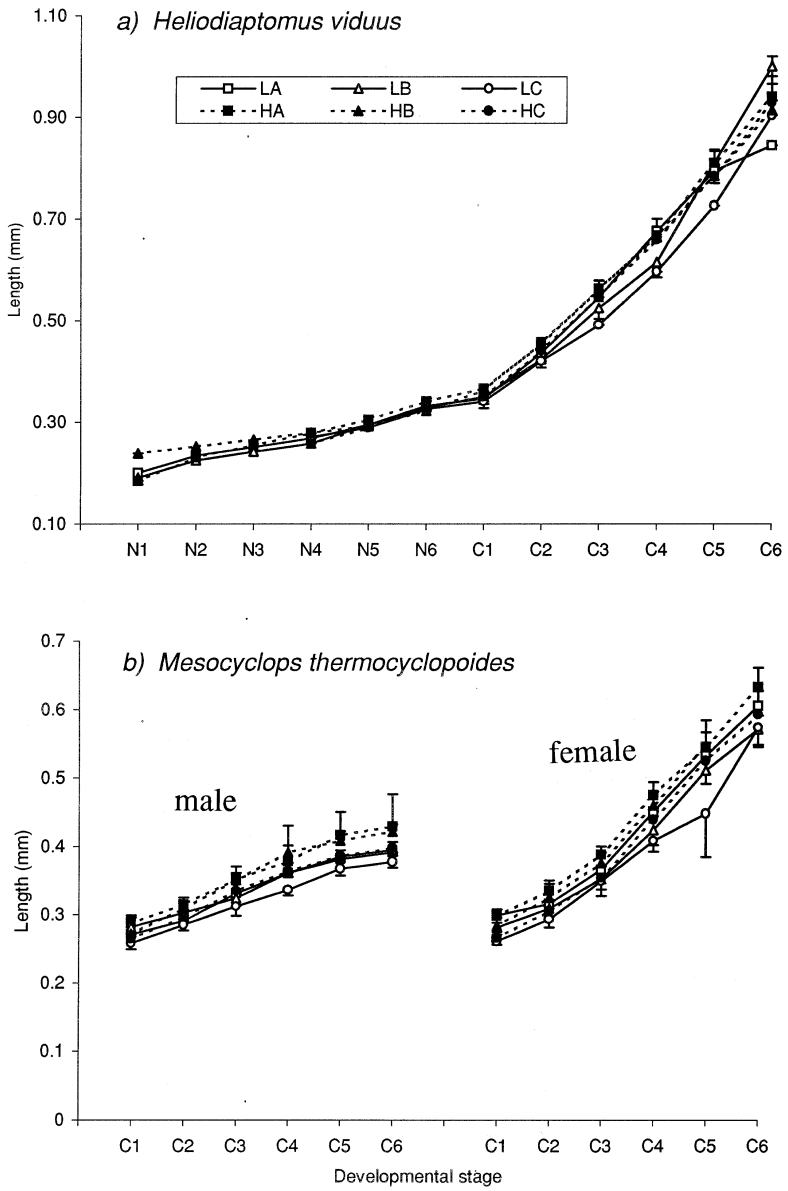

Figure 5. Length increment per instar of *Heliodiaptomus viduus* and *Mesocyclops thermocyclopoides*, at three temperatures (A: 22.5, B: 27.5, C: 32.5 °C) and two food levels (L: 10, H: 50 μg chlorophyll l^{-1}). The error bars in the graphs indicate the standard deviations of the measurements.

Table 6. Summary table of analyses of variance for the three different cladoceran species, with food level and temperature as the independent variables and split-r values as the dependent ones.

