

Response of life history traits to food conditions in a cyclopoid copepod from an oligotrophic environment

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Abstract

The *Cyclops* sp. responses of life history traits (growth, development, and reproduction) to food conditions from oligotrophic Lake Toya were analyzed by rearing animals individually under four food regimes: three densities of the alga *Cryptomonas tetrapyrenoidosa* and nauplii of the calanoid copepod *Eurytemora affinis* as prey in an algal suspension. *Cyclops* sp. grew successfully from newborn nauplii to adults when algal density was $\geq 4 \times 10^3$ cells ml^{-1} . Development time was shorter and body length larger in the high-density (H) treatment (4×10^4 cells ml^{-1}) than in medium-density (M) treatment (4×10^3 cells ml^{-1}) until the early copepodite stages. In late copepodite stages, females tended to grow more slowly but tended to be larger in the M treatment than in the H treatment. However, stage-specific growth rates were similar among treatments during development, suggesting a trade-off between body size and development time. Although the egg production rate was higher in the M treatment than in the H treatment, it depended on female body size regardless of the food regime. Furthermore, the weight-specific egg production rate was the same among treatments, indicating egg production was not affected by the algal concentrations tested. Addition of copepod prey influenced neither postembryonic development time nor body size, and egg production rates were comparable to those reared with an algal diet. These results reveal that even older copepodites and adults, which are generally thought to be predacious, do not necessarily require animal prey for growth and reproduction, and that *Cyclops* sp. can complete its life cycle on an algal diet alone. For female *Cyclops* sp., reduction of algal density resulted in slow development and large body size, which might be an adaptation to the oligotrophic environment, the original habitat of the copepod.

Copepods are distributed in many kinds of habitats, including freshwater, brackish, and saltwater environments, and play an important role in aquatic food webs both as primary and secondary consumers and also as a major source of food for many larger invertebrates and vertebrates (Williamson 1991). Copepods are exposed to fluctuating environmental factors such as water temperature and food condition, which are known to affect their life history traits, i.e., growth, development, and reproduction. Many experimental studies on calanoid copepods (e.g., Ban 1994 and citations therein) have emphasized the following two phenomena: increased water temperature causes more rapid development, smaller body size, and higher egg production; and increased food density causes more rapid development, larger body size, and higher egg production. Until recent years, information about the effect of food conditions on growth, development, and reproduction of cyclopoid copepods was scarce compared with the knowledge of the effect of water temperature (Maier 1990 and citations therein), but such in-

formation is now accumulating (Hopp et al. 1997; Kumar and Rao 1998 and citations therein).

Copepod diets appear to vary with developmental stage: nauplii and early copepodites are generally more herbivorous, and the transition to more predatory feeding occurs in the late copepodite stage in both calanoid and cyclopoid copepods (McQueen 1969; Maly and Maly 1974; Gophen 1977; Jamieson 1980). Some field observations have emphasized the carnivorous feeding habit of adult cyclopoid copepods and have revealed that in lakes, they can have a substantial impact on their prey, such as cladocerans and rotifers (Brandl and Fernando 1978, 1979; Karabin 1978; Plaßmann et al. 1997). Therefore, cyclopoid copepods are often treated as predators in analyses of aquatic food webs (e.g., Williamson 1983).

However, the importance of carnivory for cyclopoids remains controversial. In studies of natural populations, there is much evidence that cyclopoids, even older copepodites and adults, are omnivorous, consuming as much phytoplankton as zooplankton (Tóth and Zánkai 1985; Adrian 1987; Tóth et al. 1987). Experimental studies have recently indicated that carnivorous feeding is not required to complete postembryonic development and reproduction. A striking example is *Cyclops vicinus*, which can develop from hatching to adulthood and reproduce on an exclusively algal diet (*Chlamydomonas*, *Cryptomonas*, or both; Santer and van den Bosch 1994; Hansen and Santer 1995). *Acanthocyclops robustus*, *Cyclops abyssorum*, and *Thermocyclops crassus* can also produce eggs on a solely algal diet (Hopp et al. 1997). On the other hand, *Mesocyclops leuckarti* can neither develop to adulthood nor reproduce on a purely algal diet but requires animal prey (Hansen and Santer 1995; Hopp et al.

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Table 1. Stage duration (days) of each developmental stage of *Cyclops* sp. raised with three different algal concentrations (H, M, and L). Within the same food concentration, differences between male and female were tested by *U*-test.

Develop- mental stages	4 × 10 ⁴ algal cells ml ⁻¹ (H)			4 × 10 ³ algal cells ml ⁻¹ (M)			1 × 10 ³ algal cells ml ⁻¹ (L)			
	Male (N = 11)		U	Male (N = 7)		U	Female (N = 11)		U	N
	Median	Range		Median	Range		Median	Range		
N1	3	2-5	35.0	3	3-4	40.5	3	3	2-5	24
N2	3	3-5	45.0	4	4-5	45.0	5	5	8-24	22
N3	4	3-5	48.5	6	6-7	48.5	7	7	16-53	12
N4	3	2-4	45.0	7	6-8	43.5	7	7		
N5	3	3-6	39.0	6	6-7	55.5	6	6		
N6	4	4-7	50.0	6	5-7	31.5	6	6		
C1	4	3-7	42.5	6	5-7	37.0	6	6		
C2	4	3-9	46.0	5	4-6	27.5	5	5		
C3	4	4-9	23.5*	6	5-33	26.5	8	8		
C4	9	4-26	33.0	19	13-30	39.5	19	19		
C5	10	4-29	36.0	14	8-24	25.0	18	18		

* *P* < 0.05; no asterisk, *P* > 0.05.

Table 2. Summary of statistical significance of each stage duration of *Cyclops* sp. between all combinations of algal concentration (H, M, L) (ANOVA and Fisher's PLSD). In the case of C3, the difference among male in H (Hm), female in H (Hf), and M was analyzed. Symbols connected by underline are not significantly different from each other (*P* > 0.05). H; 4 × 10⁴ cells ml⁻¹, M; 4 × 10³ cells ml⁻¹, L; 1 × 10³ cells ml⁻¹.

Develop- mental stages	df	<i>F</i> ratio	Multiple comparison
N1	2, 60	0.89	
N2	2, 58	165.62***	H M L
N3	2, 47	253.89***	H M L
N4	2, 36	37.13***	H M
N5	1, 37	16.95***	H M
N6	1, 37	11.00**	H M
C1	1, 37	16.67***	H M
C2	1, 37	4.33*	H M
C3	2, 36	4.86*	Hm <u>Hf M</u>
C4	1, 37	3.28	
C5	1, 37	4.54*	H M

* Levels of significance: *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; no asterisk, *P* > 0.05.

