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Combined effects of temperature and food concentration on growth and reproduction of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan)

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SUMMARY

1. Life history traits of the freshwater calanoid copepod *Eodiaptomus japonicus* from Lake Biwa were examined in the laboratory. Four different food concentrations (FC, 10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1}) and two temperature conditions (15 and 25 °C) were used to clarify the combined effects of those two factors on life history traits.

2. A survival rate of more than 70% was observed at the two medium FCs at 15 °C, although survival was <42% at all six of the other food–temperature combinations. Post-embryonic development times to adult stage in males and females were affected by both FC and temperature; median development times ranged from 28.7 to 37.3 and 31.4 to 35.0 days at 15 °C and 13.7 to 23.9 and 14.3 to 27.7 days at 25 °C, respectively, for males and females. An interaction between the two experimental factors was found only for females, with food shortage being most acute at 25 °C.

3. Clutch sizes also increased with FC at both temperatures and interaction occurred between those two factors. Egg production rates increased with increasing FC similarly at both temperatures without an interaction effect.

4. Adult body size increased with increasing FC at both temperatures: for example, average female prosome length increased from 0.865 mm to 0.922 mm at 15 °C and from 0.799 mm to 0.904 mm at 25 °C. Somatic and population growth rates calculated from the experimental data increased with FC, but the increase was more important at 25 °C. These responses to FC and temperature suggest that both growth and population dynamics of this copepod might be more influenced by food shortage at temperatures >15 °C.

5. Adult body sizes under food-limited conditions in this study are at the lower end of the range of those observed *in situ*, while those predicted from *in situ* temperatures, assuming non-limiting food conditions, were always larger than those of natural populations. Therefore, food shortage appears to be the most important factor affecting both growth and reproduction of *E. japonicus* in Lake Biwa.

Keywords: copepod dynamics, egg production, *Eodiaptomus japonicus*, individual growth rates, temperature–food effects

Introduction

Copepods are a key component in zooplankton communities and play a major role in aquatic food webs as both primary consumers and secondary producers. Identifying the drivers of copepod population dynamics is thus an important goal in lake ecosystem research. Temperature and food concentration (FC) are known to strongly affect copepod life history traits such as development time, survival rate, hatching success (HS) and clutch size (CS, Herzig, 1983; Huntley & Lopez, 1992; Ban, 1994; Jiménez-Melero, Parra & Guerrero, 2012). Temperature

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is one of the most important factors determining the geographical distribution, metabolism and lifespan of copepod species. The metabolic theory of ecology accurately predicts how the lifespan of ectotherms varies with temperature within species and, consequently, that global warming might substantially shorten lifespan (Munch & Salinas, 2009).

Along with temperature, food condition is the most important factor determining development and egg production rates (EPRs) in copepods (Ban, 1994; Klein Breteler, Gonzalez & Schogt, 1995; Jiménez-Melero *et al.*, 2012). CS (number of eggs laid by a female) is generally related to female body size (Deevey, 1960; Klein Breteler & Gonzalez, 1988; Ban, 1994); body size in copepods depends on both temperature and FC (Ban, 1994; Lee *et al.*, 2003; Jónasdóttir *et al.*, 2005; Beyrend-Dur *et al.*, 2011). CS can also be directly affected by food conditions. Jiménez-Melero *et al.* (2012) observed an increase in the CS and stabilisation in the third clutch when females from a food-limited environment were exposed to new *ad libitum* food conditions. Some studies of marine copepods have shown that copepods were always limited by food in the field (Checkley, 1980; Durbin *et al.*, 1983) and egg production was immediately limited by phytoplankton availability (Checkley, 1980).

Many studies have focused on the effect of single environmental factors, such as temperature, food or salinity, on estuarine copepods (Vuorinen *et al.*, 1998; Cervetto, Gaudy & Pagano, 1999; Ishikawa, Ban & Shiga, 1999; Lee & Petersen, 2002; Beyrend-Dur *et al.*, 2009), or even on the combined effect of temperature and salinity (Roddie, Leakey & Berry, 1984; Chinnery & Williams, 2004; Devreker, Souissi & Seuront, 2004; Holste & Peck, 2005; Devreker *et al.*, 2007, 2009; Beyrend-Dur *et al.*, 2011). Although previous studies have shown that growth and egg production of some copepod species were more sensitive to food shortage than to temperature variation (Ban, 1994; Koski & Kuosa, 1999), few studies have dealt with the combined effect of these environmental factors on copepod development, growth and reproduction (Klein Breteler & Gonzalez, 1986; Koski & Kuosa, 1999; Cook *et al.*, 2007; Jiménez-Melero *et al.*, 2012).

We assessed the combined effects of temperature and FC on growth and reproduction of *Eodiaptomus japonicus*, the sole calanoid copepod living in Lake Biwa, the largest lake in Japan. In Lake Biwa, *E. japonicus* plays a crucial role in transport of energy through the food chain, being an important food source for fish with high economic value (Kawabata *et al.*, 2002). Over the past five decades, the Lake Biwa ecosystem has experienced drastic changes in trophic status, shifting from oligotrophic to eutrophic/mesotrophic as a result of anthropogenic activities, and an increase in water temperature due to global warming (Tsugeki, Oda & Urabe, 2003; Hsieh *et al.*, 2011). Despite the large fluctuations in such environmental conditions, *E. japonicus* has been well adapted to this highly variable environment, dominating the zooplankton community in the lake (Kawabata, 1987; Yoshida *et al.*, 2001). Recently, Liu *et al.* (2014) determined the effects of temperature on the life history traits of *E. japonicus* under sufficient food supply. The authors suggested that this copepod was warm-water adapted and did not grow below 10 °C during winter, even with sufficient food supply. Kawabata (1987) suggested that natural populations of this copepod are exposed to severe food shortages in the lake, especially in summer. However, how temperature influences the effects of food availability is still unknown. Therefore, we determined the effects of FC on the life history traits of *E. japonicus* reared at different temperatures to clarify how climatic and anthropogenic environmental changes, such as global warming and eutrophication, can be expected to affect the *in situ* growth and population dynamics of this copepod.

Methods

Field collection and stock cultures

Eodiaptomus japonicus females with an egg sac were sorted from zooplankton samples collected with vertical plankton net hauls (diameter, 45 cm; mesh size, 200 μm) from 30 m to the surface at a sampling site in the north basin of Lake Biwa (35°19′05.3″N, 136°09′67.8″E) on 17 May and 11 September 2013. The copepods were then cultivated in 1-L jars filled with autoclaved and filtered (Whatman GF/F) tap water as stock cultures. The stock cultures were maintained at a constant temperature of 15 °C under a photoperiod of 12L:12D with a light intensity of 15.4 μmol m^{-2} s^{-1} and were fed on a 1 : 1 (cell : cell) fresh algal mixture of *Chlamydomonas reinhardtii* (IAM C-9) and *Cryptomonas tetrapyrenoidosa* (NIES 282) at *c.* 10^5 cells mL^{-1} total cell concentration. Culture medium was changed weekly, and fresh food suspensions were provided every 2 days. Algal cultures were grown in 1-L flasks at 20 °C under a photoperiod of 12L:12D with a light intensity of 125 μmol m^{-2} s^{-1}. Prior to the experiments, we maintained the stock cultures in the laboratory for at least two or three generations to avoid biases due to inherent wild population variability (Laabir *et al.*, 1995).

Experimental conditions

Prior to the experiments, animals were acclimatised for at least one generation at 15 and 25 °C in autoclaved and filtered tap water with the same algal mixture as the stock culture at each of the four FCs (i.e. 10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1}) in each of the 1-L jars. Food algae were provided daily. Culture medium was changed twice and three times per week at 15 and 25 °C, respectively. Frequency of water renewal was adapted to limit bacterial growth and to keep water clean from waste matter. All experiments were conducted under the same light conditions as those of the stock cultures.

Experiments on post-embryonic development

To determine post-embryonic development time (post-EDT), we sorted 36–72 newly hatched nauplii (N1) within 12 h from a minimum of six females acclimatised at each experimental condition of the 2 × 4 factorial design of temperature and FC as described above. Each nauplius was placed in a 10-mL well of a polystyrene tissue-culture plate (TR5000; Trueline, Romeoville, Illinois, U.S.A.) at the same food and temperature conditions to which the mothers had been exposed. Each one was observed under a dissecting microscope (SZX12; Olympus, Tokyo, Japan) at *c.* 200× magnification twice per day at 15 °C and four times per day at 25 °C to check exuviae or dead animals, until the adult stage was reached. Time zero was defined as the time when N1 hatched from an egg.