Species		Food		Temperature		Food × Temp	
	df	F	P	F	P	F	P
Moina micrura	6	7.62	0.033	28.45	<0.001	1.03	0.412
Ceriodaphnia cornuta	6	0.32	0.591	32.28	<0.001	1.94	0.224
Diaphanosoma excisum	6	8.12	0.029	21.04	0.002	3.28	0.109

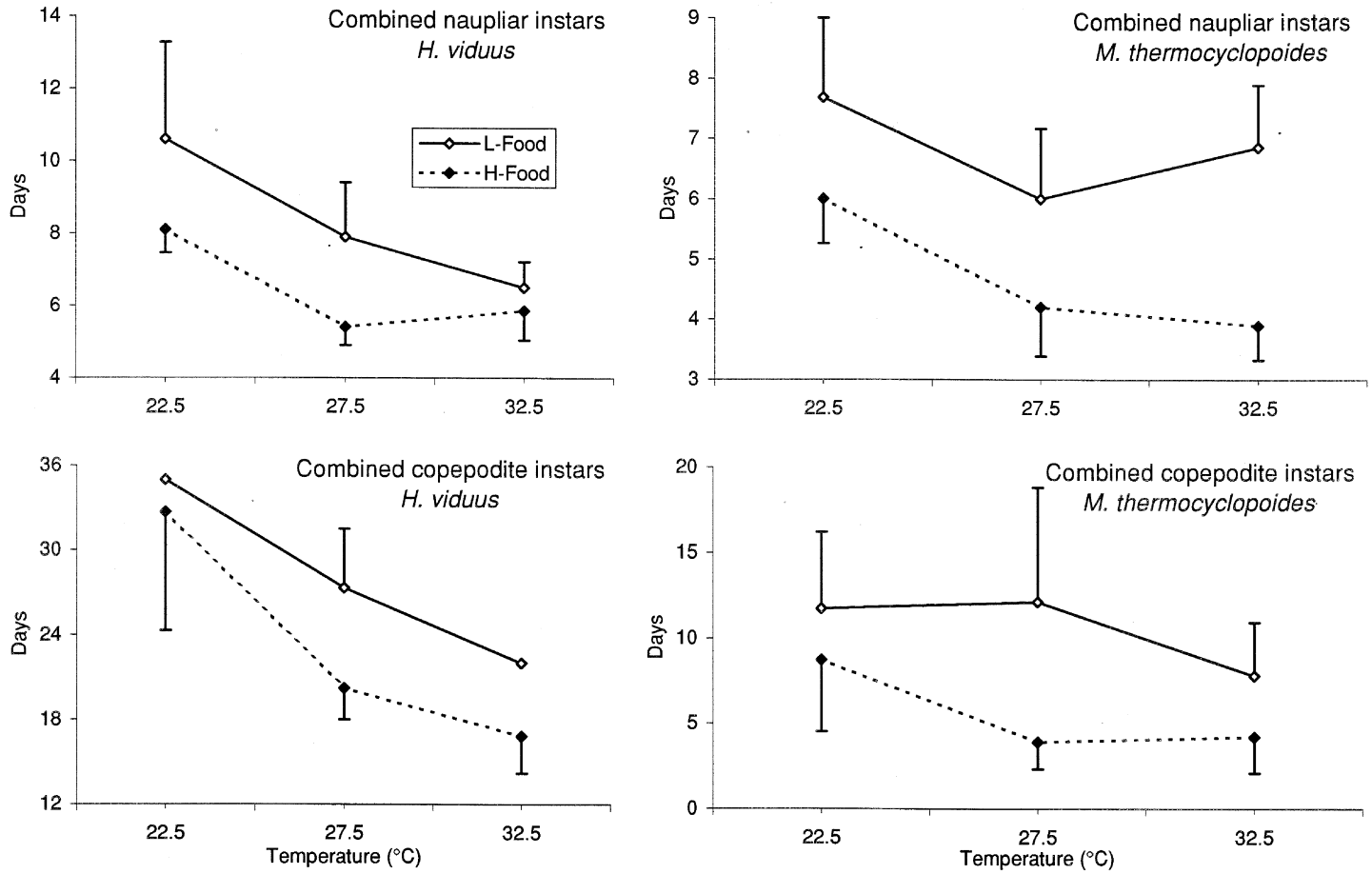

Figure 6. Duration of the combined naupliar instars, and of the combined copepodite instars of *Heliodiaptomus viduus* (left panels), and *Mesocyclops thermocyclopoides* (right panels) at three temperatures (°C) and two food levels (L-Food: 10, H-Food: 50 μg chlorophyll l^{-1}). The error bars in the graphs indicate the standard deviations of the measurements.