1997). These species-specific differences in food requirements are important when we consider their life history strategies, so this issue requires further study.

We studied the life history of *Cyclops* sp. in oligotrophic Lake Toya, Japan, where it occurs year-round and where it is the only copepod found in the plankton (Makino 1998). The range of lake temperatures in which *Cyclops* sp. occurs is 4–10°C, and the temperature experienced by the copepod is 8 ± 2°C during May to November (Makino 1998). The amount of available food, evaluated by chlorophyll *a* concentration and/or the density of small cladocerans and rotifers, is low in Lake Toya (Makino 1998; Makino and Ban 1998). Thus, the low-food condition of the lake may affect the life history traits of *Cyclops* sp.—that is, the postembryonic development and egg production—more strongly than does water temperature. In addition, adult *Cyclops* sp. in the lake are omnivorous (Makino and Ban 1998), implying that phytoplankton may be important in its diet. In the present study, we examined the effect of food conditions on post-embryonic development and egg production of *Cyclops* sp. from Lake Toya under four food regimes using both algae and copepods as prey, and we discuss the relevance of these life history traits to the oligotrophic environment. One might expect that individual fitness will decrease with decreasing food quantity because increased food density is known to cause rapid development, larger body size, and higher egg production in calanoid copepods. However, we show that *Cyclops* sp. may realize a high fitness by a trade-off between body size and development time, even in an oligotrophic environment.

In Japan, most freshwater planktonic cyclopoids are still identified as cosmopolitan species or those with wide geographical ranges, despite current doubts about cosmopolitanism in freshwater zooplankton (see Ohtsuka and Ueda 1999). *Cyclops* sp. in Lake Toya was formerly identified as *C. strenuus* (Fischer) (Makino 1998; Makino and Ban 1998), thought

Table 3. Summary of multiple comparison between stage durations within the same algal concentrations (ANOVA and Fisher's PLSD). Stages connected by the same underline are not significantly different from each other, at $P < 0.05$ level.

Food concentration (cells ml ⁻¹)	ANOVA		Multiple comparison											
	df	F ratio	C4	C5	C3f	N6	C3m	C1	C2	N5	N3	N4	N2	N1
4 × 10 ⁴ (H)	11, 219	33.03*	<u>C4</u>	<u>C5</u>	<u>C3f</u>	<u>N6</u>	<u>C3m</u>	<u>C1</u>	<u>C2</u>	<u>N5</u>	<u>N3</u>	<u>N4</u>	<u>N2</u>	<u>N1</u>
4 × 10 ³ (M)	10, 187	51.45*	<u>C4</u>	<u>C5</u>	<u>C3</u>	<u>N3</u>	<u>N4</u>	<u>N6</u>	<u>C1</u>	<u>N5</u>	<u>C2</u>	<u>N2</u>	<u>N1</u>	
1 × 10 ³ (L)	2, 55	244.97*	<u>N3</u>	<u>N2</u>	<u>N1</u>									

* Significant difference at $P < 0.00001$ level.

to be distributed in Japan as well as in Eurasia, North America, and Africa (Mizuno and Takahashi 1991). Such a "cosmopolitan" species may be closely related species or a complex of closely related species. For example, *Mesocyclops leuckarti* (Claus) was considered cosmopolitan with a single representative of the genus in Japan, but now it has been shown that *M. leuckarti* has a restricted distribution and does not occur in east Asia (see Ueda et al. 1997). The Japanese *M. "leuckarti"* has been reexamined and reallocated into three species (Defaye and Kawabata 1993; Ueda et al. 1997; Ohtsuka and Ueda 1999). Recently, Einsle (1996) pointed out the possibility that *C. strenuus* is distributed only in Europe. The ongoing taxonomic study for specimens formerly identified as *C. strenuus* from Lake Toya and other Japanese lakes reveals that their morphology differs from European *C. strenuus*, but further investigations are still needed to determine whether the Japanese *C. "strenuus"* is *C. strenuus* or another species (T. Ishida pers. comm.). Given the current state of taxonomy for this species, we identify it here as simply *Cyclops* sp.

Materials and methods

Adult females and males of *Cyclops* sp. for laboratory culture were collected by vertical net hauls at a fixed station in Lake Toya (see Makino et al. 1996). Before the start of experiments, animals were maintained in 1,000-ml jars containing filtered (Whatman GF/C) lake water and the cultured alga, *Cryptomonas tetrapyrenoidosa* Skuja (strain no. 282, National Institute for Environmental Studies; >10⁴ cells ml⁻¹) in a controlled chamber (8°C, light:dark [LD] = 14:10) for two generations. All experiments were performed at the same light and temperature conditions to the stock culture. The alga was maintained in modified MBL medium (Stemberger 1981) at 20°C, LD = 14:10, and exponential growth-phase cells were given to the copepod.

To examine the effect of food concentration on growth and development of *Cyclops* sp., 17–23 nauplii that hatched from ovigerous females isolated from the stock culture were collected within 12 h after hatching and placed individually in wells of a polystyrene tissue-culture plate filled with food suspension (2 ml per well). The animals were reared under three food concentrations (4 × 10⁴, 4 × 10³, and 1 × 10³ cells ml⁻¹ in H, M, and L treatment, respectively) of *C. tetrapyrenoidosa*. All individuals were transferred daily with a Pasteur pipette to wells of a new plate filled with fresh

food suspension, and exuvia or dead animals were collected and examined under a binocular microscope at ×40 magnification. When a molt was recognized, the animal was moved to a small drop of food suspension on a slideglass, and its body length (total length for nauplii or cephalothorax length for copepodites) was measured using a microscope with a digital micrometer (Mitutoyo Digi-Matic) at ×100 magnification within a few minutes. The animals were then transferred to a new food suspension. None of the animals treated in this way died from handling. The experiments lasted until all animals developed into adults. Using these data, we calculated the stage duration and body length of each developmental stage. We also estimated the stage-specific growth rate for each developmental stage i (g_i , d⁻¹) using the following equation:

$$g_i = \ln(M_{i+1}/M_i)/SD_i,$$

where SD_i is the stage duration (days) and M_i is the individual body weight (μ g dry weight) calculated from body length (L_i , μ m) and the length-weight relationship given in Makino (1998), $\ln(M) = 4.05 \ln(L) - 22.40$.