Reproduction experiments

For the experiments on reproduction, females with an egg sac were sorted from the zooplankton samples and incubated under each of the eight treatments (*c.* 50 females per treatment). Then, N1s hatched within 12 h in each treatment were reared in 1-L jars (*c.* 500 ind. in a jar) under the same temperature and food conditions as their mothers. When the animals reached the pre-adult copepodid 5th stage (C5), a female and a male were transferred to a 30-mL jar filled with 20 mL of the same medium and food algae as described above. Reproductive parameters were recorded daily until death of the female. Duration from moulting to adult female (AdF) death was expressed as female longevity. Dead males were removed and replaced by a new male from the stock cultures acclimatised at the same food and temperature conditions as the female. The culture medium and food algae were changed in the same manner as in the post-EDT experiments.

We determined the following reproductive parameters: HS (percentage of nauplii hatched to number of eggs in a clutch), embryonic development time (EDT, time taken from egg laying to hatching of the nauplii), CS (number of eggs per clutch), interclutch duration (ICD, time between spawning of clutch 'x' and spawning of clutch '$x + 1$'), latency time (LT, time between hatching or fall of clutch 'x' and spawning of clutch '$x + 1$') and EPR (number of eggs produced by a female per day) calculated from CS/ICD in each clutch. The few clutches that included unfertilised eggs (i.e. presenting no delimitation of the egg membranes; <6.1% of total clutches produced) were not taken into account for estimation of HS.

Body size measurements

The prosome lengths (PLs) of individuals from the first (C1) to the last (C5) juvenile stage were estimated by measuring the exuviae with an eyepiece micrometre under a dissecting microscope (SZX12; Olympus) at 900× magnification. Using exuviae is a very convenient method to distinguish individual stages and to measure the body size of these live small animals (Twombly & Burns, 1996; Lee *et al.*, 2003). The PL of adults was measured after death on individuals preserved in neutral 5% formalin.

Data transformation and statistical analysis

The gamma density function (GDF) allowed us (I) to calculate median development time (MDT_i, days) of each developmental stage in copepods (Souissi & Ban, 2001), that is time at which 50% of individuals have moulted to a certain stage i; and (II) to predict moulting probability. The cumulative proportion of individuals moulting to each stage was plotted against days from hatching using the *gamcdf* [x/α, β] function included in the curve fitting toolbox of MATLAB software (MathWorks Inc., 2009) to obtain estimates of the maximum-likelihood and confidence limits of the GDF parameters, that is the shape parameter (α) and the scale parameter (β).

Development variability in each stage was estimated with the coefficient of variation (CV %) for each experimental condition. CV was calculated as follows:

$$CV = \sigma/\mu \times 100$$

where σ is standard deviation and μ is the mean value of the development time.

To evaluate somatic growth from C1 to adult stage, the body dry weight (W, μg) was calculated from the PL

(mm) using the following exponential equation (Kawabata & Urabe, 1998):

$$W = e^{(2.59 \ln PL + 2.6995)}.$$

The body dry weight was plotted against MDT (days) and was fitted with a von Bertalanffy's function (Fitzhugh, 1976):

$$W_{Ber} = A(1 - Be^{-k\mathrm{MDT}})^m$$

where A is the asymptotic value for body weight at time $t \to \infty$, interpreted as average value of body weight, B is a scaling parameter, k (day^{-1}) is the growth coefficient, and m is the inflection parameter for Richards function ($m = 3$ in this case). The values of B and k were estimated by nonlinear least squares as a loss function in the curve fitting toolbox of the MATLAB software (MathWorks Inc., 2009).

Life table parameters were calculated from life history traits of the copepod, and the population growth rate (r, day^{-1}) was derived from Euler–Lotka's equation and calculated iteratively:

$$\sum_{x=i_a}^{\varpi} l_x m_x e^{-rx} = 1$$

where i_a is the age at maturity, l_x is the proportion of individuals surviving at day x, m_x is the number of offspring produced by a female at day x, and ω is female longevity.

Generalised linear models (GLM) were used to test the differences between each life history trait, that is the dura-

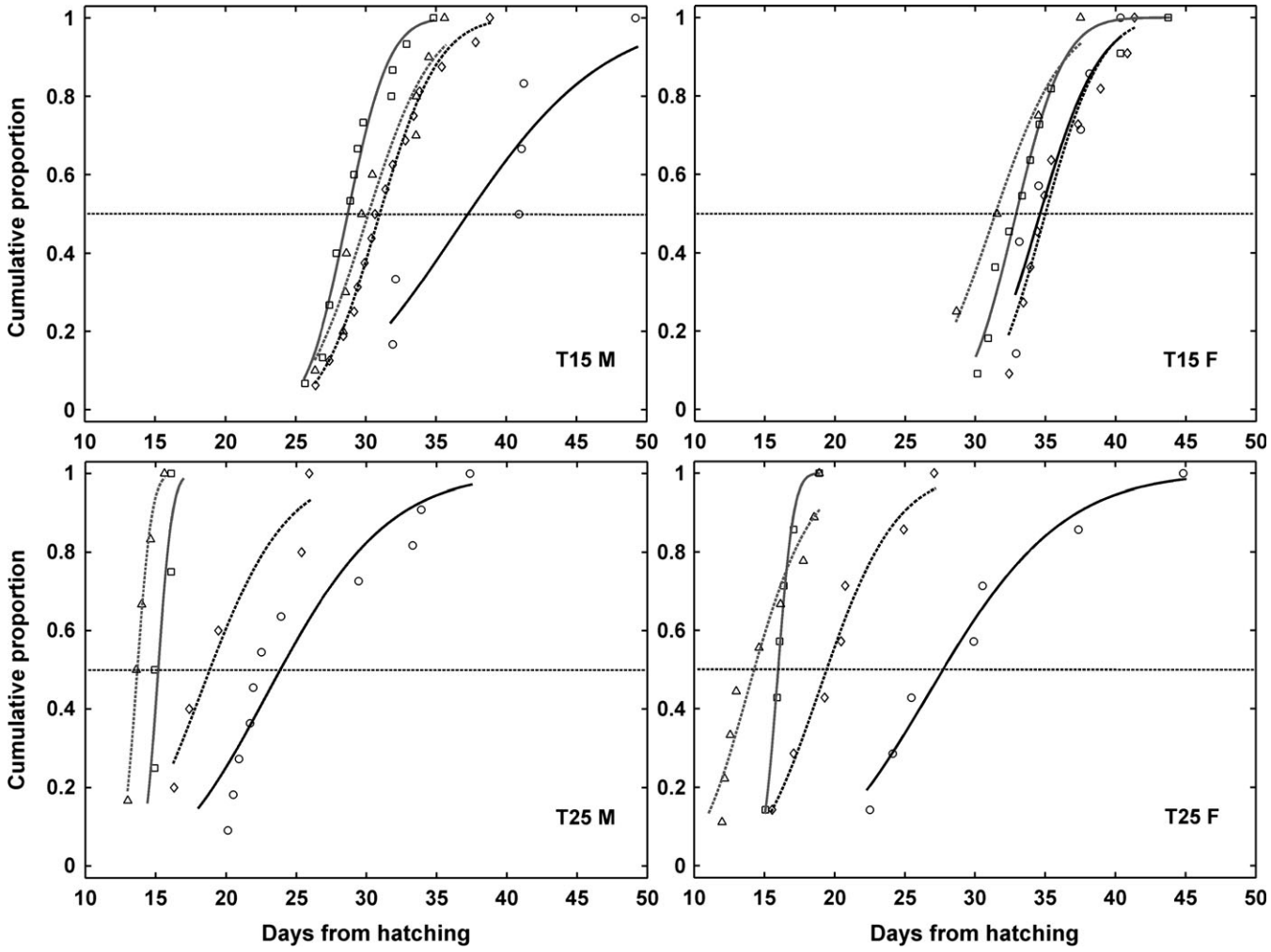

Fig. 1 Cumulative proportions of *Eodiaptomus japonicus* individuals moulting to adult stage against days from hatching. *Dots* represent observed data at food concentrations (FCs) of 10^3 (*circles*), 5×10^3 (*diamonds*), 10^4 (*squares*) and 5×10^4 (*triangles*) cells mL^{-1} at 15 °C (T15) and 25 °C (T25). Males (M) and females (F) are shown separately. The lines represent the expected data from the gamma density functions at FCs of 10^3 (*solid black*), 5×10^3 (*black dots*), 10^4 (*solid grey*) and 5×10^4 (*grey dots*) cells mL^{-1}. Function parameter data are shown in Table 1.

tion of aggregated stages (N1–N6, C1–C3 and C4–C5), post-EDT until adult, PL, EDT, CS, EPR, ICD, LT and longevity, among the eight temperature and food treatments. When the GLM showed an interaction between the two factors, we performed a Kruskal–Wallis test to evaluate the differences among the food treatments at each temperature. A log-linear model was employed for testing the effects of temperature (Temp), food concentration (Food) and developmental stage (Stage) on frequency of dead and live individuals (Survival). This log-linear model included four main effects (Temp, Food, Stage and Survival), six two-variable interactions (Temp × Food, Temp × Stage, Temp × Survival, Food × Stage, Food × Survival and Stage × Survival), four three-variable interactions (Temp × Food × Stage, Temp × Food × Survival, Temp × Stage × Survival and Food × Stage × Survival) and one four-variable interaction (Temp × Food × Stage × Survival). The variable 'Temp' had two categories (15 and 25 °C), the variable 'Food' had four categories (10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1}), the variable 'Stage' had 11 categories (N1–C5), and the variable 'Survival' had two categories (dead and alive). The saturated (full) model included all main effects, all two-way, three-way and four-way interactions. Potential differences among developmental stages in the response to increasing food on mean PL (i.e. homogeneity of slopes) were tested with analysis of covariance (ANCOVA). Post hoc tests (Tukey–Kramer test) were conducted when ANCOVA showed a significant difference of the slopes among the stages. Regression analysis against the log-transformed FCs was carried out for growth coefficient (k) and population growth rate (r) to test the differences between the slopes and zero. The differences in k between sexes were tested using ANCOVA. All statistical analyses were performed with IBM SPSS Statistics software (IBM Inc., 2011) and MATLAB software (MathWorks Inc., 2009).