Table 7. Summary table of the analyses of variance with the duration of the different instars included as repeated measurements.

Effect	Error	*H. viduus*		Error	*M. thermocyclopoides*	
	df	*F*	*P*	df	*F*	*P*
Food	20	4.49	0.047	44	8.31	0.006
Temperature	20	11.29	<0.001	44	1.37	0.264
Instar	80	3.01	0.023	176	9.57	<0.001
Food × Temp	20	0.42	0.666	44	1.89	0.163
Food × Instar	80	2.89	0.027	176	4.00	0.004
Temp × Instar	80	1.05	0.405	176	1.29	0.251
3-way interaction	80	3.75	<0.001	176	0.94	0.487

Table 8. Summary table of differences in size between males and females of the two copepod species under study. As no significant differences in sex ratio between the treatments were found all adults were analysed in one ANOVA.

Effect	Error	*H. viduus*		Error	*M. thermocyclopoides*	
	df	*F*	*P*	df	*F*	*P*
C1	24	0.10	0.758	108	15.38	<0.001
C2	24	0.96	0.338	112	19.72	<0.001
C3	24	0.01	0.929	117	52.41	<0.001
C4	24	0.32	0.577	132	279.48	<0.001
C5	24	2.83	0.105	150	758.02	<0.001
Adult	24	10.15	0.004	173	1413.15	<0.001

er than for temperate species. However, she compared large-bodied *Daphnia* species from the temperate region with smaller bodied cladocerans from the tropics, and most likely large-bodied forms are able to reach lower threshold levels than small-bodied forms (Gliwicz & Lampert, 1990). Therefore, it is not clear what the causing factors for the observed difference in threshold values are, elevated temperature or size. An other study (Hardy & Duncan, 1994), however, in *Daphnia gessneri*, shows, that although a temperature increase from 22 up to 27 °C did not affect threshold concentration values, a temperature increase from 27 to 32 °C caused a marked increase (2.5—5.0×) in the threshold concentration. Apparently temperature affects the threshold food level non linearly, depending also on the body size. This may explain the increased number of juvenile instars, even at the highest experimental food level, in two of the three cladoceran species tested and the poor development rate of *H. viduus*. Such a poor growth performance at the highest food level (50 μg chlorophyll^{-1}) was not expected. This food level corresponds with ca. 1.25 mg C l^{-1} (Bailley-Watts, 1974), which is well above the *Incipient Limiting Concentration* (ILC) generally observed for temperate herbivorous zooplankton in feeding experiments (Persson, 1985).

Instar duration time

At 27.5 °C we observed for *C. cornuta* a mean duration time of combined juvenile instars of 3.3 d, which is some what longer compared with the duration times of 2.0 and 2.5 d reported by Gras & Saint-Jean (1978) at 30 and 25 °C for this species. However, the mean duration time of the adult instars for *C. cornuta* in our culture is very similar to the value given by Gras & Saint-Jean (1976).

Our duration times for combined juvenile instars of *M. micrura* at 27.5 °C compare well with those of Gras & Saint-Jean (1978) who used natural lake seston as food and a temperature of 25 and 30 °C. In contrast, Murugan (1975) observed a relative longer duration time for this species of 2.0 d at 28–30 °C using pond seston as food, whereas Bonou et al. (1991) observed at 26 and 30 °C, using seston from eutrophic fish ponds somewhat shorter duration times. Our adult instar duration times for *M. micrura* are similar to the values reported by Gras & Saint-Jean (1976),

Hart (1985), and Bonou et al.(1991) at corresponding temperatures.

Our duration times for combined juvenile instars for *D. excisum* are similar to those reported at corresponding temperatures by Duncan (1989) and Mavuti (1994), and Hardy & Duncan (1994) for the closely related species *D. sarsi* at a food concentration above ILC. But, our juvenile duration times of *D. excisum* are ca 50% longer than reported by Gras & Saint- Jean (1978) although the number of juvenile instars was similar. Furthermore, our observations on the duration time of the adults were similar to earlier studies (Gras & SaintJean, 1976; Duncan, 1989; Mavuti, 1994), as well as of Hardy & Duncan (1994) for the closely related *D. sarsi*.