In the H treatment, body size of adult female *Cyclops* sp. was smaller than in the M treatment despite the higher algal quantity in the H treatment. Considerable individual variation in development also occurred, as well as generally poorer food condition (see Results). We hypothesized that the lack of an animal diet supply to the copepod may have affected its development under the H treatment, because *Cyclops* sp. is omnivorous in nature (see above). Therefore, we added an animal supplement to the algal regime in the H treatment and investigated its effect on growth and development (HP treatment). Twenty-three individuals at the first copepodite stage (C1) isolated from the stock culture were placed individually in wells of the tissue-culture plate filled with algal suspension (4 × 10⁴ cells ml⁻¹ of *C. tetrapyrenoidosa*). Seven nauplii of the calanoid copepod *Eurytemora affinis* obtained from a laboratory culture were added to the algal suspension daily as "animal" diet. All *Cyclops* sp. individuals were transferred daily to the wells of a new plate filled with a fresh food suspension of algae and nauplii, and the exuvia of *Cyclops* sp. and the number of nauplii of *E. affinis* were checked under the binocular microscope at ×40 magnification. When a molt was observed, body length of the animal was measured in the same manner described above. The experiment lasted until all animals developed into adults.

In the H, M, and HP treatments, when the copepods reached adulthood, a male–female pair was transferred to a 10-ml beaker with the same food suspension as in the growth experiments. Oviduct condition, the presence or absence of egg sacs attached to the abdomen of the female, and the number of newly hatched nauplii were then checked daily. We regarded the number of newborn nauplii as the clutch size of each brood. The experiments lasted until nauplii hatched from the fifth brood. In the L treatment, egg production was not observed because all animals died by the fourth naupliar stage (*see Results*).

Variations among treatments in development time from hatching to each developmental stage, stage duration, body length of each developmental stage, and reproductive parameters were analyzed using one-way analysis of variance (ANOVA; development time and body length were \log_{10} transformed to equalize variances). Multiple comparisons were then conducted using Fisher's protected least significant difference method when the ANOVA indicated a significant difference among the treatments (Steel and Torrie 1980).

Results

Effects of food concentration on postembryonic development—In the H and M treatments, all *Cyclops* sp. reached adulthood, but all died by the fourth naupliar stage (N4) in the L treatment (Table 1). The stage-specific death rates in the L treatment were 8, 55, and 100% in N2, N3, and N4 stages, respectively.

In the L treatment, sexual differences in stage duration were not observed because all individuals died before reaching the copepodite stage, and sexual dimorphism usually appears after the third copepodite stage (C3) in cyclopoid copepods (Dussart and Defaye 1995). In the H and M treatments, there were no significant differences in stage duration between males and females, except for C3 in the H treatment (Table 1). There were no significant differences among treatments in the stage duration of N1 (Tables 1, 2). However, higher algal densities resulted in shorter stage durations after N2; that is when feeding begins (Dussart and Defaye 1995) (Table 2). In the L treatment, the stage durations of both N2 and N3 were the longest, and the stage-specific death rates of N3 and N4 were very high (Tables 1, 2), implying that food shortage was critical. The stage duration of C3 was significantly longer than that of the younger stages for the females in the H treatment and for the both sexes in the M treatment (Tables 1, 3). In both the H and M treatments, the stage duration of C4 was longer than that of C3 but did not differ from that of C5 (Table 3).

There were no significant differences in development time between sexes in the M treatment through their life cycle (Fig. 1). In the H treatment, males developed about 2 weeks sooner than females after C4. In the L treatment, the copepods required 40 d to develop to N4. Development times were always shorter in the H treatment than in the M treatment until C5, although the duration from hatching to adult female did not differ significantly between the two treatments (Fig. 1 and Table 4).

Reduction in body length from N6 to C1 reflects the dif-

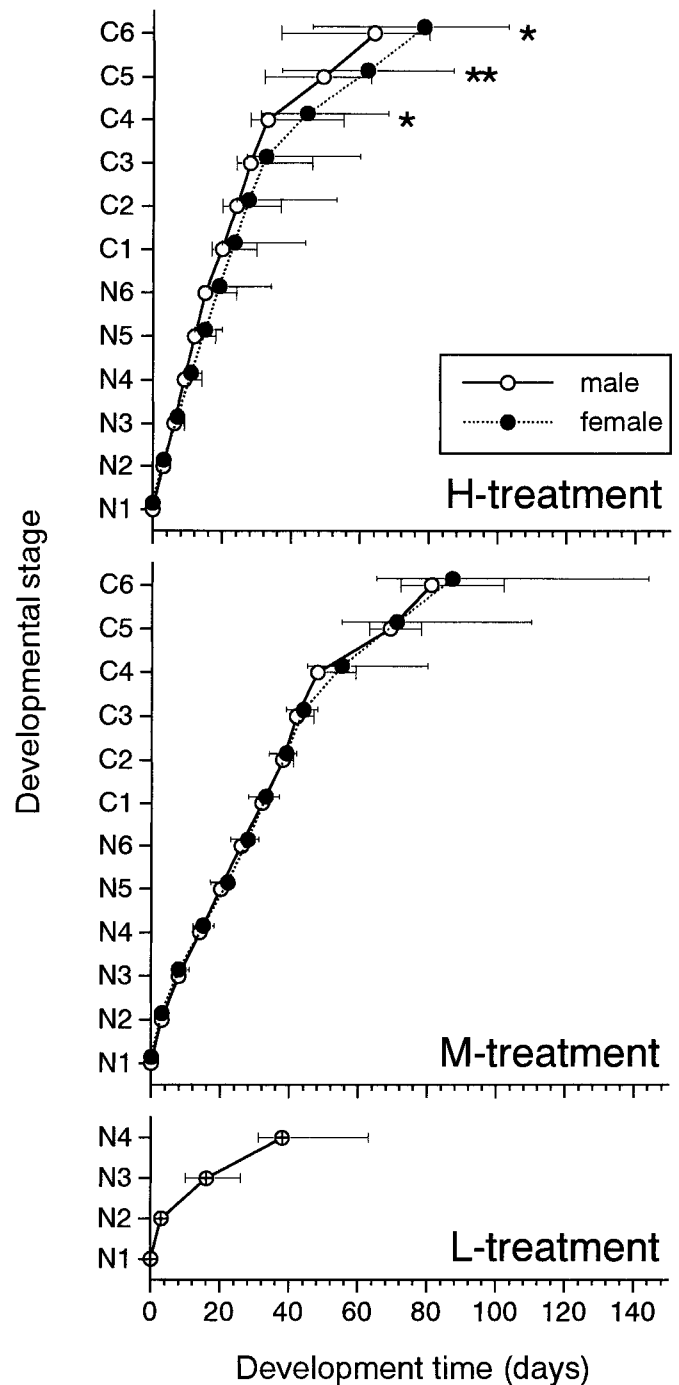


Fig. 1. Cumulative development time (days) of each developmental stage of *Cyclops* sp. raised at three different algal concentrations. Upper panel, 4×10^4 cells ml^{-1} (H treatment); middle panel, 4×10^3 cells ml^{-1} (M treatment); lower panel, 1×10^3 cells ml^{-1} (L treatment). Symbols and horizontal bars denote median and range, respectively. Statistical significance of the differences between males and females was determined by using *U*-test. Level of significance: * $P < 0.05$; ** $P < 0.01$; no asterisks, $P > 0.05$. In the L treatment, the sexual difference was not clear (*see text*).