Results

Post-embryonic development

The cumulative proportion for each developmental stage against the days from hatching was well fitted by the GDF for all temperature and food treatments ($R^2 > 0.8$) (e.g. adult stage shown in Fig. 1, Table 1). At 15 °C, the MDTs of adult males (AdMs) reared at $\geq 5 \times 10^3$ cells mL^{-1} ranged between 28.7 and 31.0 days, whereas at 10^3 cells mL^{-1} the MDT was 37.3 days. In contrast, the MDTs of AdFs were similar at all food treatments, being 31.4–35.0 days (Table 2). At 25 °C, the MDTs of AdMs and females strikingly decreased with increasing FC, ranging from 13.7 to 23.9 days and from 14.3 to 27.7 days, respectively (Table 2). GLMs showed that temperature and FC significantly influenced post-EDT for both males and females (Table 3). On the other hand, interactions between the two factors were found in females, but not in males; the post-EDTs in females were significantly different among the food treatments at 25 °C (Kruskal–Wallis test, d.f. = 3, $H = 19.42$, $P < 0.001$), but not at 15 °C (Kruskal–Wallis test, d.f. = 3, $H = 3.48$, $P = 0.323$).

The CV tended to decrease towards older stages (Table 2). The highest variation of post-EDT was always

Table 1 Parameters (α, β and R^2) with 95% confidence limits at of the gamma density function (*gamcdf* [x/α, β]) fitted against days from hatching to adult male (M) and female (F) *Eodiaptomus japonicus* at each temperature and food treatment

Temp. (°C)	Food conc. (cells mL^{-1})	Sex	Gamma density function parameter (confidence limits)		
			α	β	R^2
15	10^3	M	24.46 (−13.94, 62.87)	1.545 (−0.8797, 3.969)	0.855
		F	110.20 (−46.51, 266.9)	0.315 (−0.1339, 0.7642)	0.888
	5×10^3	M	89.61 (77.14, 102.1)	0.347 (0.2986, 0.3958)	0.995
		F	129.20 (52.58, 205.9)	0.272 (0.1096, 0.4334)	0.957
	10^4	M	155.60 (92.48, 218.8)	0.185 (0.1098, 0.26)	0.975
		F	150.60 (85.4, 215.7)	0.219 (0.124, 0.3141)	0.977
	5×10^4	M	73.91 (30.82, 117)	0.410 (0.1695, 0.6511)	0.944
		F	68.46 (−19.66, 156.6)	0.461 (−0.1322, 1.055)	0.980
25	10^3	M	15.53 (1.994, 29.06)	1.570 (0.1403, 2.999)	0.892
		F	17.16 (6.197, 28.12)	1.648 (0.5678, 2.728)	0.972
	5×10^3	M	19.68 (−10.46, 49.81)	0.974 (−0.5554, 2.502)	0.935
		F	25.05 (9.16, 40.93)	0.786 (0.2819, 1.29)	0.973
	10^4	M	375.40 (−1143, 1894)	0.040 (−0.1232, 0.204)	0.800
		F	347.50 (156.4, 538.5)	0.046 (0.02077, 0.0713)	0.989
	5×10^4	M	284.30 (116.3, 452.2)	0.048 (0.01974, 0.07663)	0.991
		F	20.43 (8.059, 32.81)	0.710 (0.2712, 1.149)	0.943

Table 2 Median development time (MDT, days), its coefficient of variation (CV, %), stage duration (SD, days), number (*n*) and proportion of survivors (%) for different stages of *Eodiaptomus japonicus* reared in eight different treatments. Sex ratios (female/male) of adult stage and the survival rates from hatching to each developmental stage are also indicated

Stage and sex	15 °C, 10^3 cells mL^{-1}					15 °C, 5×10^3 cells mL^{-1}					15 °C, 10^4 cells mL^{-1}					15 °C, 5×10^4 cells mL^{-1}				
	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%
No. of eggs				36					36					36					36	
N1			1.2	36	100			0.9	36	100			1.0	36	100			1.2	36	100
N2	1.2	49.3	2.4	36	100	0.9	74.3	2.3	36	100	1.0	66.6	2.1	36	100	1.2	39.6	2.0	36	100
N3	3.5	33.5	1.8	34	94	3.2	30.4	1.9	34	94	3.1	40.9	1.8	35	97	3.2	21.1	2.2	29	81
N4	5.3	37.8	3.0	24	67	5.0	35.4	2.7	34	94	4.9	39.2	2.4	33	92	5.5	17.8	2.1	22	61
N5	8.3	40.8	3.5	15	42	7.7	32.1	2.9	32	89	7.3	30.6	3.7	31	86	7.5	13.1	2.2	21	58
N6	11.8	35.9	3.1	14	39	10.6	26.8	3.0	29	81	10.9	21.5	2.7	29	81	9.8	17.8	2.4	19	53
C1	14.8	19.5	3.1	13	36	13.6	17.0	3.0	27	75	13.6	20.5	2.8	27	75	12.2	8.4	3.2	18	50
C2	17.9	20.9	3.4	13	36	16.5	15.0	3.2	27	75	16.4	20.6	2.9	27	75	15.4	11.3	4.3	17	47
C3	21.4	19.8	3.7	13	36	19.8	13.8	4.4	27	75	19.4	18.3	3.3	27	75	19.7	11.9	3.2	16	44
C4	25.1	17.5	5.0	13	36	24.1	12.2	3.8	27	75	22.7	17.0	3.4	26	72	23.0	11.5	3.7	16	44
C5 M	31.9	17.1	5.4	6	36*	26.6	12.2	4.5	16	75*	24.8	9.7	3.9	15	72*	26.0	11.5	4.2	10	39*
C5 F	28.5	8.7	6.1	7		29.2	7.7	5.8	11		27.3	15.5	5.6	11		27.8	9.0	3.7	4	
C6 M	37.3	16.6		6		31.0	11.1		16		28.7	8.5		15		30.2	10.1		10	
C6 F	34.6	8.3		7		35.0	8.6		11		32.9	12.2		11		31.4	11.5		4	
Sex ratio	1.17					0.69					0.73					0.40				
Stage and sex	25 °C, 10^3 cells mL^{-1}					25 °C, 5×10^3 cells mL^{-1}					25 °C, 10^4 cells mL^{-1}					25 °C, 5×10^4 cells mL^{-1}				
	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%	MDT	CV	SD	*n*	%
No. of eggs				72					36					36					36	
N1			0.7	72	100			0.5	36	100			0.4	36	100			0.4	36	100
N2	0.7	30.3	1.0	72	100	0.5	40.0	1.3	36	100	0.4	53.5	0.8	36	100	0.4	26.5	1.1	34	94
N3	1.7	18.9	1.2	64	89	1.8	14.4	0.9	30	83	1.2	24.6	1.4	29	81	1.5	19.0	1.0	29	81
N4	2.9	13.0	1.4	45	63	2.6	15.1	1.6	30	83	2.6	15.8	1.7	23	64	2.5	16.8	1.0	25	69
N5	4.3	20.2	1.7	28	39	4.3	26.7	1.3	18	50	4.3	23.4	1.4	19	53	3.5	15.1	1.2	21	58
N6	5.9	30.5	1.9	22	31	5.5	32.4	1.3	17	47	5.7	17.2	1.2	17	47	4.7	16.4	1.2	19	53
C1	7.8	30.2	2.4	20	28	6.8	37.2	2.1	16	44	6.9	16.4	1.5	11	31	5.9	16.7	1.2	15	42
C2	10.2	28.9	3.0	19	26	8.9	21.5	2.3	15	42	8.4	12.8	1.3	11	31	7.1	16.0	1.8	15	42
C3	13.3	27.6	4.4	19	26	11.2	17.5	3.0	14	39	9.7	11.9	1.7	11	31	8.9	14.1	1.3	15	42
C4	17.6	25.1	4.0	19	26	14.2	14.6	2.0	13	36	11.4	13.5	1.8	11	31	10.3	15.4	1.6	15	42
C5 M	20.4	25.8	3.4	11	25*	16.4	21.2	2.4	5	33*	12.5	6.2	2.7	4	31*	11.6	7.9	2.1	6	42*
C5 F	22.6	21.0	5.1	7		15.9	11.4	3.5	7		13.4	9.2	2.6	7		12.1	18.9	2.2	9	
C6 M	23.9	24.3		11		18.8	21.5		5		15.2	4.3		4		13.7	6.6		6	
C6 F	27.7	26.0		7		19.4	19.7		7		16.0	7.5		7		14.3	18.7		9	
Sex ratio	0.64					1.40					1.75					1.50				