The development times of *H. viduus* are extraordinary long as compared with other tropical calanoid copepods (*Phyllodiaptomus annae, Paradiaptomus africana, Thermodiaptomus galebi, Tropodiaptomus incognitus*). Our estimates of the combined naupliar instars of *H. viduus* are generally 2–3 times longer than the literature values on other tropical copepod species. The difference was even larger for the combined copepodite instars, a duration time 2–8 times longer than the literature values (Gras & Saint-Jean, 1981; Vareschi & Jacobs, 1984; Piyasiri, 1985). Apparently, food quality in our culture were poor for *H. viduus* and limited growth and reproduction at both food levels and all three temperatures.

Our development times of *M. thermocyclopoides* are better comparable with the literature on tropical cyclopoid species: both for the combined naupliar and combined copepodite instar durations (Burgis, 1971; Gophen, 1976; Gras & Saint-Jean, 1981; Bonou et al., 1991). Clearly, the cyclopoid *M. thermocyclopoides* coped much better than the calanoid *H. viduus* with the ambient food conditions in the culture.

Sex differentiation

In most copepods, sex is indistinguishable using morphological criteria until at least copepodite stage four (Vijverberg, 1977; Hicks & Coull, 1983). We know only of two studies reporting early sex determination in copepods. Fahrenbach (1962) could determine the sex of the harpacticoid *Diarthrodes cystoecus* from copepodite stage three onwards; also Abraham & Gopalan (1975) state that sex of the harpacticoid copepod *Nitocra spinipes* could be determined from copepodite one onwards, but provided no convincing evidence. Therefore, in our study the early sex differentiation based on size distinction from copepodite one onwards observed for *M. thermocyclopoides* is exceptional. Because of this early differentiation, the observed size differences among the adult males and females are much larger than usually observed among copepod populations.

Population growth rate

The population growth rate '*r*' of all the three cladoceran species were positively affected by temperature, though increased food level had a positive effect for two of the three species. Thus, although food appeared to limit growth and reproduction to some extent, this food effect was not so strong that it confounded the positive effect of temperature.

In *C. cornuta r*-values fluctuated between 0.20 and 0.40, i.e. a range found also earlier for small bodied cladocerans in a temperature range of 20–25 °C (Montu, 1973a; Goulden et al., 1978; Pace et al., 1983; Anderson & Benke, 1994). In our study *M. micrura* with its highest *r*-values, 0.30–0.60, compared well with the '*r*' of 0.27 for *M. reticulata* at 20 °C (Montu, 1973b), but much less so with the 0.56 for *M. micrura* at 20 °C (Montu, 1973c). Our *r*-values for *D. excisum* are low varying between −0.06 and +0.15, being the combined effect of low fecundity and high mortality. It is also lower than the *r*-value of 0.19 estimated by Jana & Pal (1984) using natural seston from a pond and a temperature of 29–32 °C. The low population growth rate caused by these factors indicate that *D. excisum* experienced poor food conditions in our culture.

Food limitation

As we tested only two different food levels, using natural seston (alga plus detritus and bacteria) of undefined food quality we cannot conclude that food was not limiting growth and development rate at the highest food level. Obviously, however, the relative effect varied substantially among the study species, and food limited generally growth and development of study species at all three temperatures. Besides, compared with other published works *H. viduus* and *D. excisum* performed very poorly, exhibiting long instar duration times (*H. viduus*) or high mortality and low fecundity (*D. excisum*). Probably, the food quality of the seston used in our cultures was poor food for these two species. The growth and development times of the remaining three study species (*C. cornuta, M. micrura, M. thermocyclopoides*) compared favourably with

other published studies. This does not mean that our cultures were not food limited, but it would imply that food limited growth and development of crustacean zooplankton are a general feature for the natural conditions prevailing in the tropics.

In conclusion

Culture studies as the present one are useful to improve our understanding about the functioning of herbivorous zooplankton in the food webs of tropical lakes and reservoirs. There is certainly a great need, never the less, for more detailed studies on tropical cladocerans and copepods using defined culture media comprising algae of known origin and with high food value.

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