Table 4. Summary of statistical significance of development time from hatching to each developmental stage in *Cyclops* sp. between all combinations of algal concentration (H, M, L) (ANOVA and Fisher's PLSD). After C4, the difference among male in H (Hm), female in H (Hf) and M was analyzed. Symbols connected by underline are not significantly different from each other ($P > 0.05$), H; 4×10^4 cells ml⁻¹, M; 4×10^3 cells ml⁻¹, L; 1×10^3 cells ml⁻¹.

Developmental stages	df	F ratio	Multiple comparison
N2	2, 60	0.89	
N3	2, 58	50.01***	H M L
N4	2, 47	62.25***	H M L
N5	1, 37	63.71***	H M
N6	1, 37	48.78***	H M
C1	1, 37	41.62***	H M
C2	1, 37	39.06***	H M
C3	1, 37	34.33***	H M
C4	2, 36	16.82***	Hm Hf M
C5	2, 36	16.42***	Hm Hf M
C6	2, 36	12.59***	Hm <u>Hf M</u>

*** $P < 0.001$, no asterisk, $P > 0.05$.

ferences in the body parts measured (see *Materials and Methods*). The body lengths of female were greater than those of males after C3 and C4 in both the M and H treatments, whereas there were no significant differences between the sexes at the younger stages (Fig. 2). N1 females were significantly larger than N1 males in the H treatment for an unknown reason; this difference may have been due to differences in the size of eggs from which these nauplii were born. Guisande and Harris (1995) found that the egg size in *Calanus helgolandicus* is not constant under experimental conditions and that larger eggs produce larger nauplii. Body length also differed significantly among treatments; giving more algae resulted in greater body length from N3 through C3, except for C3 females in the M treatment (Fig. 2; Table 5). After C4, male body lengths did not differ between the

Table 5. Summary of statistical significance of individual body length of *Cyclops* sp. between all combinations of algal concentration (H, M, L) (ANOVA and Fisher's PLSD). In the case of N1, the difference among male in H (Hm), female in H (Hf), M, and L was analyzed. In the case of C3, the difference among H, male in M (Mm), and female in M (Mf) was analyzed. After C4, the difference among Hm, Hf, Mm, and Mf was analyzed. Symbols connected by underline are not significantly different from each other ($P > 0.05$), H; 4×10^4 cells ml⁻¹, M; 4×10^3 cells ml⁻¹, L; 1×10^3 cells ml⁻¹.

Developmental stages	df	F ratio	Multiple comparison
N1	3, 59	17.56***	Hf M Hm L
N2	2, 60	25.05***	<u>H M L</u>
N3	2, 58	127.03***	<u>H M L</u>
N4	2, 48	183.76***	H M L
N5	1, 37	98.27***	H M
N6	1, 37	52.06***	H M
C1	1, 37	55.47***	H M
C2	1, 37	16.28***	H M
C3	2, 36	7.82**	H Mf Mm
C4	3, 35	19.30***	<u>Mf Hf Hm Mm</u>
C5	3, 35	60.15***	<u>Mf Hf Hm Mm</u>
C6	3, 35	89.38*	Mf Hf <u>Hm Mm</u>

** $P < 0.01$, *** $P < 0.001$, no asterisk $P > 0.05$.

H and M treatments, whereas females in the M treatment were larger than those in the H treatment.

During development, the animals showed individual variations under the same experimental conditions. Coefficients of variation for stage duration were 10–37% until C2 and tended to increase after C3, reaching 89% (Fig. 3). Individual variations in body length were smaller than those in stage duration; coefficients of variation were mostly less than 5%, reaching 7% after C3.

The above-mentioned patterns in growth and development from hatching to adulthood were summarized by calculating

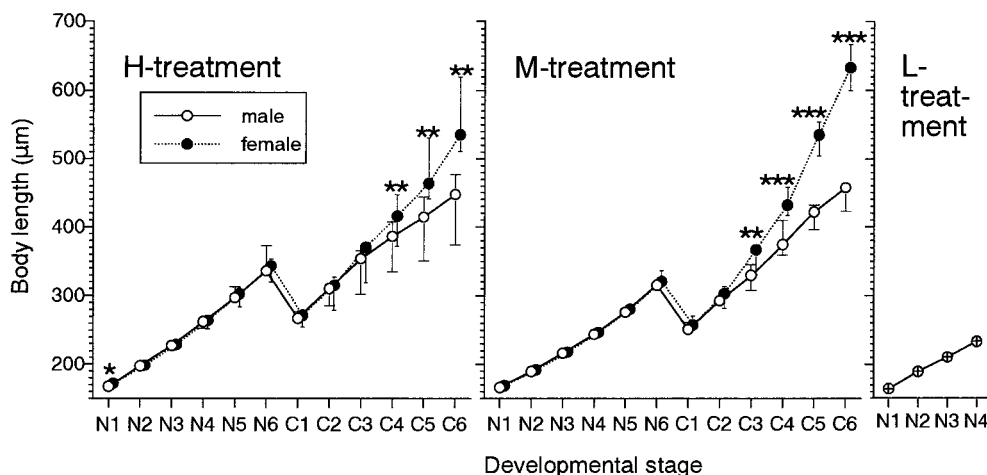


Fig. 2. As for Fig. 1 but for body length (μm) of each developmental stage. In the L treatment, the sexual difference was not clear (see text).