M, male; *F*, female; *n*, number of individuals developed to the stage
*Survival rate of total individuals at C5 and adult stages.

observed in the second naupliar stage (N2) (Table 2). CV of adult post-EDT strongly depended on FC at both experimental temperatures, especially at 25 °C, ranging from 4.3 to 24.3% for males and from 7.5 to 26.0% for females. The highest variation was observed at the lowest FC for males, but with females, it occurred at 15 °C at the medium FC, that is 10^4 cells mL^{-1} (Table 2).

The shortest stage duration (SD) was observed in the N1 stage among developmental stages of all treatments (Table 2). SD tended to increase with developmental stage, especially under low FCs at both temperatures. GLM of SD showed that all SDs in three aggregated stage groups were significantly different among temperature and food treatments (Table 3). The interaction between the two factors was significant in early (C1–C3) and late (C4–C5) copepodid stages of females but not in naupliar stages (N1–N6) and late copepodid stages (C4–C5) of males (Table 3). Because of the interaction between the two factors, the SD differences in C1–C3 and C4–C5 females among FCs were tested with the Kruskal–Wallis test. A significant effect was found at each temperature (d.f. = 3, $H = 17.73$ and 42.71 in

Table 3 Generalised linear models (GLM) show the effect of temperature (Temp) and food concentration (Food) on stage durations (*N1–N6*, *C1–C3* and *C4–C5*), post-embryonic development time (post-EDT) and prosome length (PL) of *Eodiaptomus japonicus* for each experimental condition. Males (M) and females (F) were considered separately in stage duration of *C4–C5*, post-EDT and PL

Factor	d.f.	Chi-square	*P* value
N1–N6			
Temp	1	284.027	<0.001
Food	3	22.596	<0.001
Temp × Food	3	2.147	0.542
C1–C3			
Temp	1	124.503	<0.001
Food	3	70.722	<0.001
Temp × Food	3	44.660	<0.001
C4–C5 (M)			
Temp	1	47.276	<0.001
Food	3	32.788	<0.001
Temp × Food	3	4.073	0.254
C4–C5 (F)			
Temp	1	33.245	<0.001
Food	3	47.594	<0.001
Temp × Food	3	10.976	0.012
Post-EDT (M)			
Temp	1	188.935	<0.001
Food	3	71.236	<0.001
Temp × Food	3	4.784	0.188
Post-EDT (F)			
Temp	1	192.159	<0.001
Food	3	44.981	<0.001
Temp × Food	3	27.131	<0.001
PL (C1)			
Temp	1	132.403	<0.001
Food	3	55.808	<0.001
Temp × Food	3	6.491	0.090
PL (M)			
Temp	1	32.627	<0.001
Food	3	116.543	<0.001
Temp × Food	3	16.256	0.001
PL (F)			
Temp	1	26.031	<0.001
Food	3	80.265	<0.001
Temp × Food	3	8.826	0.032

d.f., degrees of freedom.

C1–C3, 12.82 and 15.85 in C4–C5 female, at 15 and 25 °C, respectively, $P < 0.01$), indicating that the effect of FC on SD differed with temperature in C1–C3 and C4–C5 females, but not in N1–N6 and C4–C5 males.

Sex ratios (female/male) at each treatment are shown in Table 2. The proportion of males was higher than the proportion of females except at the lowest FC at 15 °C (Table 2). At 25 °C, the opposite occurred; the proportion of females was higher than that of males except at the lowest FC (Table 2). Survival rates until adult stage did not exceed 42% in any treatment, except for those at 5×10^3 and 10^4 cells mL^{-1} at 15 °C where a survival rate of >70% was recorded (Table 2). In all the treatments, the death of individuals mostly occurred during the naupliar stages. Our log-linear model showed that temperature, FC and stage affected the frequency of dead and live individuals. Interaction was also observed, indicating that food effect was different at different temperatures for each stage (Table 4, $P < 0.001$). The most severe mortalities were found at the lowest FC, especially at 25 °C (Table 2).

Table 4 Results of the log-linear model for a four-way analysis: effect of temperature (Temp), food concentration (Food) and developmental stage (Stage) on frequency of dead and live individuals (Survival) of *Eodiaptomus japonicus*

Model		Goodness of fit tests		
		G^2	d.f.	*P* value
1	Temp + Food + Stage + Survival	470.683	160	<0.001
2	Temp × Food	379.536	157	<0.001
3	Temp × Stage	439.720	150	<0.001
4	Temp × Survival	446.505	159	<0.001
5	Food × Stage	435.382	130	<0.001
6	Food × Survival	450.314	157	<0.001
7	Stage × Survival	292.502	150	<0.001
8	Temp × Food × Stage	313.003	87	<0.001
9	Temp × Food × Survival	327.641	150	<0.001
10	Temp × Stage × Survival	236.925	129	<0.001
11	Food × Stage × Survival	181.042	87	<0.001
12	Saturated (full) model	0	0	

PL of C1 increased with increasing FC similarly at both temperatures (Fig. 2), although the differences were quite small: just 6% larger in the highest FC compared to the lowest one (Fig. 2, Table 3). Adult body size of both males and females also increased with increasing FC at both temperatures, being 11 and 10%, respectively, larger in the highest FC than those in the lowest one (Fig. 2, Figure S1). GLM showed an interaction between food and temperature in adults, but not in C1 (Table 3). The Kruskal–Wallis test showed that the differences of PL in adults were statistically significant among FC at each temperature (d.f. = 3, $H = 21.8$ and 19.95 in males, 14.38 and 20.22 in females, at 15 and 25 °C, respectively, $P < 0.01$). The relationship between FC and PL was not the same at all stages (Figure S1, Table S1). ANCOVA showed that the slopes at every developmental stage were not homogeneous at 15 and 25 °C (d.f. = 6 and 20, $F = 4.45$, $P < 0.01$ for 15 °C; and d.f. = 6 and 20, $F = 4.67$, $P < 0.01$ for 25 °C). Post hoc tests showed that at 15 °C the slopes for AdM and AdF were significantly different from those of C3 (Figure S2a). At 25 °C, the slope for C1 was significantly different from those of AdM and AdF (Figure S2b).

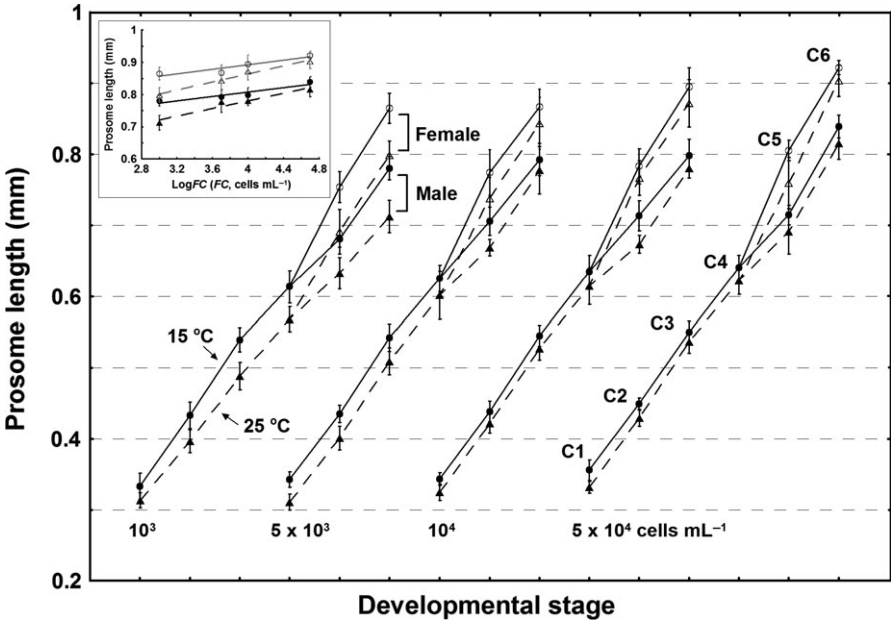

Fig. 2 Prosome length (PL, mm) of developmental stages from C1 to adult in *Eodiaptomus japonicus* males (*solid symbols after C5*) and females (*open symbols after C5*) at food concentrations of 10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1} at 15 (*circles* and *solid lines*) and 25 °C (*triangles* and *dashed lines*). *Vertical bars* indicate standard deviations. Inset: PL (mm) of adult *E. japonicus* males (*filled circles* and *black solid line*) and females (*open circles* and *grey solid line*) at 15 °C and males (*filled triangles* and *black dashed line*) and females (*open triangles* and *grey dashed line*) at 25 °C reared under different food treatments. *Vertical bars* indicate standard deviations.