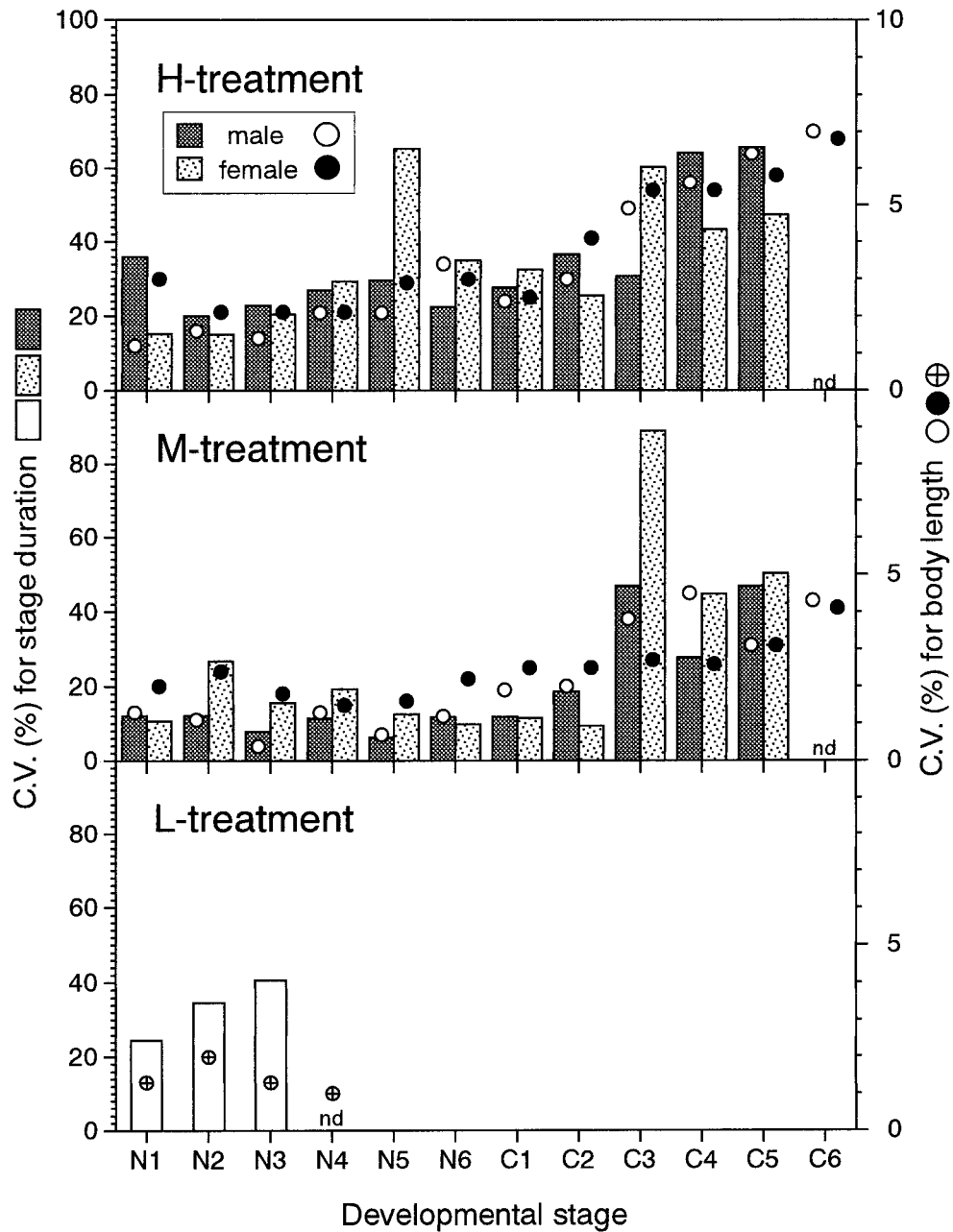


Fig. 3. Coefficients of variation (CV, %) for stage duration and body length of each developmental stage of *Cyclops* sp. raised on various concentrations of algal diet. Upper panel, 4×10^4 cells ml^{-1} (H treatment); middle panel, 4×10^3 cells ml^{-1} (M treatment); lower panel, 1×10^3 cells ml^{-1} (L treatment). In each panel, columns and circles denote the CV for body length and stage duration, respectively. nd denotes that the stage duration could not be determined because no animals entered the next developmental stage. In the L treatment, the sexual difference was not clear (see text).

stage-specific growth rates and then comparing them between the H and M treatments (Fig. 4). Stage-specific growth rates were higher in the H treatment throughout the naupliar stages, suggesting that the rate was food dependent. However, it was not so for later stages. Stage-specific growth rate did not differ between the treatments after C1, though female

body length from C3 through C6 was larger in the M treatment than in the H treatment (see Fig. 2 and Table 5).

Effects of adding an animal component to the diet—As in the H treatment, all *Cyclops* sp. in the HP treatment reached adulthood. They began to prey on the nauplii of *E. affinis* at

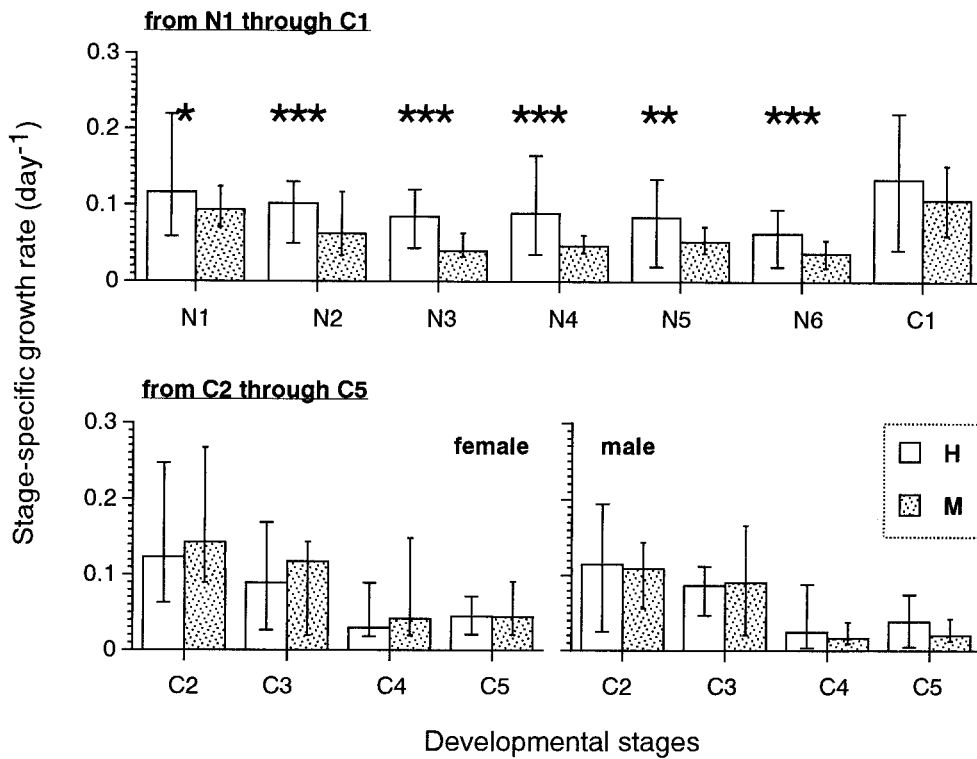


Fig. 4. Comparison for stage-specific growth rates (d^{-1}) of *Cyclops* sp. reared under two different algal concentration. Open columns, 4×10^4 cells ml^{-1} (H treatment); hatched columns, 4×10^3 cells ml^{-1} (M treatment). Sex of the animal was taken into consideration from C2 to C5, since body length of females was larger than that of males after C3 at M treatment (see Fig. 2). Columns and vertical bars represent median and range, respectively. Differences between the treatments were tested by using ANOVA. Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; no asterisk, $P > 0.05$.