Somatic growth rate

Body dry weight changes in both males and females of *E. japonicus* from the time of hatching were well described by the von Bertalanffy's function for all treatments (Fig. 3, Table 5). Regression analyses showed that the growth coefficient (k, day^{-1}) increased significantly with log-transformed FCs (cells mL^{-1}) for both males and females at 25 °C ($n = 4$, t for the slope = 3.472 in males and 4.302 in females, $P < 0.05$), but not at 15 °C ($n = 4$, t for the slope = 0.343 in males and 1.278 in females, $P > 0.05$) (Fig. 4). At 25 °C, ANCOVA showed that the differences of k between the sexes were not statistically significant (d.f. = 1 and 13, $F = 0.13$, $P = 0.727$).

Reproduction

Hatching success of *E. japonicus* exceeded 97% in all experimental treatments, except for a value of 78.9% at 10^3 cells mL^{-1} at 15 °C (Table 6). EDTs were significantly different between the two temperatures, but not among FCs, averaging 3.9 and 1.7 days at 15 and 25 °C, respectively (Fig. 5, Tables 6 & 7). CS increased with FC at both temperatures (Fig. 5), being 2.2-fold higher in the highest FC than those in the lowest one. Although GLM showed that CS was significantly different among FCs and temperatures with interactions between them, the increasing trends were quite similar between the two temperatures (Fig. 5, Table 7). The Kruskal–Wallis test also showed significant CS differences among the FCs at both temperatures (d.f. = 3, $H = 151.56$ and 124.36 at 15 and 25 °C, respectively, $P < 0.05$).

Interclutch duration ranged from 5.91 to 9.36 days and from 2.48 to 2.85 days among the FCs at 15 and 25 °C, respectively (Fig. 5, Table 6). Both temperature and FC significantly affected ICD with interactions between the two factors (Table 7). The Kruskal–Wallis test showed that ICDs were significantly different among the FCs at both 15 and 25 °C (d.f. = 3, $H = 36.31$ and 13.02 at 15 and 25 °C, $P < 0.01$), although the variations were larger at 15 °C than at 25 °C.

Latency times showed a similar trend as ICD, varying between 2.52 and 6.12 days at 15 °C and between 1.22 and 1.96 days at 25 °C (Fig. 5, Table 6). The Kruskal–Wallis test showed that LTs were significantly different among the FCs at both temperatures (d.f. = 3, $H = 42.88$ and 24.74 at 15 and 25 °C, respectively, $P < 0.05$).

Finally, EPR, calculated from CS and ICD, increased with increasing FC at both temperatures (Fig. 5), increasing by 4.0-fold and 2.2-fold at 15 and 25 °C, respectively, from the lowest FC to the highest one. No interaction between the two factors on EPR was found (Table 7). Average longevity of *E. japonicus* females always exceeded 2 months at 15 °C and was about a month at 25 °C regardless of FC (Fig. 5, Table 6). Longevity was significantly different between the temperatures, but not among the FCs (Table 7).

Population growth rate

Population growth rate (r, day^{-1}) in each experimental treatment, calculated from life history parameters of *E. japonicus* with Euler–Lotka's equation, increased with increasing FC at both temperatures (Fig. 6). Regression

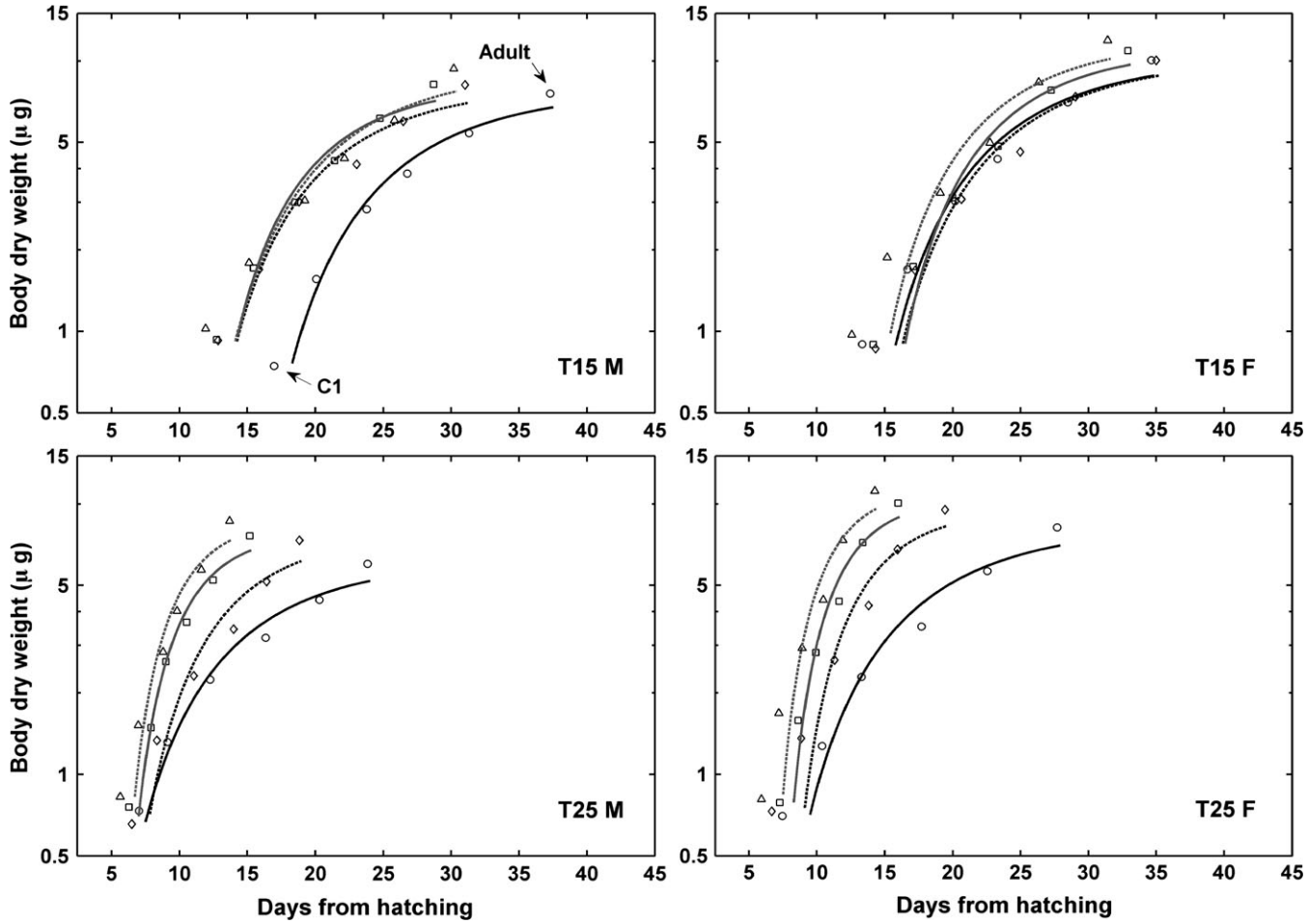

Fig. 3 Body dry weights of *Eodiaptomus japonicus* in copepodid stages as a function of the cumulative development time of each stage. *Dots* represent observed data at food concentrations (FC) of 10^3 (*circles*), 5×10^3 (*diamonds*), 10^4 (*squares*) and 5×10^4 (*triangles*) cells mL^{-1} at 15 (T15) and 25 °C (T25). Males (M) and females (F) are shown separately. The lines represent the expected data from the von Bertalanffy's functions at FCs of 10^3 (*solid black*), 5×10^3 (*black dots*), 10^4 (*solid grey*) and 5×10^4 (*grey dots*) cells mL^{-1}. Function parameter data are presented in Table 5.

analysis showed that r significantly increased with log-transformed FCs at 25 °C ($n = 4$, $R^2 = 0.877$, t for the slope = 3.783, $P < 0.05$), but not at 15 °C ($n = 4$, $R^2 = 0.435$, t for the slope = 1.241, $P > 0.05$).

Discussion

In this study, we demonstrated that growth and reproduction of *E. japonicus* were significantly influenced by FC and temperature. Kawabata (1989) suggested the probability of food shortage for *E. japonicus* from Lake Biwa through quasi-*in situ* enrichment experiments. The present study using factorial-designed laboratory experiments supports the hypothesis of Kawabata (1989) and additionally demonstrates temperature-mediated food effects.

Embryonic development time and duration of the first naupliar stage (N1) of *E. japonicus* were not affected by FC, whereas they both depended on temperature as shown in previous studies (e.g. Landry, 1975), including ours under sufficient food supply (Liu *et al*., 2014). Probably food shortage does not influence yolk investment in an egg, although reproductive investment decreased with decreasing food uptake (Guisande & Harris, 1995). In general, early naupliar stages of calanoid copepods survive on their oil sacs or yolk until the mandible is sufficiently developed to start feeding (Mauchline, 1998). The early feeding naupliar stages vary between species (Sekiguchi, 1974). In *E. japonicus*, the N1 stage is a non-feeding stage (Kawabata, 1989; Liu *et al*., 2014), as confirmed by complementary observation of empty guts in the N1 stage in the course of the present study. There-

Table 5 Parameters (A, B, k and R^2) with 95% confidence limits at of von Bertalanffy's function for *Eodiaptomus japonicus* in copepodid male (M) and female (F) individuals under different experimental conditions

Temp. (°C)	Food conc. (cells mL^{-1})	Sex	Parameters of von Bertalanffy's function (confidence limits)			
			A	B	k	R^2
15	10^3	M	7.827	5.342 (−1.329, 12.01)	0.1252 (0.0739, 0.1766)	0.965
		F	10.215	4.283 (−2.152, 10.72)	0.1291 (0.06015, 0.1981)	0.951
	5×10^3	M	8.146	3.663 (−1.683, 9.008)	0.1377 (0.06314, 0.2123)	0.938
		F	10.279	4.479 (−2.099, 11.06)	0.1279 (0.06236, 0.1934)	0.950
	10^4	M	8.302	5.027 (−2.7, 12.75)	0.1599 (0.07865, 0.2411)	0.946
		F	11.154	6.924 (−4.76, 18.61)	0.1516 (0.0739, 0.2293)	0.956
	5×10^4	M	9.449	3.388 (−2.888, 9.664)	0.1301 (0.03903, 0.2211)	0.902
		F	12.056	5.236 (−5.901, 16.37)	0.1443 (0.04453, 0.244)	0.922
25	10^3	M	6.183	1.439 (0.2521, 2.626)	0.1347 (0.07182, 0.1976)	0.947
		F	8.321	1.85 (−0.189, 3.889)	0.1259 (0.05581, 0.1959)	0.941
	5×10^3	M	7.755	2.222 (−0.7701, 5.213)	0.1789 (0.07027, 0.2876)	0.919
		F	9.573	5.062 (−5.033, 15.16)	0.2394 (0.08343, 0.3954)	0.935
	10^4	M	7.827	4.296 (−1.257, 9.849)	0.2929 (0.1583, 0.4276)	0.959
		F	10.435	7.84 (−5.887, 21.57)	0.3143 (0.1529, 0.4758)	0.954
	5×10^4	M	8.774	4.589 (−3.841, 13.02)	0.3177 (0.1194, 0.516)	0.929
		F	11.439	7.365 (−10.7, 25.43)	0.3384 (0.09503, 0.5819)	0.921

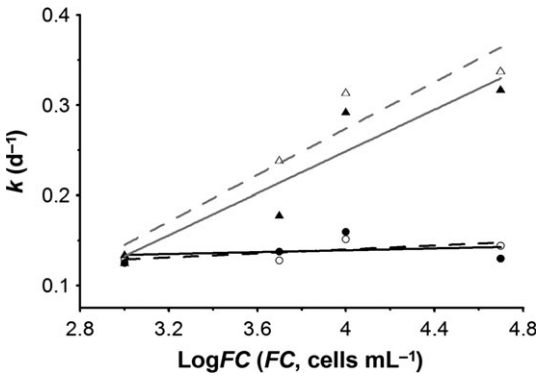

Fig. 4 Growth coefficient (k, day^{-1}) of *Eodiaptomus japonicus* of males (*filled circles* and *black solid line*) and females (*open circles* and *black dashed line*) at 15 °C and males (*filled triangles* and *grey solid line*) and females (*open triangles* and *grey dashed line*) at 25 °C reared under different food treatments.

fore, EDT and the duration of the N1 stage in this copepod are simply a function of temperature regardless of FC.

In this study, *E. japonicus* showed high mortality rates in the naupliar stages, especially at lower FCs, while mortality was quite low in copepodid stages, as reported for other copepod species (Kimmerer & McKinnon, 1987; Lee *et al.*, 2003). Williamson, Butler & Forcina (1985) showed that adults of the freshwater copepod *Diaptomus pallidus* exhibited a higher survival rate than that of nauplii under food-limited conditions. The marine copepod *Pseudocalanus newmani* exhibits higher survivals in later developmental stages under starved conditions (Tsuda, 1994). In general, clearance rates in naupliar stages are lower than those in copepodid stages (Berggreen, Hansen & Kiørboe, 1988; Merrell & Stoecker, 1998), and nauplii cannot capture food as efficiently as copepodites since grazing ability is improved ontogenetically with the development of feeding appendages and swimming behaviours (Paffenhöfer & Lewis, 1989). Energy storage, as lipid and wax esters, is greater in copepodites than in nauplii and allows them to moult successfully even under food-limited conditions (Kattner & Krause, 1987; Hagen, 1988; Lee, Hagen & Kattner, 2006). Furthermore, nauplii do not seem to accumulate lipid stores even in the presence of excess phytoplankton (Håkanson, 1984).

In the present study, the highest survival rates observed during development until adulthood were observed at medium FCs, that is 5×10^3 and 10^4 cells mL^{-1} at 15 °C, equivalent to *c.* 1.29 and 2.57 mg C L^{-1}, respectively, while the variation over all the experimental FCs ranged from 0.26 to 12.86 mg C L^{-1}. Survival rates exceeded 80% at the medium food levels (i.e. 1.0 mg C L^{-1} in the experimental ranges from 0.05 to 2.5 mg C L^{-1}), as have been observed in other freshwater copepods (Hart, 1996). On the other hand, fungal parasitism has been shown to be an important contributor to mortality of copepods cultured in the laboratory (Burns, 1984; Kimmerer & McKinnon, 1990; Hart, 1996). Zeller, Jiménez-Melero & Santer (2004) suggested that two freshwater copepods, *Eudiaptomus gracilis* and *Eudiaptomus graciloides*, suffered from fungal infection and exhibited low survival rates at

Table 6 Mean and standard deviations (SD) of reproductive parameters in *Eodiaptomus japonicus* reared under eight different treatments

	15 °C, 10^3 cells mL^{-1}			15 °C, 5 × 10^3 cells mL^{-1}			15 °C, 10^4 cells mL^{-1}			15 °C, 5 × 10^4 cells mL^{-1}		
Parameters	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
No. of pairs			18			18			18			10
HS (%)	78.87		–	97.60		–	99.26		–	97.50		–
EDT	4.02	0.80	94	3.90	0.61	130	3.82	0.69	121	3.87	0.54	57
CS	7.55	3.05	94	13.47	4.69	130	14.56	4.59	121	16.84	4.64	57
EPR	0.81	0.32	15	2.11	0.73	17	2.50	0.94	14	3.20	1.70	8
ICD	9.36	6.88	79	6.84	5.07	113	6.50	5.99	107	5.91	2.50	48
LT	6.12	6.63	94	3.31	4.74	130	2.89	5.62	121	2.52	2.43	57
Longevity	85.54	15.57	15	70.99	25.99	17	82.96	24.54	14	68.83	21.43	9
	25 °C, 10^3 cells mL^{-1}			25 °C, 5 × 10^3 cells mL^{-1}			25 °C, 10^4 cells mL^{-1}			25 °C, 5 × 10^4 cells mL^{-1}		
Parameters	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
No. of pairs			15			14			13			15
HS (%)	98.82		–	98.09		–	98.45		–	97.51		–
EDT	1.68	0.38	62	1.65	0.31	106	1.66	0.34	110	1.65	0.45	99
CS	6.84	3.32	62	10.33	3.81	106	13.45	4.22	110	14.98	3.52	99
EPR	2.62	1.36	13	4.31	1.62	14	5.14	2.14	11	5.75	1.89	13
ICD	2.85	1.32	49	2.48	1.34	62	2.80	2.68	98	2.85	1.81	84
LT	1.96	1.91	62	1.29	1.77	106	1.22	2.54	110	1.39	1.64	99
Longevity	31.38	8.76	13	30.94	12.63	14	30.43	14.39	12	30.48	18.85	15

HS, hatching success (%); EDT, embryonic development time (days); CS, clutch size (eggs clutch^{-1}); EPR, egg production rate (eggs female^{-1} day^{-1}); ICD, interclutch duration (days); LT, latency time (days); Longevity, longevity of adult females (days).

high temperature (i.e. 24 °C). However, we did not find any evidence of parasitic infections on or in the experimental animals during the present study. An increase in mortality with temperature has often been observed in copepods (Jamieson, 1986; Jamieson & Burns, 1988; Amarasinghe, Boersma & Vijverberg, 1997; Devreker *et al.*, 2004; Zeller *et al.*, 2004; Devreker, Souissi & Seuront, 2005). Williams & Jones (1994) showed that mortality of the copepod *Tisbe battagliai* increased with reduction in food supply and was enhanced with a rise in temperature from 15 to 25 °C. Similar results were obtained with *E. japonicus* in the present study, suggesting that increases in water temperature might induce high mortality when *E. japonicus* faces severe food shortages in the field.