C3, and the predation rate increased at later developmental stages (0.27 ± 0.28 , 0.74 ± 0.52 , 2.46 ± 1.48 individuals. copepod⁻¹ d⁻¹ for C3, C4, and C5 stages, respectively). However, neither cumulative development times nor the degree of its individual variation differed from those in the H treatment (Fig. 5).

Body lengths of male *Cyclops* sp. in the HP treatment were significantly smaller than those in H treatment except for C5. We found no differences in the lengths of females between treatments (Fig. 6). However, in males, differences in body length among treatments appeared even in C2 that did not prey on *E. affinis* nauplii, implying that the body lengths of C1 males isolated from the stock culture were already smaller than those in H treatment. Therefore, these observations suggest that the animal diet affected neither the body length nor the development time of *Cyclops* sp. for either sex.

Reproduction—Some female *Cyclops* sp. produced unusual egg sacs that contained only a few eggs of irregular shape. This occurred in 10 of the 44 clutches in the H treatment and in 8 of the 33 clutches in the HP treatment. These eggs dropped from the egg sac within 2 d after spawning, and sometimes the egg sac itself separated from the animals. Maier (1992) found similar eggs in unmated *Cyclops vicinus*.

Since irregular-shaped eggs may be infertile, we excluded them from the following analyses.

In the H, M, and HP treatments, females had transparent oviducts, i.e., “nongravid” condition in the terms of Watras and Haney (1980), immediately after molting to become adults. Their oviducts gradually became dark 3–4 d after molting. In all treatments, it took 11 d (median value) from molting into adults to produce the first clutch (Table 6). We continued the experiment until a fifth clutch was produced and found no significant differences among the clutches for all other reproductive parameters, including interclutch duration (number of days between the extrusion of egg sacs and the extrusion of the next), egg development time, clutch size, egg production rate and weight-specific egg production rate (ANOVA, $P > 0.05$). Hence, these parameters were pooled for the first to fifth clutches and compared among treatments.

Interclutch duration did not differ significantly either among treatments (Table 6) or from the time between molting into adults and producing the first clutch (ANOVA, $df = 2, 112$, F ratio = 0.547, $P = 0.58$). Egg development time was 9 d in all treatments. It took 2 d from the hatching of one clutch of eggs to the extrusion of the next clutch, since the eggs did not finish ripening in oviducts while the

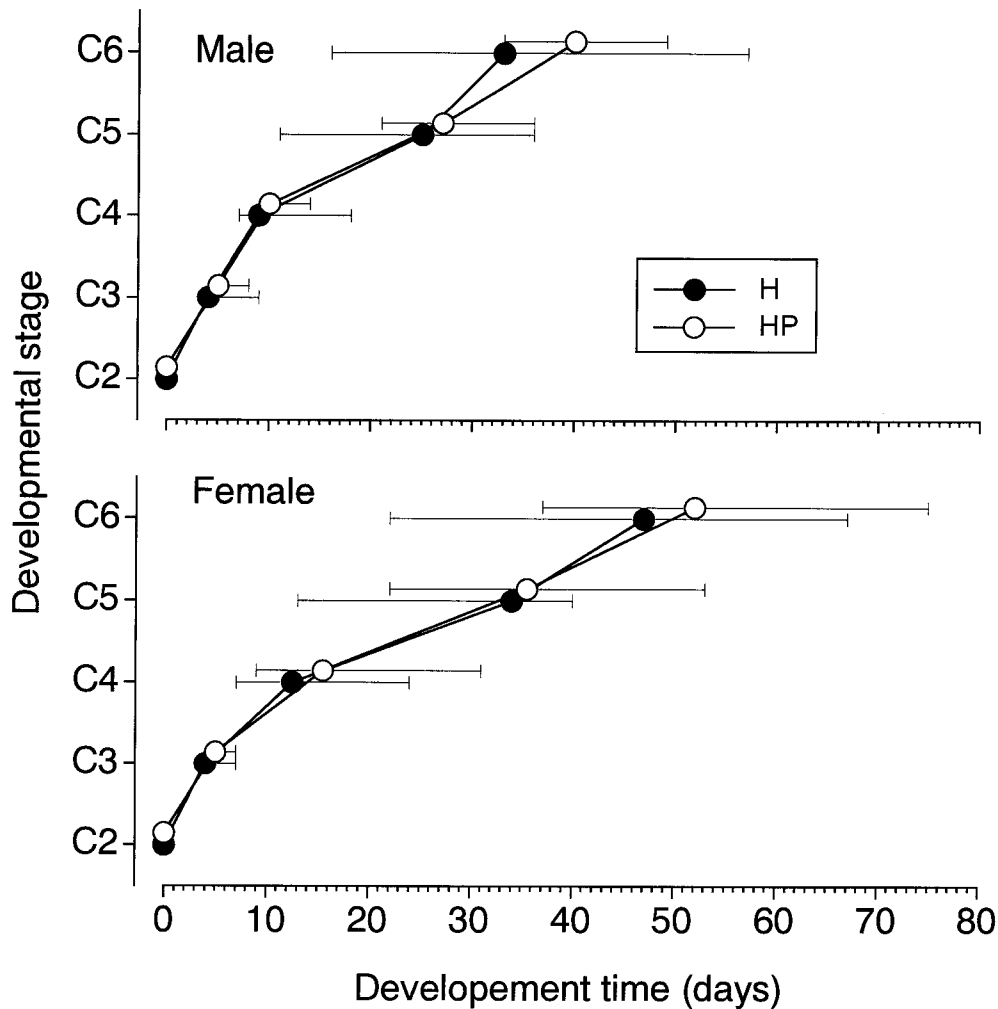


Fig. 5. Cumulative development time (days after molt to C2) of *Cyclops* sp. reared with algal diet (*Cryptomonas tetrapyrenoidosa*; 4×10^4 cells ml^{-1}) alone (closed circles; H treatment) and with a mixture of the same algal diet and seven nauplii of *Eurytemora affinis* (open circles; HP treatment). Symbols and horizontal bars denote median and range, respectively.

previous eggs were developing. Females produced two egg sacs per brood, and the number of newborn nauplii out of both sacs was treated as clutch size. Clutch size and egg production rate, calculated by dividing clutch size by inter-clutch duration, in the M treatment were about 1.5 times higher than in the H and HP treatments (Table 6). This was explained by the larger body size of adult females in the M treatment compared to the H and HP treatments (see Table 5 and Fig. 6). The number of eggs in a sac (ES , $0.5 \times$ clutch size) was a function of cephalothorax length (L in mm) of adult females ($N = 113$, $r^2 = 0.39$, $P < 0.001$; Fig. 7). Therefore, weight-specific egg production rate was not significantly different among the treatments (Table 6).