Adult body size of copepods has been shown to increase asymptotically with increasing FC (Hart, 1996) and also to increase with decreasing temperature in the presence of excess food (Jamieson & Burns, 1988; Ban, 1994; Lee *et al.*, 2003). Nevertheless, the combined effects of these two factors are poorly understood. According to results of laboratory experiments using a mixture of algae with more than a 20-fold difference in FC at four temperatures from 5 to 20 °C, adult size of *Temora longicornis* was more influenced by temperature than by FC, while that of *Pseudocalanus elongatus* was equally affected by both factors for the same ranges of temperature and FC (Klein Breteler & Gonzalez, 1988). It has been shown that the food effect on adult body size and weight of *Calanus chilensis* is greater than that of temperature without an interaction between food and temperature ranges experienced *in situ* (Escribano, Irribarren & Rodriguez, 1997). Ban (1994) showed that female body size in *Eurytemora affinis* decreased by up to 32% under food-limited conditions at 15 °C, but only declined by 10% when temperature increased from 10 to 20 °C with sufficient food supply, suggesting that FC was more influential than temperature with respect to copepod body size. A temperature increase from 10 to 25 °C, which is typical of the range occurring in Lake Biwa, induced only a 5% decrease of adult *E. japonicus* PL under excess food supply (Liu *et al.*, 2014), while, in the present study, food limitation induced a 4% and 16% reduction of body size under sufficient food supply at 15 and 25 °C, respectively. This suggests a temperature-mediated food effect on body size of the copepod, implying that body size of adult *E. japonicus* is potentially more influenced by food shortage at temperatures >15 °C.

Adult body sizes of *E. japonicus* reared under sufficient food supply in the present study were larger than those of individuals collected from Lake Biwa, while those reared under limiting food levels were similar in

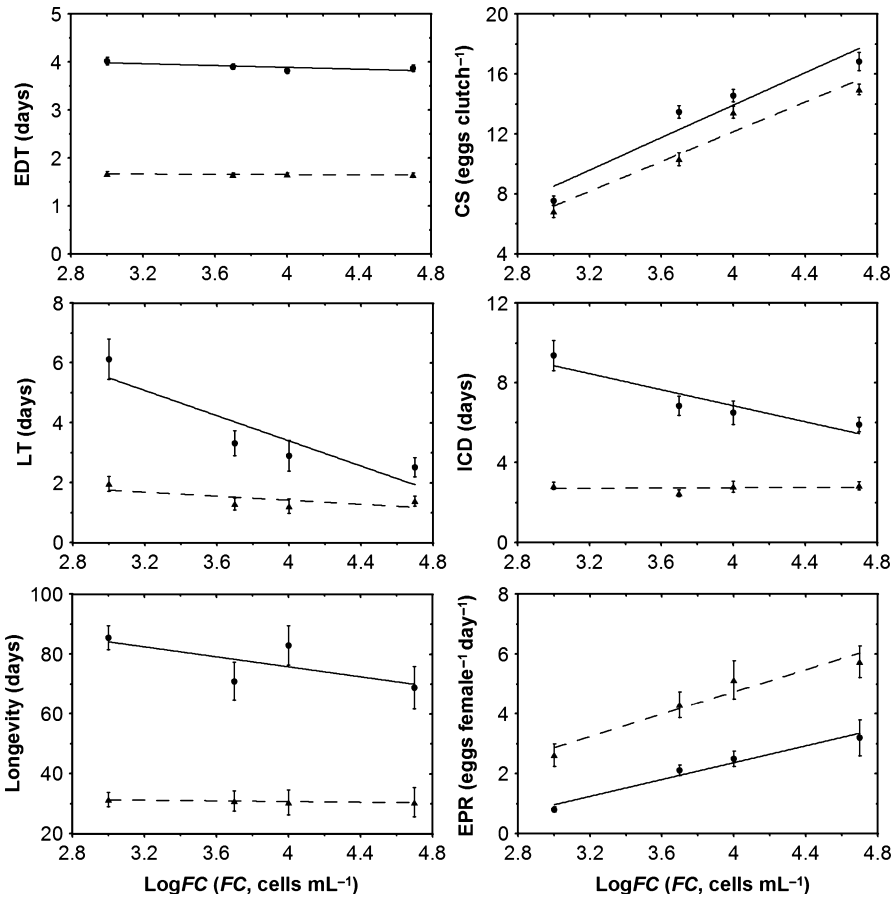

Fig. 5 Reproductive parameters of *Eodiaptomus japonicus* reared under four food concentrations (FC, cells mL^{-1}) at 15 (*circles*) and 25 °C (*triangles*). The regression lines are for the experiments at 15 (*solid lines*) and 25 °C (*dashed lines*), respectively. Acronyms are as follows: EDT (embryonic development time, days), CS (clutch size, eggs clutch^{-1}), LT (latency time, days), ICD (interclutch duration, days), *Longevity* (longevity of females, days), EPR (egg production rate, eggs female^{-1} day^{-1}). *Vertical bars* indicate standard error.

size (Kawabata, 1987; Kawabata & Urabe, 1998). Therefore, natural populations may experience a limited food supply at times as suggested by Kawabata (1989). On the other hand, large zooplankton have been shown to be selected by visually oriented predators, such as planktivorous fish (Svensson, 1997), and copepods usually represent the principal prey for small planktivorous fish (Plounevez & Champalbert, 1999; Turner, 2004). Thus, predation by fish might affect the body size distribution of the copepods in the field. *E. japonicus* is known to be an important food resource for the dominant planktivorous fish, *Plecoglossus altivelis*, in Lake Biwa (Kawabata *et al*., 2002). Stomach content analysis of *P. altivelis* showed an almost 90% occurrence of *E. japonicus* (Kawabata *et al*., 2002). Although *E. japonicus* in our study grew larger at higher FCs, the occurrence of the larger individuals, which are more easily perceived by fish (Mahjoub *et al*., 2011), in the lake might be limited by this top-down control even under excess food. Unfortunately, we do not have any reliable data for the impact of fish predation on the copepods in the lake. According to long-term analysis over the last four decades, total zooplankton abundance has shown a positive correlation with phytoplankton biomass in Lake Biwa (Hsieh *et al*., 2011). These results and those of previously published studies indicate that food availability may control zooplankton community dynamics in the lake.

In the present study, the growth coefficient (k) increased significantly with increasing FC at 25 °C but not at 15 °C, which may be attributed to temperature-mediated metabolic cost (Lampert, 1977b; Gillooly *et al*., 2001; Alcaraz *et al*., 2013). In several marine copepods, it has been shown that oxygen consumption rates increase with increasing temperature (i.e. 1–25 °C) (Castellani *et al*., 2005; Alcaraz *et al*., 2013; Cruz *et al*., 2013). For example, oxygen consumption in *Oithona similis* was 0.03 and 0.42 μL O_2 μg C^{-1} day^{-1} at 4.6 °C and 25 °C, respectively (Castellani *et al*., 2005), while in *Centropages chierchiae*, it was *c*. 0.03 and 0.15 μL O_2 μg C^{-1} day^{-1} at 8 and 24 °C, respectively (Cruz *et al*., 2013). It has also been shown that the Q_{10} of metabolic rates in several marine copepods was 2–3 (Lee, Ikeda & Ban, 2001; Isla & Perissinotto, 2004; Castellani *et al*., 2005). As a result of physiological processes, temperature mediates food effects on carbon assimilation, and it plays an important role in the efficiency of a diet supplied to animals (Lam-

Table 7 Generalised linear models (GLM) show the effect of temperature (Temp) and food concentration (Food) on embryonic development time (EDT), clutch size (CS), egg production rate (EPR), interclutch duration (ICD), latency time (LT) and Longevity of female *Eodiaptomus japonicus* reared in different experimental conditions

Factor	d.f.	Chi-square	*P* value
EDT			
Temp	1	3037.434	<0.001
Food	3	3.747	0.290
Temp × Food	3	2.532	0.469
CS			
Temp	1	30.489	<0.001
Food	3	392.579	<0.001
Temp × Food	3	9.511	0.023
EPR			
Temp	1	81.775	<0.001
Food	3	63.770	<0.001
Temp × Food	3	1.695	0.638
ICD			
Temp	1	166.357	<0.001
Food	3	13.019	0.005
Temp × Food	3	12.064	0.007
LT			
Temp	1	55.934	<0.001
Food	3	27.950	<0.001
Temp × Food	3	12.444	0.006
Longevity			
Temp	1	162.890	<0.001
Food	3	6.019	0.111
Temp × Food	3	5.550	0.136

d.f., degrees of freedom.