Median hatching success of eggs (HS) was 76.0% and 84.6% in the H and HP treatments, respectively (Table 6). In these treatments, individual variability of HS was high; some hatched completely, but some did not hatch at all. On the other hand, HS in the M treatment was very high (97.2% and 76% in median and minimum value, respectively), and

was significantly higher than in the H and HP treatments (Table 6).

Discussion

In this study, *Cyclops* sp. developed completely from hatching to adulthood on an exclusively algal diet of *Cryptomonas tetrapyrenoidosa* when the amount of the alga was $\geq 4 \times 10^3$ cells ml^{-1} . Cannibalism did not occur during development because we reared the copepods individually. In addition, *Cyclops* sp. produced eggs on the algal diet alone, and adding nauplii prey improved neither postembryonic development nor reproduction. These results clearly indicate that *Cyclops* sp. can complete its life cycle without animal prey; even the older copepodites and adults, which are often regarded as "predatory" stages (e.g., Jamieson 1980), do not necessarily need animal food to grow and reproduce. Similar results have been reported by Santer and van den Bosch (1994) in *Cyclops vicinus*; however, the food requirement

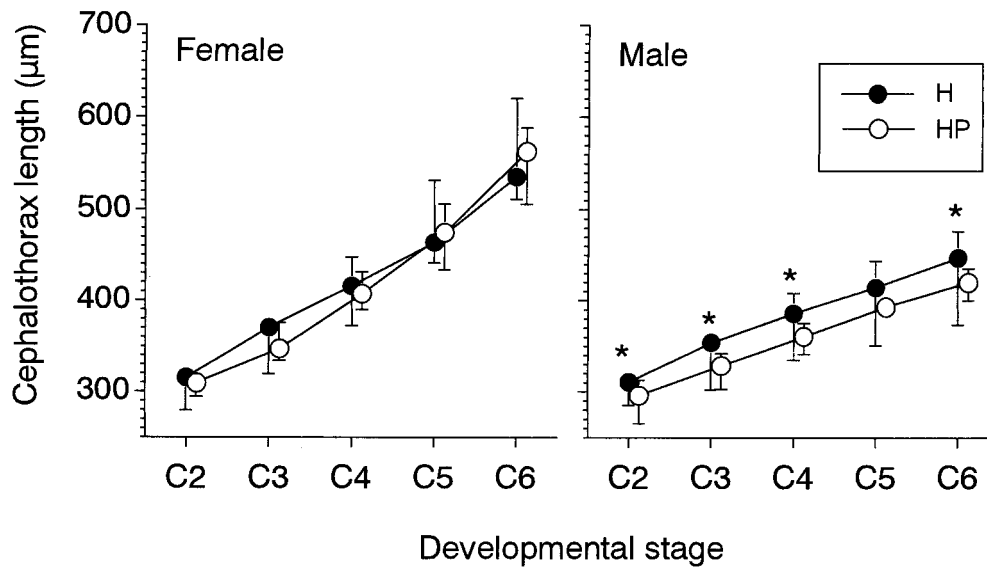


Fig. 6. As for Fig. 5, but for cephalothorax length of each developmental stage. Asterisks denote significant different between the two treatments at $P < 0.05$ level with U -test.

for postembryonic development and reproduction of cyclopoids can differ among species (*see above*). Larger cyclopoid species tend to be more carnivorous than smaller ones (Fryer 1957; Brandl 1998), and female body size has been shown to be a critical factor related to the importance of carnivorous nutrition for survival and reproduction in some species (Adrian and Frost 1993). However, this feature does not hold for all cyclopoids. Hopp et al. (1997) examined the egg production of five different-sized species and found that small *Mesocyclops leukarti* did not reproduce when fed with algae alone, whereas larger species (*Acanthocyclops lobstus*, *Cyclops vicinus*, and *C. abyssorum*) did. Hence, Hopp et al. (1997) concluded that factors other than body size are important regulators of feeding habits and of reproductive output.

The pattern of postembryonic development characterized by developmental stages of equal duration is referred as isochronality (Miller et al. 1977) and has been shown to occur under conditions of sufficient food in marine calanoids inhabiting shallow waters (e.g., Uye 1980). However, strictly speaking, most copepod development is anisochronal; the stage duration of the prefeeding N1 stage is the shortest of all developmental stages, whereas the duration of the first feeding N2 stage and preadult C5 stage in females are slightly longer than those of other stages (Landry 1983; Ban 1994). In the present study, stage duration of prefeeding N1 of *Cyclops* sp. was not affected by food regime and was the shortest of all developmental stages in all treatments. However, the stage duration of first-feeding N2 was not particularly long and the durations of stages after C3 gradually increased, indicating that true isochronality does not occur in *Cyclops* sp. Rather, our results seem to be identical to those of Whitehouse and Lewis (1973). They raised *Cyclops abyssorum* at 15°C and found that its stage duration increased over successive stages: 2–4 d from N1 through C2, about 6.5 d for C3 and C4, and about 15 d for C5. Hart's (1990) review of the duration of postembryonic development

in copepods also pointed out that isochronal development occurs in only two species and that in general, the later copepodite stages are likely to be protracted. A similar trend was recently observed in *Mesocyclops thermocyclopoides* (Kumar and Rao 1998). This type of development may be a common feature in the postembryonic development of cyclopoids.