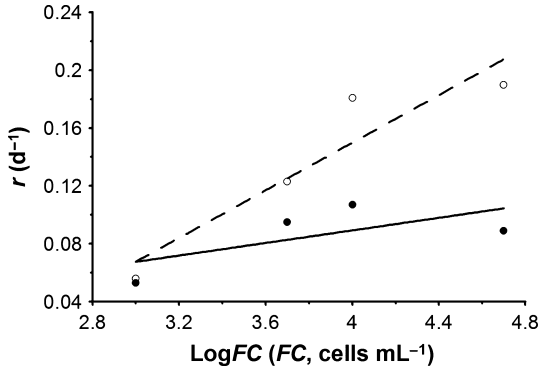

Fig. 6 Estimated population growth rate (r, day^{-1}) of *Eodiaptomus japonicus* reared under different food treatments at 15 (*filled symbols* and *solid line*) and 25 °C (*open symbols* and *dashed line*).

pert, 1977a; Jamieson, 1986; Klein Breteler & Gonzalez, 1986; Jamieson & Burns, 1988; Klein Breteler *et al*., 1995; McKinnon, 1996). Net production efficiencies (NPEs) in *Daphnia pulex* were more influenced by food shortage at higher temperature; when FCs decreased from 2.0 to 0.1 mg C L^{-1}, the NPEs decreased from 85 to 60% and from 75 to 10% at 15 and 25 °C, respectively (Lampert, 1977b). Therefore, food effects on individual growth might only rarely be found in cold water due to the low metabolic cost. Further study of the metabolism of *E. japonicus* (e.g. respiration rate) will be required to clarify this kind of a relationship.

The CSs of *E. japonicus* exhibited similar trends against FC at the two tested temperatures, whereas ICDs were significantly influenced by the two factors and their interactions. Consequently, EPR increased with increasing FC at both temperatures, always being higher at 25 °C than at 15 °C. Probably, this difference may be attributed to prolonged and more variable ICDs at 15 °C due to delayed spawning from the previous hatching of nauplii, that is longer LT as EDT was independent from FC. Since LT represents a part of oocyte maturation time (Watras & Haney, 1980; Williamson & Butler, 1986), prolonged LT was attributed to longer maturation time due to less food uptake and low temperature (Castellani *et al*., 2005; Jiménez-Melero *et al*., 2012).

Finally, population growth rate (r) of *E. japonicus* calculated from the life history parameters increased significantly with increasing FC at 25 °C, but not at 15 °C. This implies that r is more influenced by food shortage at higher temperatures than at 15 °C, as was observed for somatic growth. Considering the probable food limitation of this copepod species in Lake Biwa deduced from the comparison between body sizes of adults in our laboratory studies (Liu *et al*., 2014, this study) and the field, both growth and population dynamics of this copepod might be affected by food shortage in the lake.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Parameter estimates for regression of prosome length of *Eodiaptomus japonicus* on food concentration for each post-larval stage at 15 and 25 °C.

Figure S1. Prosome length of juvenile and adult stages of *Eodiaptomus japonicus* over a food concentration gradient at 15 and 25 °C.

Figure S2. Results of the multiple comparison of slopes following the ANCOVA testing the potential differences between stages in the response of prosome length to increasing food concentration at 15 and 25 °C.

(Manuscript accepted 2 June 2015)

Table S1. Parameter estimates (Estim.) for regression of prosome length of *Eodiaptomus japonicus* on food concentration for each post-larval stage (see Figure S1) at 15 and 25 ºC. For every regression, the intercept (a) and coefficient of linear regression (b) are provided. Values of $p < 0.05$ are shown in bold.

Temperature	Stage		Estim.	SE	*t*	*p*
15 ºC	C1	a	293.83	28.135	54.98	**< 0.001**
		b	13.00	7.219	7.73	0.315
	C2	a	402.53	28.135	60.42	**< 0.001**
		b	9.47	7.219	7.04	0.105
	C3	a	519.28	28.135	66.27	0.094
		b	6.36	7.219	6.43	**0.034**
	C4	a	565.87	28.135	68.60	0.599
		b	16.30	7.219	8.37	0.695
	C5	a	682.20	28.135	74.42	**< 0.001**
		b	13.71	7.219	7.86	0.381
	AdM	a	671.11	28.135	73.87	**< 0.001**
		b	34.19	7.219	11.86	**0.008**
	AdF	a	750.94	28.135	77.86	**< 0.001**
		b	35.41	7.219	12.10	**0.005**
25 ºC	C1	a	274.84	45.057	29.13	**< 0.001**
		b	11.81	11.560	8.06	**0.007**
	C2	a	331.84	45.057	30.91	**0.002**
		b	20.90	11.560	9.16	0.063
	C3	a	402.49	45.057	33.12	0.134
		b	29.22	11.560	10.18	0.333
	C4	a	477.29	45.057	35.46	0.467
		b	32.29	11.560	10.55	0.539
	C5	a	529.09	45.057	37.08	**0.033**
		b	46.35	11.560	12.26	0.297
	AdM	a	544.01	45.057	37.54	**0.013**
		b	59.12	11.560	13.82	**0.019**
	AdF	a	613.95	45.057	39.73	**< 0.001**
		b	62.52	11.560	14.23	**0.008**

Figure S1. Prosome length (μm) of juvenile (C1−C5) and adult stages (AdM, male; AdF, female) of *Eodiaptomus japonicus* over a food concentration gradient at 15 (A) and 25 ºC (B). Lengths are mean values and lines represent statistically significant regressions (see Table S1).

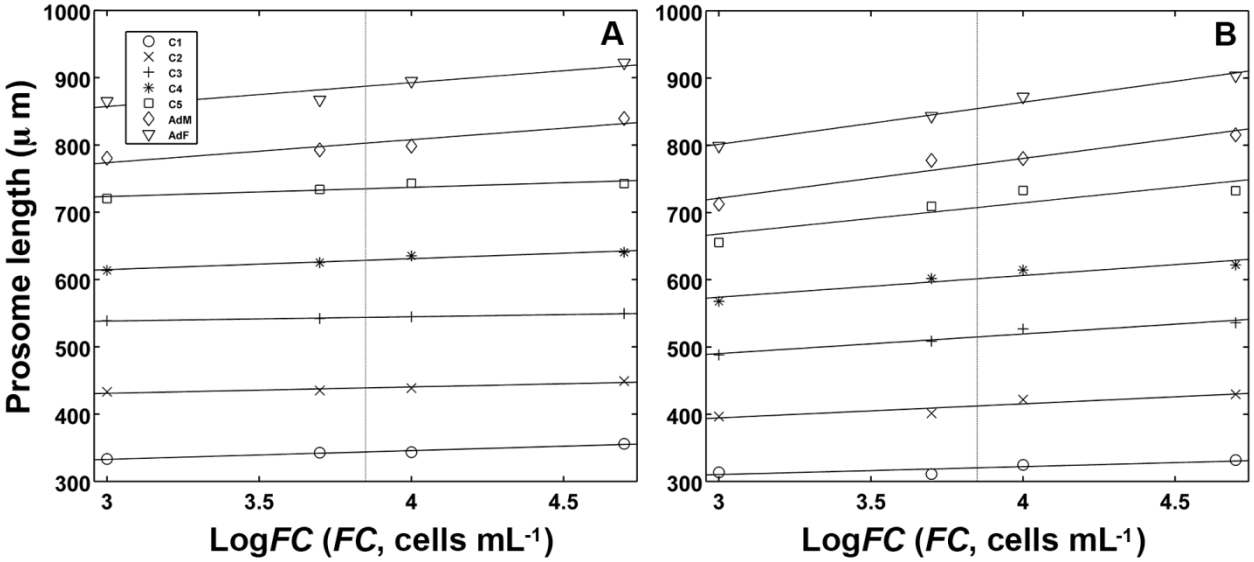

Figure S2. Results of the multiple comparison of slopes following the ANCOVA testing the potential differences between stages in the response of prosome length to increasing food concentration at 15 (A) and 25 ºC (B). Estimated slope values and standard deviations (*horizontal bars*) are indicated. The same letters indicate statistically non-significant differences between the developmental stages with post-hoc test at $p < 0.05$.