Egg production rate is the product of two components: clutch size and interclutch duration. Clutch size varies with food supply (Smyly 1970; Santer and van den Bosch 1994) and is a function of body size of adult females when food supply is sufficient (Smyly 1973; Ban 1994). Interclutch duration also varies with food concentration for *Eurytemora affinis* (Ban 1994), and food concentration influences the interclutch duration much more than the clutch size for some marine calanoids (Corkett and McLaren 1969; Runge 1984; Kimoto et al. 1986). In the present study, clutch size was larger in the M treatment than in the H and HP treatments because it depended on the body size of adult females, and body size was larger in the M treatment. In contrast, interclutch duration was the same among treatments. Therefore, weight-specific egg production rates were also the same among treatments. This indicates that the lower algal concentration in the M treatment did not affect the egg-production ability of *Cyclops* sp. and that adult females always reproduced at their maximum potential rate.

van den Bosch and Gabriel (1994) proposed a model for copepod growth and development based on the assumption that a fixed fraction of assimilated energy is allocated to the development of a new carapace, and that copepods molt into the next stage when the weight of the accumulated carapace building blocks reaches a fixed fraction of the body mass at the beginning of the current stage. In their model, the carapace building-block storage compartment is filled sooner if more food is available and predicts decreasing stage duration and increasing body size with increasing food quantity, as observed for copepod growth and development (e.g., Ban

Table 6. Summary of reproductive parameters of female *Cyclops* sp. raised with (HP) and without nauplii (H, M), including duration between molt to adult and producing first clutch (DBMFC; days), interclutch duration (ICD; days), egg development time (EDT; day), clutch size (CS; eggs clutch⁻¹), egg production rate (EPR; eggs female day⁻¹), weight-specific egg production rate (WSEPR; eggs $\mu\text{g dry wt}^{-1} \text{day}^{-1}$) and hatching success (HS; %). For each parameter, data for the first to fifth clutch are pooled, because there was no significant difference among clutches. Differences between three feeding regimes were tested by using ANOVA and Fisher's PLSD.

Reproductive parameters	Food regime						ANOVA		
	Without nauplii (H, N = 34)		With nauplii (HP, N = 25)		4 × 10 ³ cells ml ⁻¹ (M, N = 57)		df	F ratio	Multiple comparison by Fisher's PLSD
	Median	Range	Median	Range	Median	Range			
DBMFC	11	8-16	11	8-35	11	8-43	2, 25	0.21	
ICD	11	8-18	11	8-43	11	7-35	2, 83	0.68	
EDT	9	8-10	9	8-10	9	8-10	2, 102	0.87	
CS	25	8-63	28	8-63	46	16-74	2, 112	21.36*	H HP M
EPR	2.27	0.73-7.88	2.62	0.35-6.30	4.27	1.15-10.14	2, 112	26.26*	H HP M
WSEPR	0.17	0.07-0.50	0.24	0.03-0.49	0.21	0.05-0.42	2, 112	1.28	
HS	76.0	0-100	84.6	0-100	97.2	76-100	2, 112	31.03*	H HP M

* Significant difference at P < 0.00001 level.

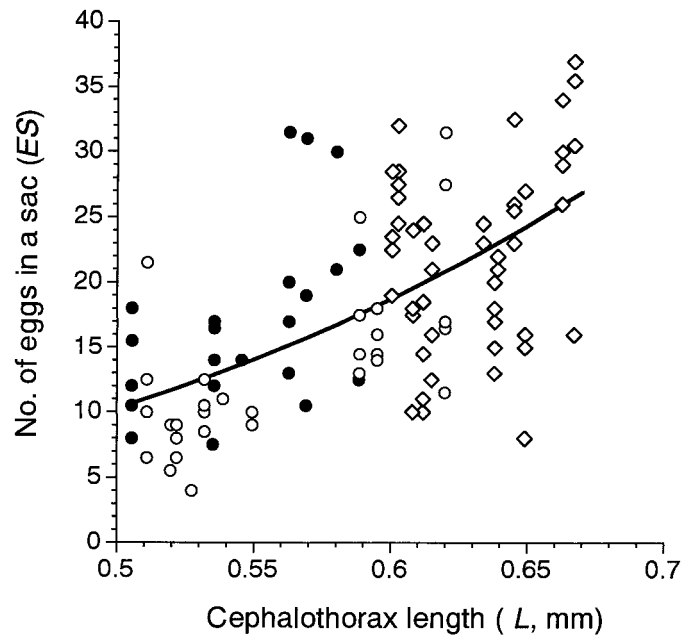


Fig. 7. The relationship between cephalothorax length and the number of eggs in a sac of adult female *Cyclops* sp. reared under three different feeding regimes. Open diamonds, 4 × 10³ cells ml⁻¹ (M treatment); open circles, 4 × 10⁴ cells ml⁻¹ (H treatment); closed circles, 4 × 10⁴ cells ml⁻¹ adding seven nauplii of *Eurytemora affinis* per day (HP treatment). The curve was estimated from the data of the three treatments expressed as an equation: $ES = 100.42 \times L^{3.28}$ ($r^2 = 0.39$, $P < 0.001$, $N = 113$).

1994). In our study of *Cyclops* sp., increased algal concentration caused more rapid development and larger body size prior to the early copepodite stages, which agrees with their model. However, after C3, algal density had no effect on male body size, and female body size in the M treatment was larger than that in the H treatment, although the stage duration tended to be shorter in the H treatment than the M treatment. Therefore, stage-specific growth rate was higher in the H treatment than in the M treatment until C1, but was not significantly different between the two treatments after C2. These responses cannot be explained by the building-block model. The lack of difference in stage-specific growth rate could mean that the rate might be saturated at the two food quantities used in our study, which also suggests that the energy allocation did not vary with treatments.

In the laboratory, we observed that females growing under reduced food conditions (M treatment) grew larger and had a longer development time than females grown under high food conditions (H treatment). This response to reduced food may be adaptive. If the female longevity is the same between the two treatments, lifetime reproductive success in the M treatment would be 1.8 times greater than that in H treatment. However, fitness is the product of reproduction and survivorship of individuals. Longer juvenile development would cause higher mortality due to various factors during development (e.g., predation). How might a trade-off between juvenile development rate and adult fecundity affect *Cyclops* sp. at the population level? We estimated the pop-

ulation growth rate (r , d^{-1}) in the H and M treatments using the Lotka–Euler equation:

$$1 = \sum_{x=0}^k \exp(-rx) l_x m_x,$$

where x is the age at reproduction, l_x is the probability of surviving to age x , and m_x is the fecundity at age x . The parameters used for calculation, selected from the median values, included the age at first reproduction (89.5 and 98 d for the H and M treatments, respectively), clutch size (25 and 46 eggs), and interclutch duration (11 d for both), and the calculation was made through the fifth clutch. We calculated r for the M and H treatments at zero mortality (i.e., $l_x = 1$), and found the results were similar: 0.045 and 0.047 in the H and M treatment, respectively. If, however, mortality rates are higher for longer developing individuals (the M treatment), population growth rates under the lowered food condition would be lower.

In conclusion, reduction of algal quantity resulted in slower development and larger body size for female *Cyclops* sp. This response may be an adaptation to increase the fitness of individuals in the oligotrophic environment, the original habitat of the copepod. This mechanism may act to keep the population growth rate relatively high despite low food supply.

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